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Guidance Document on Estimating Persistence and Degradation Kinetics from Environmental Fate Studies on Pesticides in EU Registration

The Final Report of the Work Group on Degradation Kinetics of FOCUS
(FOrum for the Co-ordination of pesticide fate models and their USe)

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FOREWORD BY THE FOCUS STEERING COMMITTEE

Since its beginning in 1993, FOCUS (FOrum for the Co-ordination of pesticide fate models and their USe) has established a number of work groups to develop procedures for estimating concentrations of plant protection products and their metabolites in various environmental compartments (ground water, surface water, soil, sediment, and air). One of the most important parameters in these environmental assessments is the degradation of these compounds. Although the procedures for conducting the laboratory and field studies measuring degradation are specified in study guidelines, the procedures for calculating degradation rates have not been standardised. The general procedures for calculating degradation rates are well known but the assumptions made during this process can appreciably affect the results. Therefore FOCUS established a work group of experts from regulatory authorities, research institutes, and industry to develop recommendations for calculating degradation kinetics in the EU registration process. This FOCUS group met nine times between September 2002 and January 2005, carefully considered the comments of the member states to an initial draft of its findings, and prepared version 1.0 of this report in February 2005 outlining its recommendations for calculating degradation kinetics of parent and metabolites in soil, water, and water sediment systems.

The EFSA PPR Panel reviewed version 1.0 of this guidance document and adopted its opinion on this matter in December 2005. The summary of this opinion stated that the Panel sees this guidance document as a significant step forward in the risk-assessment process and that the Panel supports and endorses the document's overall conclusions and recommendations. Furthermore this summary stated that the Panel recommends the Commission to adopt this document including consideration of the following issues:

(1a) The Panel recommended adding a new chapter on uncertainties that systematically summarizes the potential sources of uncertainty and that discusses their combined effect on the uncertainty of the assessment procedure as a whole.

(1b) The guidance document should recommend including an evaluation of uncertainties in the report of each assessment.

(2) The Panel recommends holding training courses for MS authorities.

(3) The Panel recommends organising comparison exercises and/or ring tests to ensure that the proposed procedures are intelligible, robust and precise enough.

(4) The Panel recommends developing appropriate software tools to perform the kinetic analyses as proposed in the guidance document.

(5) The Panel recommends that the Commission reviews experiences on the conservativeness of the procedures gained by the practical use of recommended procedures as a basis for regular updates of the guidance document.

In response to the comments made by the EFSA PPR Panel, the FOCUS Steering Committee (SC) asked the work group to produce this final version (version 2.0) of the report. The SC reacts to the recommendations by the Panel as follows:

(1a) This chapter was not part of the remit of this work group and would lead to a considerable delay in the finalisation of this guidance document which the SC considers undesirable. Therefore the SC decided not to follow this recommendation. However, new Section 11.5 was added to the guidance document that gives some reflections on this matter.

(1b) This recommendation was included in the document (see Chapter 12, point 4 in the list of aspects to be addressed).

(2) The FOCUS Degradation Kinetics Work Group organised a training course for all Member State authorities in January 2005 in Brussels (and additionally a delegation from the workgroup organised in January 2006 training for USEPA and the Canadian registration authorities in Washington). The training material will be made available at the FOCUS website in spring 2006.

(3) The SC agrees that such comparison exercises are useful but decided not to give priority to this activity in view of limited resources for travel budgets and in view of other urgent needs for developing guidance.

(4) Several companies have taken the initiative to develop such software, which is expected to become available in 2006. However, the guidance described in the report has been designed to be generally applicable, independent of specific software tools.

(5) The SC agrees that reviewing such experiences is necessary. However, the first step is to collect and report these experiences systematically. The SC cannot commit a FOCUS work group to this task because such experiences are gained in the EU risk assessment procedure co-ordinated by the EFSA PRAPeR team. The SC suggests therefore that this team collects and reports these experiences.

The calculation of kinetic parameters is a fundamental component of environmental risk assessments of plant protection products. The recommendations of this report can and will impact the evaluation process. These impacts include more complex evaluation processes, more detailed documentation of calculations, and potentially the need to conduct additional experimental studies. Therefore, the FOCUS Steering Committee recommends the following

phased approach to the introduction of the recommendations: All regulatory studies that include estimation of degradation kinetics completed later than nine months after the adoption of this report by the Working Group Plant Protection Products – Legislation should follow the recommendations in this report. Generally, exposure assessments in dossiers submitted nine months after this adoption should be based on degradation parameters derived with procedures in agreement with the principles of this report. If kinetic analyses in degradation studies completed prior to nine months after adoption of this report by the EU adhere to the main principles of this report, then the kinetic analyses do not need to be repeated even if they do not conform exactly to the procedures recommended by the report.

Table of Contents

EXECUTIVE SUMMARY

Why is the work of the FOCUS Work Group on Degradation Kinetics important?

Degradation rates of active substances in crop protection products and their metabolites are among the most important parameters for assessing environmental exposure. Differences in approaches can substantially affect the degradation rates calculated from experimental data obtained in laboratory and field studies.

Currently, degradation rates calculated by registrants are usually re-calculated by regulatory agencies and different regulatory agencies can propose different degradation rates. A harmonised approach can reduce the need to re-calculate degradation rates and provide more certainty for these important parameters in increasingly complex risk assessments.

What regulatory endpoints does this report address?

The work group has proposed approaches for calculating degradation kinetics for parent and metabolites for laboratory soil and water studies, field studies, and water-sediment studies. For parent and metabolites, this includes DT50 and DT90 values for triggering additional studies and degradation rates for use in models for estimating environmental exposure. For metabolites, an additional endpoint is the rate of formation (often described using parent or precursor metabolite kinetics and a formation fraction). The same endpoints are addressed for water-sediment studies, but these endpoints are calculated for the overall system as well as the water column and sediment separately so that the degradation parameters required in the FOCUS surface water scenarios can be obtained.

What are the recommendations regarding calculation of degradation kinetics?

Parent

The work group has divided its recommendations depending on the use of the kinetic description. For calculation of DT50 and DT90 values that trigger additional studies, the best available model should be used. When calculating degradation rates or corresponding half-lives to be used in models for calculating Predicted Environmental Concentrations for ground and surface water, single first-order kinetics are used when the fit is acceptable because of the limitations of existing mechanistic models. Alternative approaches have been outlined for

cases in which the decline in degradation rate is not due to a decline in microbial activity or similar experimental artefacts.

Metabolites

One of the most important factors in determining the kinetics of metabolites is to correctly describe the degradation of the parent or predecessor metabolite. Because each metabolite undergoes both formation and decline, the uncertainty associated with most metabolite kinetics is usually greater than for parent compounds. When several metabolites are involved, often a stepwise approach may be useful to ensure that the resulting kinetic descriptions are reasonable. In most cases, single first-order kinetics are suitable for describing the degradation of metabolites, but bi-phasic approaches are presented and can be used when appropriate.

Water-Sediment Studies

Because of the complexity of the water-sediment system, a complete description requires a number of degradation and transfer parameters. Therefore, the procedures for determining system parameters must make certain that the description is not the result of unrealistic combinations of the large number of parameters. A two level process, for both parent and metabolites, has been developed to evaluate the kinetic parameters from water-sediment studies. In the first level, the degradation of parent and the degradation or dissipation of metabolites in the overall system are estimated, plus parent and metabolite dissipation from the water column and from the sediment separately. In the second level, the degradation rates in the water column and sediment are estimated.

What other recommendations are included in this report?

A list of recommendations has been provided for dealing with general data issues such as data quality, replicates, data weighting and transformation, concentrations below the limits of quantification and detection, outliers, starting concentrations, and experimental conditions that might influence the observed kinetics (experimental artefacts).

When used as input to environmental models, field data should be normalised to standard conditions either before or during the calculation of kinetic parameters.

If an average of results from several studies is desired for either modelling or a trigger value, usually an average of the kinetic parameters will suffice. However, in some circumstances,

such as when degradation rates are strong functions of soil properties such as pH, averaging is not appropriate. The geometric mean should normally be used as the average of degradation parameters because it provides the best representation of the average of different first order degradation curves over the entire time period. Using the geometric mean also has the advantage that the same result is obtained from averaging first-order degradation rates and averaging the corresponding half-lives.

In order to assess the fit of predicted and observed concentrations and to compare fits obtained with different models the work group has recommended certain visual and statistical evaluations. The work group recommends that steps be taken to develop tools to help implement the recommendations for kinetic assessments that are described in this report. In the short term some simple tools for calculating relevant statistics should be developed. In the long term the development of a suitable software tool for fitting data to kinetic models should be explored, although the development of software is usually a lengthy and expensive process.

What other accomplishments are described in this report?

The report contains descriptions of the software packages most commonly used to determine degradation kinetics and the results of standard test cases run with many of these models. The report contains the equations for calculating Predicted Environmental Concentrations in soil for all of the kinetic models that have been recommended for describing degradation in soil. Previously the work of the FOCUS Soil Modelling Work Group presented equations only for single first-order kinetics.

The report describes methods for incorporating bi-phasic kinetics into soil models used to assess movement of parent and metabolites to ground and surface water. The report also describes an example approach for assessing compounds with bi-phasic kinetics resulting from increasing sorption.

1 INTRODUCTION

In the EU registration process for crop protection products, degradation rates of active substances and their metabolites in the environment are among the most important parameters for assessing environmental exposure as well as potential to move to ground water. Although the procedures for conducting laboratory and field studies have been specified in study guidelines, the procedures for calculating degradation rates have not been standardised. The general procedure for calculating degradation rates of first-order reactions is well known, but various assumptions such as whether to transform the data or how values below the detection limit are treated can sometimes make appreciable differences in the calculated degradation rates. Less straightforward are the procedures for calculating kinetic parameters for bi-phasic degradation, degradation rates of metabolites, and the kinetic parameters from water-sediment studies. However, the increasing importance of metabolites in regulatory assessments has resulted in increasing importance of metabolite degradation rates. Also, the surface water assessments proposed by the FOCUS Surface Water Scenarios Workgroup and the Med-Rice work group require the degradation rates from the water column and sediment in water-sediment studies.

Several groups have issued instructions on calculating degradation rates (for example, the EU in the soil persistence paper, the U.S. EPA, and a joint German working group of the IVA, BVL, and UBA for calculating degradation rates of metabolites). However, a more comprehensive document is needed to cover all of the current issues. Several commonly used software packages have functions for calculating degradation rates, but these differ slightly.

Because of the uncertainty associated with degradation rates in study reports, many regulatory agencies re-calculate the degradation rate as a part of their review. This can add appreciably to the time required for review of studies. In addition, the results of risk assessments are often sensitive to the value of degradation rates used in these assessments. As these risk assessments have become more complex, changes in the degradation rates can also result in substantial additional effort as well as delay the approval of a registration. Definitive guidelines would facilitate and standardise calculation of degradation rates and reduce the work required by both regulatory agencies and registrants. Such guidelines would also promote harmonisation between countries, if they were adopted throughout the world.

The objective of this FOCUS work group on degradation kinetics was to prepare a guidance document for calculating degradation rates of parent and metabolites from laboratory and field studies. One special area included was the calculation of both parent and metabolite degradation rates and degradation rates from water-sediment studies. The work group also performed a review of the differences between various existing software packages and their associated advantages and disadvantages. Additional areas which also became important included development of a glossary of terms, normalisation of field data, development of appropriate statistical measures to determine adequate fits of kinetic models and experimental data, and implementation of higher-tier assessments including increasing sorption with time and bi-phasic kinetics.

The emphasis of the work group was on analysing data sets from existing regulatory studies, rather than on developing strategies for conducting these regulatory studies. Of course the information presented in this report could be used in the development of such strategies.

Throughout the report, the examples (unless otherwise noted) are from actual studies. This explains why some of the kinetic fits are less than perfect due to natural variability and also demonstrates that the recommendations of the work group are directly applicable to real-life situations. The mention of software packages in the report, outside of Chapter 13 and Appendix 11, reflects only their use in a particular situation or example and should not be considered as an endorsement of the software package.

2 GLOSSARY

Aerobic degradation

Degradation occurring in the presence of molecular oxygen.

Alpha risk

The probability of accepting the alternate hypothesis when, in fact, the null hypothesis is true.

Anaerobic degradation

Degradation occurring under exclusion of molecular oxygen.

Arithmetic mean

A term used in descriptive statistics to describe the location of a distribution.

$$\bar{x} = \frac{(x_1 + x_2 + ... + x_n)}{n}$$

with $\bar{x}$ = arithmetic mean

x_i= i th observation

n= total number of observations

Beta risk

The probability of accepting the null hypothesis when, in fact, the alternate hypothesis is true.

Bi-phasic

The term bi-phasic kinetics is used pragmatically to describe changes in dissipation or degradation rates over time that are not proportional to the concentration or amount of compound remaining, for example, not single first order, particularly when these rates slow down to produce a residual tail in the dissipation or degradation pattern.

Bound residues

Definitions from three different sources, which are similar but not identical, are presented here:

Chemical species in soil, plant or animal tissue originating from a pesticide, (generally radio labelled) that are unextracted by a standard method, such as Soxhlet solvent extraction, which does not substantially change the chemical nature of the residues. These unextractable residues are considered to exclude small fragments recycled through metabolic pathways into natural products. (after Roberts, T.R. (1984), Non-extractable pesticide residues in soils and plants. Pure Applied Chem., 56, 945-956.)

Bound residues represent compounds in soil, plant or animal, which persist in the matrix in the form of the parent substance or its metabolite(s)/transformation products after extraction. The extraction method must not substantially change the compounds themselves or the structure of the matrix. The nature of the bond can be clarified in part by matrix-altering extraction methods and sophisticated analytical techniques. To date, for example, covalent ionic and sorptive bonds, as well as entrapments, have been identified in this way. In general, the formation of bound residues reduces the bioaccessability and the bioavailability significantly (from OECD, 2002: OECD guideline for the testing of chemicals 307: Aerobic and anaerobic transformation in soil. 24th April 2002, 17p.).

Non-extractable residues (sometimes referred to as 'bound' or 'non-extracted' residues) in plants and soils are defined as chemical species originating from pesticides used according to good agricultural practice that cannot be extracted by methods which do not significantly change the chemical nature of these residues. These non-extractable residues are not considered to include fragments through metabolic pathways leading to natural products (from Council Directive 97/57/EC (1997) establishing Annex VI to Directive 91/414/EEC concerning the placing of plant protection products on the market. Official Journal of the European Communities, Series L 265, p87-109, 27.September 1997. Please note that this Directive is currently in the process of a revision).

Breakdown products: see degradation products

Chi-square Test

The χ^2-test considers the deviations between observed and calculated values (numerator) for each separate model relative to the uncertainty of the measurements (denominator). The latter term describes the measurement error with a common error model. *Err* is a term of proportionality scaled with the mean observed which describes the dependence on the measured values. The overall measurement error term is thus constant throughout the measurement period.

$$\chi^2 = \sum \frac{(C - O)^2}{(err/100 \text{ x } \overline{O})^2}$$

where

C = calculated value

O = observed value

$\overline{O}$ = mean of all observed values (element of scale in error model (denominator))

err = measurement error percentage(element of proportionality in error model (denominator))

The calculated χ^2 for a specific fit may be compared to tabulated $\chi^2_{m,\alpha}$

where

m = degrees of freedom = number of measurements minus number of model parameters
α = probability that one may obtain the given or higher χ^2 value by chance.

Compartment

In this Guidance Document, the term compartment may refer to one of three different aspects: environmental, kinetics and chemical aspects, all of which are interrelated. The environmental aspect refers to the test system under consideration. If the test system as a whole is considered, the test system comprises one environmental compartment. However, sometimes the test system can be considered to consist of two or more compartments, e.g. a water-sediment system can be considered to have separate compartments for the water column and sediment. The kinetics aspects refer to how the kinetics are related to these environmental compartments, e.g. a single kinetics compartment may be used to describe a single environmental compartment, e.g. soil in an aerobic degradation study. However, the environmental compartment may be subdivided into more than one kinetics compartment, such as in biphasic kinetics. Finally, the chemical aspect refers to which chemical is being considered in the kinetics for a given environmental compartment, e.g. a parent compound or a metabolite.

Conditional parameter

Result of a parameter estimation procedure, if one or several other parameters are kept fixed during the estimation procedure.

Confidence interval

Estimate of the uncertainty in a model parameter; the interval denotes a particular probability that the 'true' value of the model parameter lies within this confidence interval.

Constituting autonomous differential equation

Constituting autonomous differential equations are differential equations in which the right hand side only contains state variables (variables such as concentration describing the state of the system at some instant of time). Autonomous differential equations must, therefore, not contain dose (initial concentration or application rate) and time.

Constraint equation

Not all parameter values of a model are admissible, e.g. degradation rate constants have to be greater than or equal to zero (In this case the constraint equation $k \geq 0$ applies). Other possible constraints are upper boundaries for parameter estimates, e.g. the parameter g in the bi-exponential model (fraction of compound placed in one of the two compartments) cannot exceed the value 1.

Convergence steps

Iteration procedures stop if either the prescribed accuracy is reached or a maximum number of iteration steps is surpassed. Usually, a procedure stops if in several (typically 5) subsequent iteration steps the convergence criterion is fulfilled.

Decline phase

Time period with an observable decrease of the concentration/amount of the metabolite. The dissipation/degradation of the metabolite may be accompanied with formation processes, but the degradation rate is higher than the formation rate.

Degradation

Degradation processes, such as microbial degradation, hydrolysis and photolysis, break down substances in different environmental compartments by transforming them into degradation products. Degradation also includes processes such as oxidation and transformation into microbial biosynthetates or polymerization products, which may result in larger molecules than the parent substance.

Degradation products

All substances resulting from biotic or abiotic transformation reactions of the test substance including CO_2, microbial biosynthetates, and products that are in bound residues.

Degrees of freedom

Used in slightly different senses throughout the study of statistics, Degrees of Freedom (DF) were first introduced by Fisher (Statistical methods for research workers. Edinburgh: Oliver & Boyd, 1925) based on the idea of degrees of freedom in a dynamical system (for example, the number of independent co-ordinate values which are necessary to determine the system). In some circumstances the term degrees of freedom is used to denote the number of independent comparisons that can be made between the members of a sample. In the context of this report, to calculate DF for the chi2 test use DF = number of observations

minus number of parameters. See descriptions on the F-test (Section 6.3.2.4) and t-test (Section 6.3.1.3) for details on how to calculate DF for these tests.

Deg T50/90

Term with no association to any particular type of kinetics to describe the time taken for a 50/90% decline in mass or concentration of a substance to occur by degradation from the environment or an environmental compartment after it has been applied to, formed in, or transferred to, an environmental compartment. The first half-life of a substance may be identical to the DegT50. But for the purposes of this document, the term half-life has been restricted to mean the half-life from fitting single first-order (SFO) kinetics to data, due to its familiar association with the "half-life concept" of SFO kinetics, and to avoid confusion in the use of terminology.

Differential equation

Degradation curves are generally derived from mass balance equations in differential form, i.e. as differential equations (when possible, constituting autonomous differential equations are preferred and must be used in environmental fate models). For example, the single first-order model is derived from the differential equation:

$$\frac{dMP}{dt} = -k_p\, MP \quad MP(0) = MP_0$$

with MP = mass of the parent compound

MP_0 = initial mass of the parent compound

k_P = rate constant for the parent compound

This is in contrast to the analytical solution of the preceding equation, which is:

$$MP_t = MP_0 e^{-k_p t}$$

with MP_t = mass of the parent compound at time t

If a metabolite is included, one obtains a further differential equation for the metabolite:

$$\frac{dMM}{dt} = k_p MP - k_m MM \qquad \text{(assuming 100\% formation of the metabolite)}$$

with MM = mass of the metabolite

k_m = rate constant for the metabolite

Disappearance: see dissipation

Disappearance/Dissipation time (DTx)

Term with no association to any particular type of kinetics to describe the time taken for a 50/90% decline in mass or concentration of a substance to occur by dissipation from the environment or an environmental compartment after it has been applied to, formed in, or transferred to, an environmental compartment. DTx does not differentiate between transfer processes and degradation processes. The first half-life of a substance may be identical to the DT50. But for the purposes of this document, the term half-life has been restricted to mean the half-life from fitting single first-order (SFO) kinetics to data, due to its familiar association with the "half-life concept" of SFO kinetics, and to avoid confusion in the use of terminology.

Preferred terms for description of degradation/dissipation of substance

DTx	Generic description for time taken for x percent of substance to disappear from a compartment by dissipation processes
DegTx	Description for time taken for x percent of substance to disappear from a compartment due to degradation processes alone
Half-life	Description for time taken for 50% of substance to disappear/dissipate from a compartment following single first-order kinetics

Dissipation

Overall process leading to the eventual disappearance of substances from the environment, or an environmental compartment. Dissipation comprises two main types of processes: transfer processes, such as volatilisation, leaching, plant uptake, run-off or erosion that transfer substances to different environmental compartments; and degradation processes such as microbial degradation, hydrolysis and/or photolysis transforming substances into degradation products.

Dissipation/degradation kinetics

Equation or set of equations used to describe the eventual disappearance of substances from the environment, or an environmental compartment by various dissipation/degradation processes.

Dissipation/degradation rate

The first time derivative for the dissipation/degradation for a substance, namely the relative amount per unit time by which the amount ($N\ T^{-1}$) or mass ($M\ T^{-1}$) of the substance decreases.[1]

Extrapolation

Estimation of the value of an entity, where the value is outside the boundaries of the measured data, e.g. extrapolation of endpoints beyond the duration of the experiment.

Fitting

Mathematical procedure to find optimal kinetic parameters for a kinetic model to describe measured data.

Formation fraction

Fraction of the amount of substance that is transformed from a precursor into a degradation product (the precursor may be the parent or another degradation product). The formation fraction is expressed as a molar fraction.

$f_{ij} = F_{ij}/F_{iTotal}$

f_{ij}: formation fraction of degradation product j from i

F_{ij}: flow from i to j

F_{iTotal}: total flow from i

For first-order reactions, the formation fraction of a degradation product j from parent or preceding degradation product i can be calculated from the first-order rate constants as follows:

$f_{ij} = k_{ij}/k_{iTotal}$

k_{ij}: first-order rate constant from i to j

k_{iTotal}: sum of first-order rate constants from i

The formation fraction can also be directly estimated as a free parameter in a fitting procedure. Conceivably, flows to different compartments/degradation products may obey different kinetics of formation, in which case the formation fraction of a degradation product would be a function of concentration.

[1] M: mass; N: amount of substance (i.e. number of moles); T: time

Differences in molecular weights of the precursor and the degradation product must be taken into account when calculating the mass of the degradation product.

Formation phase

Time period with an observable increase of the concentration/amount of the metabolite. The formation of the metabolite may be accompanied with degradation processes, but the formation rate is higher than the degradation rate.

Formation rate

Formation per unit time of the degradation product from the parent or from a preceding degradation product (time derivative of the amount of product formed, expressed in substance rate ($N\ T^{-1}$) or mass rate ($(M\ T^{-1})^2$. Analogous to the degradation rate of the parent or preceding degradation product to this degradation product only if all concentrations are expressed as molar fractions or percent applied radioactivity, otherwise the ratio of molar masses must be considered.

F-Test for model comparison

Test to compare suitability of different models applied to the same data set; for details see Chapter 6.3.2.4.

Generalized Likelihood Ratio Test for model comparison

Test to compare suitability of different models applied to the same data set; for details see Chapter 6.3.2.4.

Geometric mean

A term used in descriptive statistics to describe the location of a distribution. The geometric mean is the n^{th} root of the product of n numbers. It will always be less than or equal to the arithmetic mean. For details on its use in endpoint selection in a regulatory context, see Chapter 11.

$$\bar{x}_G = \left(x_1 * x_2 * \ldots * x_n\right)^{\frac{1}{n}}$$

with x_i= observation

n= total number of observations

Goodness of fit

Agreement between the model predictions and the experimental data. For applications in kinetics, see Chapter 6.

Half-life

Is the time taken for 50% degradation/dissipation of a test substance described by single first-order kinetics following the concept of radiodecay, where the decay rate constant for each radionuclide is independent of concentration and time.

Interpolation

Estimation of the value of an entity, where the value is within the boundaries of the measured data.

Inverse modelling

A mathematical procedure by which the input parameters to a complex model describing transfer and degradation processes (e.g. leaching models) are fitted by stepwise optimisation of the observed outcome to measured data, rather than vice versa(estimating the outcome based on values of model input parameters).

Kinetic model

Set of assumptions and mathematical expressions that describe the variation of the concentration of the different compounds that participate in a transformation/dissipation process.

Least squares

Principle of least squares: parameters are determined such that the sum of squared deviations between calculated and observed values (RSS= residual sum of squares) is minimal.

$$RSS = \sum_{j=1}^{n} (C_j - O_j)^2$$

C_j = jth calculated value

O_j = jth observed value

The set of values for C that give the minimum RSS is the set of values providing the best fit of the data according to least squares.

Limit of Detection (LOD)

A practical LOD is the lowest level at which an analyte can be reliably detected in matrix > ~90% of the time. An LOD should be specified if it is required by the guidelines being followed, or when estimating and reporting levels between the LOD and LOQ. The LOD can vary substantially from instrument to instrument and with time. The LOD can generally be set

[2] M: mass; N: amount of substance (i.e. number of moles); T: time

at three times the background response in the vicinity of the analyte response, normalised for average recovery at the LOQ level.

Limit of Quantification (LOQ)

A practical LOQ is justified by demonstrating acceptable recovery and precision data for control samples fortified at that level. The average recoveries should range between 70 and 110%, with a relative standard deviation (RSD) of less than or equal to 20%. Most (~70-80%) of the individual recoveries should lie within this range, as well. Typically, five or more fortifications at the LOQ are acceptable, spread over one or two sets. Also, as a general rule, the LOQ should exceed the level corresponding to the noise background of the control matrix, in the vicinity of the analyte response, by a factor of about ten (or any background peaks due to matrix by a factor of about five).

Major metabolite

A degradation product that is formed in amounts of ≥10% (molar fractions or percent applied radioactivity) of the applied amount of active ingredient at any time evaluated during the degradation studies in the compartment (i.e. soil, water and/or sediment) under consideration.

Maximum fraction of the amount of substance

The amount of a metabolite relative to the amount of applied parent (expressed as a molar fraction) at the peak of its formation phase before the start of the decline phase at which the formation rate of a degradation product is equal to its degradation rate. Non-continuous sampling schemes in experiments may result in the predicted kinetic maximum not actually being measured. The maximum fraction is not the same as formation fraction (the maximum fraction cannot exceed the formation fraction and usually is appreciably lower due to degradation of the metabolite that occurs prior to the occurrence of the peak concentration).

Measurement error

The measurement error is the net effect of all sources of measurement variability that cause an observed value to deviate from the true value.

Metabolite: see degradation product

Mineralisation

The complete degradation of an organic compound to CO_2, H_2O and inorganic substances by respiration processes, and CH_4, CO_2, H_2O and inorganic substances by fermentation and/or anaerobic processes.

Minor metabolite

All degradation products that are formed in amounts of < 10% (molar fractions or percent applied radioactivity) of the applied amount of substance at any time evaluated during the degradation studies in the compartment (i.e. soil, water and/or sediment) under consideration.

Model

Mathematical model: equation or systems of equations for simulating or predicting processes. Conceptual model (cf. compartment model): the set of variables and relationships in the natural system that are formalised in the model.
See also kinetic model.

Non-extractable residues

Equivalent to 'bound residues'.

Optimisation

Process whereby the numeric values of the parameters used in a model are systematically adjusted to obtain closer agreement between values calculated by the model and measured data provided by the user.

Optimisation equation or objective function

Measure of the deviation between model and data. Usually, the least squares criterion (cf least squares) is employed. Once the model is given, the criterion measure depends on the model parameters. Parameter estimates are required to minimise the objective function.

Optimisation parameter

Those parameters which are not fixed in an optimisation procedure.

Percent of applied (amount of substance)

The basis for a kinetic evaluation in relative terms should be the percent of the applied amount of substance (unit: mols). In studies where ^{14}C was used as a radiolabel, this corresponds to the '% of applied radioactivity' (% AR).

P-value

The probability that a variate would assume a value greater than or equal to the observed value strictly by chance.

Plateau phase

Period of time between the formation phase and the decline phase during which the dissipation of a substance from an environmental compartment appears to undergo no net change in its concentration or mass with time. The length of a plateau phase depends on how long the formation rate is approximately equal to the dissipation/degradation rate.

Rate constant

A kinetic parameter describing an aspect of the rate at which a substance dissipates from the environment or an environmental compartment. Such parameters may be non-specific, simply describing net dissipation due to degradation and transfer processes, or they may be specific, describing dissipation due to degradation, formation, or transfer. The dimensions of these rate constants can vary.

In a strict sense rate constants will only depend on the temperature. Many of the rate constants considered in this document are pseudo-rate constants, since they depend on other factors as well. However, the term rate constant is employed generically without specifying if it is a true or a pseudo rate constant.

Residuals

Deviation of each calculated (fitted) value from the corresponding measurement value.

Sensitivity analysis

The process whereby the value of a selected parameter is systematically varied to obtain an indication of how sensitive the model outputs are to this change.

Sink

In the context of kinetic analyses, a sink compartment is any compartment without an outflow, regardless of which components it represents. The sink compartment often represents CO_2, bound residues and minor unidentified residues, as well as any metabolite, identified or not, that is not included in the fit. The flow to sink describes all transformation and/or transfer processes leading to the sink components. For example, when fitting the degradation of parent only in a two-compartment model (parent and sink), the flow between parent and sink represents all degradation processes and the sink compartment represents all possible degradation products. The sink compartment may, in specific situations, be linked to measured data (including measured radioactivity levels as CO_2, bound residues, unidentified and unresolved radioactivity, from mass balance data), and this sink data may be fitted in the kinetic analysis.

Standard deviation

Statistical description of how tightly all the various samples are clustered around the mean in a normally distributed set of data.

State variable

Dependent variable of a dynamic system, e.g. concentration or mass of parent or metabolite, describing the state of the system at some instant of time. Dose (initial concentration or application rate) and time are not state variables.

Statistical outlier

Data in a sample, which do not belong to the underlying statistical distribution. Can be interpreted as measurement error.

Time derivative

Rate of change of a function f with respect to time t.

$$\frac{df}{dt} = \lim_{\Delta t \to 0} \frac{f(t + \Delta t) - f(t)}{\Delta t}$$

Transfer fraction

Net fraction of a substance that transfers from one environmental compartment to another, e.g. from water column to sediment.

Transfer phase

Period over which the dissipation of a substance is by net transfer to an environmental compartment and results in a transient increase in its concentration or mass with time in that compartment, observed as a trend to increase residues or a monotonic increase in a kinetic fit to the data with time. The length of time over which a transfer phase appears to occur depends on how rapidly transfer occurs into the environmental compartment, e.g. due to parent transfer into sediment, and how rapidly the substance dissipates/degrades on entering the environmental compartment.

Transfer rate

The first time derivative for the transfer of a substance from one environmental compartment to another, e.g. from the water column to the sediment, namely the amount per unit time by which the concentration ($N\ T^{-1}$) or mass ($M\ T^{-1}$) of the substance transfers from one compartment to another.[3]

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[3] M: mass; N: amount of substance (i.e. number of moles); T: time

Transformation: see degradation

Transformation product: see degradation product

t-Test

If the parameters are normally distributed, then the statistic

$$t = \frac{\hat{a}_i}{\sigma_i}$$

is t-distributed.

$\hat{a}_i$ = estimate of parameter i
σ_i = standard error of parameter i

The probability (p-value) corresponding to the calculated t-value is read from statistical tables or calculated with Excel (TDIST) or statistical packages (one-sided; degrees of freedom equals the number of observations minus the number of model parameters). The parameter is significantly different from zero if the probability is smaller than the selected significance level (see Chapter 6.3.1.3).

Weighting of fits

Assigning different weights to data points depending on justified criteria, like differences in precision at different time points. For example, if the variance of the errors depends on the concentration range, the terms of the sum of squares may be weighted by the error variances (see least squares).

$$RSS_{weighted} = (1/2\sigma_j) \sum_{j=1}^{n} (C_j - O_j)^2$$

C_j = jth calculated value
O_j = jth observed value
σ_j = error of jth value

3 EXISTING GUIDANCE ON EXPERIMENTAL LABORATORY AND FIELD DEGRADATION STUDIES

Several guidelines exist on how to conduct degradation experiments with either soil or water-sediment systems. The major objective of a study to be used for pesticide registration is the identification of the major individual components present during the duration of the study, thus allowing the establishment of the degradation pathway and estimates for the time taken for degradation of 50% and 90% of the active substance and metabolites.

3.1 Laboratory soil experiments

The most commonly used guidelines are SETAC (1995), US-EPA (1982, 1993) and OECD (2002a). Generally freshly sampled representative soils are characterised with regard to common soil properties and incubated under static soil moisture and temperature conditions in the dark, in either flow-through or biometer test systems, after application of the active substance. The use of ^{14}C-labeled material is preferred. During incubation soil samples are taken and analysed for active substance, metabolites, volatile components and bound residues. The time taken for degradation of 50% and 90% of the active substance and major metabolites is derived from the formation and decline curves. Details on the individual guidelines are given in Appendix 1.

Increasingly, attempts are undertaken to derive the regulatory endpoints from test systems using a dynamic, process-oriented approach by simulating pesticide transport through the unsaturated zone of the topsoil using soil columns or micro-lysimeters. The potential advantage of these systems is that the conditions of incubations are much more similar to the actual conditions present in an agricultural field after application of the active substance. However, no standardised guideline exists up to now, but a useful design is reported in Heistermann *et al*. (2003).

3.2 Laboratory water-sediment experiments

The conduct of water-sediment studies for pesticides that are non-volatile or slightly volatile is described in OECD Guideline 308 (OECD, 2002b) and by SETAC (1995). In these guidelines, a minimum of six sampling times (including zero time) is considered necessary to estimate kinetic endpoints over an experimental period not normally exceeding 100 days, or when 90% of the test substance has dissipated by transformation and/or volatilisation.

However, the guidance also acknowledges that the number of sampling times and/or the experimental period may need to be extended, to establish the degradation pathway and distribution between the water column and sediment. For example, with hydrophobic parent compounds, additional sampling points may be needed during the initial period of the study to estimate transfer rates between the water column and sediment more precisely and hence those for the degradation rates in these compartments. With metabolites, additional sampling points may be needed to estimate formation rates more precisely, to reduce the influence of the statistical correlation between estimated formation and degradation rates. Hence, in the guidance, an option is provided to conduct a preliminary study in order to establish an appropriate sampling regime and duration for the test. This is, however, only one option. Other options include using information from all other studies, e.g. adsorption and metabolism studies, to help design the study most appropriately.

3.3 Field soil dissipation studies

Guidelines for field soil dissipation studies in Europe are reported by SETAC (1995), basically referring to EPPO (1993). Typically, these studies should be carried out at four locations. The sites should be representative of the intended use of the pesticide and the soil must be characterised in different horizons. A representative pesticide formulation is applied to either cropped or bare soil with calibrated application equipment. Control samples and residues samples will be collected during the study. Typically 20 cores are taken per plot at each sampling time and split into layers of appropriate depths. Representative sub-samples are then analysed for active substance and major metabolites. The time taken for dissipation of 50% and 90% of the active substance and major metabolites is derived from the formation and decline curves.

The guidelines for field soil dissipation studies in the U.S. and Canada are in the process of being revised. Studies are typically conducted on a single plot (usually divided into subplots) in about four locations in the U.S. and an additional 2-4 locations in Canada. Typically today 15-20 cores are taken per plot down to a depth of about 1 m, divided into about six depth increments, and composite samples are analysed for parent and major metabolites. The formation and decline curves are used to derive kinetic models describing the dissipation of parent and the formation and decline of metabolites.

3.4 Higher-tier studies in aquatic systems

Guidance for the performance and interpretation of higher-tier studies in aquatic systems, such as meso- or microcosm studies, are provided by SETAC (1991, 1999, 2002). Other higher tier studies that may be useful are water sediment studies under outdoor conditions and irradiated water sediment studies.

Although the primary aim of higher-tier studies in aquatic systems, especially meso- or microcosm studies, is usually to address effects in aquatic ecosystems, the studies may sometimes provide useful information on fate and exposure endpoints. These endpoints include build-up in water, sediment and biota, and disappearance times. For determination of DT50/90, SETAC (1991) recommends sampling of the various compartments at ≥ 4 occasions in short-term studies (up to 1 month), and at 6-10 occasions in long-term studies (1-6 months). The intervals between sampling depends on expected partitioning and disappearance rate of the test substance but will usually be spaced logarithmically (SETAC, 1991).

Previously, dosing regime in micro- and mesocosm studies were often chosen to simulate the expected route of entry to natural aquatic systems (spray drift, run-off etc.). However, more recently a concentration-response approach is recommended (SETAC 1999, 2002). This means inclusion of several test concentrations in the studies, and attempts to achieve, at least initially, a uniform test concentration in the system. This approach is more likely to produce data that can be used to estimate the disappearance times than the previous "simulation" studies.

3.5 References

EPPO, 1993. Decision making scheme for the environmental risk assessment of plant protection products. EPPO Bulletin 23, Chapter 3.

Heistermann, M., Jene, B., Fent, G., Feyerabend, M., Seppelt, R., Richter, O., Kubiak, R., 2003. Modelling approaches to compare sorption and degradation of metsulfuron-methyl in laboratory micro-lysimeter and batch experiments. Pest Management Science 59:1276-1290.

ISO 10381-6, 1993. Soil Quality – Sampling – Part 6: Guidance on the collection, handling and storage of soil for the assessment of aerobic microbial processes in the laboratory.

OECD, 1995. Final report of the OECD Workshop on Selection of Soils/Sediments. Belgirate, Italy, 18-20 January 1995.

OECD, 2002a. Aerobic and Anaerobic Transformation in Soil, OECD Guideline for Testing of Chemicals 307, adopted 24. April 2002.

OECD, 2002b. Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. OECD Guideline for Testing of Chemicals 308, adopted 24 april 2002.

SETAC, 2002. Community-Level Aquatic System Studies - Interpretation Criteria (CLASSIC). Proceedings from workshop held at Fraunhofer Institute - Schmallenberg, Germany, 30 May-2 June, 1999.

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US-EPA, 1982. Pesticide Assessment Guidelines, Subdivision N, Chemistry: Environmental Fate, Series 162-1 Aerobic Soil Metabolism Studies. U.S. EPA Office of Pesticide Programs, Washington, DC.

US-EPA, 1993. Pesticide Re-Registration Rejection Rate Analysis, Environmental Fate. EPA 738-R-93-010, September 1993. U.S. EPA Office of Pesticide Programs, Washington, DC.

4 REGULATORY ENDPOINTS

The aim of this FOCUS initiative was to give guidance on how to derive kinetic endpoints for parent compounds and metabolites in soil and water-sediment systems:

Endpoints for parent compounds in soil

Regulatory endpoints for parent compounds include DT50 and DT90 values used as triggers for higher-tier experiments (see Section 4.1). In addition, information on the type of degradation kinetics and associated DT50 values or degradation rates are required for calculation of predicted environmental concentrations in soil (PEC_S), groundwater (PEC_{GW}), surface water (PEC_{SW}) and sediment (PEC_{SED}).

Endpoints for metabolites in soil

The endpoints for potentially relevant metabolites in soil are the same regulatory triggers (DT50 and DT90 for lab and field) as for parent compounds. Endpoints needed for models also include rate of formation (degradation of parent or precursor metabolite and formation fraction) and degradation kinetics and associated rate constants.

Endpoints for parent compounds and metabolites in water-sediment studies

The endpoints for the kinetic analysis of a water-sediment study are the same as discussed for parent compounds and metabolites in soil. However, the complexity of the system means that such values must be calculated for the overall system as well as in the water column and sediment. The various uses of these endpoints are discussed in Section 10.1.

Details on degradation endpoints used as triggers for higher-tier experiments as defined in EU documents are given in Section 4.1. Implications of the intended use of the endpoint on the kinetic analysis of degradation studies are outlined in Section 4.2.

4.1 Regulatory endpoints as defined in EU documents

Data on the persistence of a parent compound and its metabolites in soil and water-sediment systems are an important part of the regulatory data package. Point 2.5.1.1 of Part C of Annex VI to Dir. 91/414/EEC states:

"No authorization shall be granted if the active substance and, where they are of significance from the toxicological, ecotoxicological or environmental point of view, metabolites and breakdown or reaction products, after use of the plant protection product under the proposed conditions of use:
- during tests in the field, persist in soil for more than one year (i.e. DT90 > 1 year and DT50 > 3 months)" ...
"unless it is scientifically demonstrated that under field conditions there is no accumulation in soil at such levels that unacceptable residues in succeeding crops occur and/or that unacceptable phytotoxic effects on succeeding crops occur and/or that there is an unacceptable impact on the environment …."

A description of the determination of the significance of a metabolite concerning its toxicological, ecotoxicological or environmental point of view (relevant metabolite) is given in the Guidance Document on Relevant Metabolites and the Guidance Documents on Terrestrial and Aquatic Ecotoxicology. Therefore, all discussion on metabolites throughout this document makes no assumptions about whether they are relevant or not. To express this fact the term "metabolite" or "potentially relevant metabolite" is used.

DT50 and DT90 values are also used as trigger values for higher-tier experiments:

Annex II to Dir. 91/414/EEC:

- 7.1.1.2.2 Field dissipation studies required when $DT50_{lab}$ > 60 days (20°)/ > 90 days (10°);
- 7.1.1.2. Soil residue studies required when $DT50_{lab}$ > 1/3 of the period between application and harvest;
- 7.1.1.2. Soil accumulation studies required when $DT90_{field}$ > 1 year;
- 8.2.2 Fish life cycle test required when DT90 in water or sediment > 100 days;
- 8.4.2 Sublethal effects test on earthworms required when DT90 in soil > 100 days.

Annex III to Dir. 91/414/EEC:

- 10.6.2 Testing for effects on soil non-target macro-organisms, e.g. impact on organic matter breakdown required when $DT90_{field}$ > 365 days;
- 10.7.1 Testing for effects on soil micro-organisms required when $DT90_{field}$ > 100 days.

Draft Guidance Document on Terrestrial Ecotoxicology
(SANCO/10329/2002, 17 October 2002, rev. 2 final):

- Avian reproduction test (Annex II 8.1.3), always required for substances which are generally persistent (reference to criteria in Annex VI 2.5.1.1);
- Sublethal effects test on earthworms (Annex II 8.4.2, Annex III 10.6.1.2), requirement depends on combination of the number of applications and the $DT90_{field}$;
- Other soil non-target organisms (Annex III 10.6.2):
  a) *Collembola* reproduction test or test on gamasid mites, required when $DT90_{field}$ is 100-365 days,
  b) Litter bag test under field conditions; conditional when $DT90_{field}$ is 100-365 days, always required when $DT90_{field}$ > 365 days (or higher-tier testing).

Guidance Document on Aquatic Ecotoxicology
(SANCO/3268/2001 rev. 4 (final), 17 October 2002):

- Long-term/chronic toxicity tests on fish (Annex II 8.2.2) required when DT50 in water column ≥ 2 days, fish full life cycle (FLC) test required when DT90 in water or sediment > 100 days (among other criteria);
- Fish bioconcentration study (Annex II 8.2.3), not necessary if DT90 in the whole water-sediment system < 10 days;
- Chronic study on daphnids (Annex II 8.2.5) required when DT50 in water ≥ 2 days;
- For higher-tier exposure assessment to address potential biomagnification in aquatic food chains, the same triggers as for FLC-test is applied, among them DT90 in water or sediment > 100 days.

In general, the triggers for further study requirements listed above are applicable to (major) metabolites as for parent compounds. However, the assessment of metabolites also includes consideration of when kinetic calculations on metabolites are *not* needed, e.g. when their potential ecotoxicity is covered implicitly by higher-tier ecotoxicity studies on the parent compound.

4.2 Implications of the intended use of endpoints for kinetic analysis

Regulatory endpoints are derived by analysing data from laboratory or field dissipation studies. Kinetic models are fitted to concentrations of the pesticide measured at different points in time. Single first-order kinetics (SFO, Section 5.1) have until recently been the preferred model for estimating DT50 and DT90 values for several reasons:

- Many biotic and abiotic processes in environmental compartments such as soil effectively follow single first order kinetics (exponential decay), even though the behaviour may be controlled by several competing first order kinetic processes (e.g. if the rate limiting process follows first order kinetics in soil pore water);
- The equation is simple and has only two parameters;
- It is easy to fit the equation to experimental data;
- DT50 and DT90 values are easy to calculate
- Parameters are theoretically independent of concentration and time;
- First-order DT50 values can be used as input for pesticide leaching models.

Single first order (SFO) kinetics describe reactions with a rate-limiting step involving the concentration of only one component. If the concentrations of other components are also involved the order normally changes to a higher order, e.g. second order, if the concentrations of two components are involved in the rate limiting step. If one of the two components is present in excess and its change in concentration is negligible the second order kinetic equation collapses to the pseudo-first order equation.

Results from degradation studies may not be always well described by first-order kinetics. Some causes of such deviations are lower availability of the chemical with time, spatial variability of the degradation process, concentration dependence of degradation and/or decreasing microbial activity. When using SFO values as input for pesticide leaching models, the validity of the kinetic hypothesis that degradation rates can be sufficiently approximated by SFO kinetics must be checked. For example, if the best fit to the disappearance of a compound under laboratory conditions clearly follows a non-SFO pattern, the possibility that artefacts contributed to this behaviour pattern must be considered. This is particularly the case when the degradation pattern appears to be strongly non-SFO in some test systems but still conforms closely to SFO kinetics in other test systems. There may then be some likelihood that this non-SFO behaviour is due to certain soil properties or conditions in the test system rather than the “typical” behaviour of the compound under investigation. Hence, checks should be made to determine whether the microbial activity, temperature and moisture content were sufficiently constant over the experimental period, whether such behaviour is observed under field conditions, or whether other factors have affected the pattern or apparent decline. Such checks are important because the resulting kinetic models are used to assess behaviour under actual use conditions. If the non-SFO behaviour is the result of test system artefacts (such as declining microbial populations), then the way the

non-SFO kinetics is addressed is different than if the non-SFO kinetics are the result of changes in bioavailability (increasing sorption) over time in the test system.

From a scientific viewpoint, the model that best describes the experimental data should be used. However, the technique used to derive regulatory endpoints must not conflict with their intended use. There are two general approaches, which are outlined in more detail below:

- Use best-fit kinetics for calculation of PEC values in soil or to derive DT50/90 values for use or as a trigger for higher-tier experiments.
- Use first-order kinetics or pragmatic correction procedures to derive kinetic models for calculation of PEC values in groundwater, surface water and sediment with current standard versions of regulatory pesticide fate models.

4.2.1 Triggers for higher-tier experiments

DT50 and DT90 values that are used as triggers for higher-tier experiments should always be derived by best-fit kinetics provided the observed deviations from first-order kinetics can be expected to occur under normal usage conditions in the field. Attempts should be made to establish the underlying mechanisms. Alternative fits are not recommended when deviations from first-order kinetics can be attributed to experimental artefacts (Section 6.1.7). Appropriate models to describe degradation kinetics are listed in Chapter 5 and a stepwise approach to derive best-fit DT50 and DT90 values for parent degradation in soil is outlined in Section 7.1.1 and for water sediment studies in Chapter 10.

The DT50 value derived from bi-phasic kinetics is usually less than the first-order DT50 (with the exception of lag-phase models). The opposite is usually true for the DT90 value. First-order DT90 values are greater than the DT50 by a factor of 3.32 (ln 10 / ln 2). A much wider ratio is found for bi-phasic models. In a large number of cases, first-order kinetics will provide an acceptable fit to the data and the use of bi-phasic kinetics will be limited to cases where clear deviations from first-order kinetics occur. For less rapidly degrading substances, the study duration of 120 days in the laboratory experiment may not allow a measurement of a DT90 in the study period and extrapolation far beyond the duration of the study should be conducted with care. At present, DT90 values from laboratory soil studies are not used as triggers for additional work. However, the DT90 values from field studies are used in the terrestrial ecotoxicological risk assessment to trigger further work on terrestrial organisms (Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC, SANCO/10329/2002 rev 2 final, 17 October 2002, pp. 39).

Trigger DT90 values from field studies are usually derived by graphical analysis (actual or interpolated time by which 10% of the initial mass is reached in the study) or kinetic evaluation. Non-first-order kinetics can be used. The resulting DT90 values are usually not adjusted to standard temperature and moisture conditions, but taken directly from studies relevant to the proposed usage scenario. Chapter 9 provides guidance on how to derive field half-life values for modelling.

4.2.2 Predicted environmental concentrations in soil (PEC_S)

Predicted environmental concentrations in soil (PEC_S) are usually calculated using simple tools (e.g. ModelMaker) or spreadsheets following the procedures outlined by FOCUS (1996). These calculations are not limited to first-order kinetics so the model that fits the experimental data best should be used to derive degradation parameters. The kinetic model used for PEC_s calculations must be identical to the one used in the best-fit procedure. Note that when considering multiple applications for a parent substance in a compartment model with differential equations, only constituting autonomous differential equations may be used. Differential equations with time on the right-hand side are not appropriate in this case. Kinetic models that cannot be described with one or a set of constituting autonomous differential equations should be expressed in their integrated form for the calculation of PEC_s with multiple applications. A critical assessment must be made as to whether the kinetics observed in the experimental study is applicable to actual usage conditions of the pesticide in the field. Experimental artefacts must be taken into account before starting the kinetic analysis.

Initial PEC_S values for metabolites have often been derived from the application rate of the parent compound and the maximum observed amount of the metabolite in soil incubation studies. PEC values at later time points are calculated using first-order kinetics. Half-life values for use with this approach are derived by fitting first-order kinetics to the decline of metabolite concentrations from the maximum onwards (Figure 4-1). This approach is suitable for estimating the exposure of soil organisms to the metabolite. However, the derived DT50 value for the decline curve underestimates degradation of the metabolite.

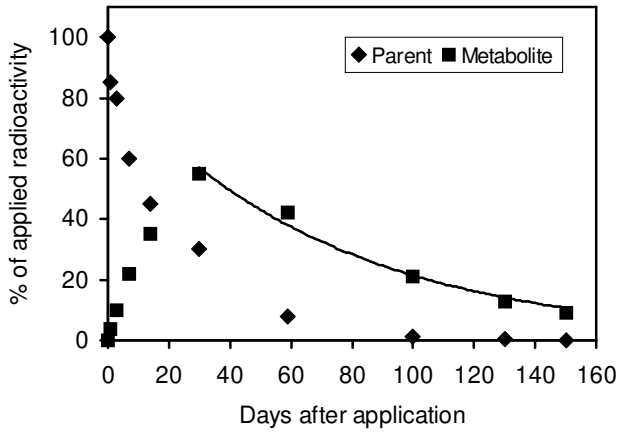


Figure 4-1: Fitting of first-order kinetics to the decline of metabolite concentrations from the maximum onwards

When possible, PEC values (soil, ground water, and surface water) for metabolites should be calculated using kinetic models that take the simultaneous formation of the metabolite from the parent compound and its degradation into account. This can be achieved by fitting kinetic models to data from standard degradation studies as illustrated for an example in Figure 4-2 (note that 100% formation of a single metabolite from the parent was assumed in this simplified example, transformation to any other substances or a sink was ignored). The analysis is not limited to first-order kinetics. The selected model and the optimised parameter values can then be used to calculate predicted environmental concentrations of the metabolite in soil. Note that in this case, the initial PEC of the metabolite is usually zero. The maximum PEC occurs at a later time. Because of the gradual formation of metabolites over time, when considering compartment models with differential equations, only constituting autonomous differential equations may be used. Differential equations with time on the right-hand side are not appropriate in this case. Kinetic models that cannot be described with one or a set of constituting autonomous differential equations should be expressed in their integrated form for the calculation of PEC_s of metabolites.

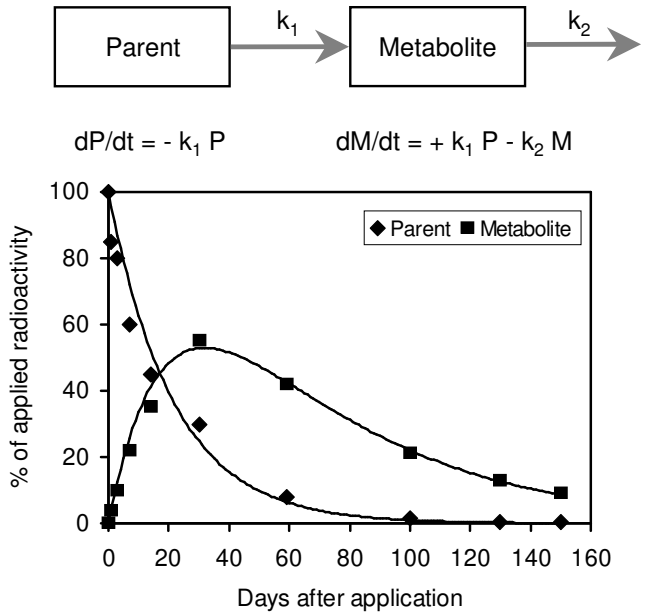


Figure 4-2: Simultaneous fitting of a kinetic model to data for the parent compound and the metabolite (assuming 100% formation of the metabolite) and SFO kinetics for parent and metabolite).

4.2.3 Predicted environmental concentrations in groundwater (PEC_{GW})

Predicted environmental concentrations in ground water (PEC_{GW}) are commonly calculated using FOCUS versions of simulation models such as MACRO, PEARL, PELMO and PRZM (FOCUS, 2000). All of these models use first-order kinetics to describe degradation rates in soil. Therefore, first-order kinetics are the most straightforward way for describing results from laboratory or field studies to be used to derive relevant degradation parameters from laboratory or field studies. Degradation parameters derived from alternative kinetics cannot generally be used as input data for these models, although different options exist in some models. For example, PRZM incorporates the hockey-stick model to calculate degradation in soil (Section 5.2.2). However, the degradation rate reverts to the faster initial rate for multiple applications, equivalent to that at time zero for a single application. The model PEARL has an option to simulate long-term sorption kinetics using a two-site approach. Substance in the liquid phase and sorbed to sorption sites that are instantaneously at equilibrium is degraded according to first-order kinetics. Pesticide sorbed to slowly reacting sorption sites is, however, protected from degradation. This results in deviations of degradation from first-order kinetics with slower degradation later in the simulation period. The current (non-FOCUS) version of MACRO (MACRO 5.0) uses a similar approach.

Future versions of pesticide fate models may overcome these current limitations. However, at this time, kinetics other than first-order are not generally recommended to derive

parameters for PEC_{GW} using first-tier approaches. Detailed guidance on how to derive relevant parameters for parent compounds within the current constraints of pesticide fate models is given in Chapter 7. The long-term sorption routine in PEARL or similar techniques can be used as a higher-tier approach to incorporate bi-phasic kinetics into leaching modelling (see Section 7.1.2.2.1). An additional, pragmatic higher-tier approach to implement bi-phasic kinetics into leaching models is described in Section 7.1.2.2.2.

4.2.4 Predicted environmental concentrations in surface water (PEC_{SW}) and sediment (PEC_{SED})

FOCUS developed standard tools and scenarios to calculate concentrations of pesticides in surface water and sediment within the EU registration process (FOCUS, 2003). Below is a short summary of the approach, focussing on issues of importance in the context of degradation kinetics.

The FOCUS Surface Water assessment proceeds step-wise:

Step 1 is a worst-case for different routes of enter into surface water, since entry from spray drift and runoff/erosion/drainage is assumed to occur at one single day, even if multiple applications occur in practice - unless interval between treatments is longer than 3 x $DT50_{wc+sed}$.
On entry into surface water, pesticide distribution between water and sediment is assumed to occur instantaneously, except for spray drift which enters surface water and is then distributed instantaneously between water and sediment after a delay of 1 day. Koc is used to estimate the fraction distributed to sediment.

The degradation half-life for the total water-sediment system is used to calculate daily concentrations in water and sediment.

No specific scenario with regard to climate, cropping, topography or soil is assumed at Step 1. The model used for the calculations is STEPS1-2 in FOCUS.

At **Step 2**, sequential entry from different routes is assumed. This means that entry from spray drift following multiple applications are separated in time, and entry from runoff/erosion/drainage is assumed to occur 4 days after the last spray drift event. The distribution of spray drift between water column and sediment is assumed to take longer than 1 day by separating the substance into two sub-compartments (available and non-available for sorption) with Koc to estimate subsequent distribution between water and sediment. As in step 1, the distribution between water and sediment from the other routes of entry is assumed to occur instantaneously, and Koc is used to estimate the fraction distributed to sediment.

To calculate daily concentrations in water and sediment, a temporary mass of the compound in each compartment is calculated using the half-lives in the water column and sediment.

No specific scenario with regard to climate, cropping, topography or soil is assumed at Step 2. The model used for the calculations is STEPS1-2 in FOCUS.

At **Step 3**, up to 10 realistic worst-case European scenarios are introduced. Sequential loading is assumed. The PRZM and MACRO models are used to calculate the flow of water and substance to a water body (ditch/pond etc.) via runoff/erosion and drainage, respectively, and spray drift loadings are calculated by a separate module (SWASH). The model TOXSWA in FOCUS is used to simulate the fate of the substance in the water body. Water concentrations are uniform over depth in the model, but vary along different horizontal compartments. In the sediment, the calculated concentrations vary both vertically and horizontally. In TOXSWA in FOCUS, transport across the water-sediment interface takes place via diffusion.

Step 4 is considered as a higher-tier exposure assessment, which is not further considered here.

In summary, at each step of the FOCUS Surface Water scenarios, the models calculate PEC in water and sediment at specified days, and Time Weighted Average (TWA) concentrations over specified time periods. First-order kinetics are assumed by the models internally for these calculations, thus the input values should also be calculated by SFO. As input parameters from water-sediment studies, FOCUS Surface Water Step 1 makes use of the half-life *in the whole water-sediment system*, whereas for Step 2 and 3 *separate DT50 values for the water column and sediment* are needed[4]. Moreover, the separate half-life values for the water column and sediment used in Step 2 and 3 should represent transformation processes only, not mass transfer processes (sorption and/or volatilisation)[5]. Thus, they should be degradation half-lives, not dissipation half-lives. Detailed guidance on how to derive relevant parameters for surface water assessments within the current constraints of pesticide fate models is given in Chapter 10.

[4] For Step 2, in case separate DT_{50} values for water column and sediment cannot be calculated, the report of the FOCUS Surface Water Workgroup recommends that the degradation rate for the whole system is used for both the water column and the sediment.
[5] Note that formation of non-extractable residues ("bound residues") is regarded as a transformation process here.

4.3 Dissipation or degradation values for triggers

Endpoints for use as trigger values can be either degradation values (characterising the inherent degradation potential of a parent compound or a metabolite) or dissipation values (characterising the overall decline of the concentration of a substance as a result of a number of processes). Endpoints for parent compounds in soil are usually degradation endpoints whereas dissipation endpoints are derived from field studies. In water-sediment systems, dissipation in each individual compartment is the result of degradation and the partitioning between the water and sediment. The overall decline in concentrations of metabolites in soil and water-sediment systems is often slower than degradation due to the continuous formation of the metabolite from the parent compound.

Which type of endpoint is most suitable depends on the intended use of the trigger value. For instance, for an assessment of parent persistence in the aquatic environment as a whole, degradation values are recommended; while dissipation values are recommended for an assessment of parent or metabolite persistence in the water column. Often, dissipation values are suitable to characterise the potential for effects to occur as a result of overall exposure over a period of time. However, these trigger values should be seen in the context of calculations of predicted environmental concentrations which are an important part of the regulatory data package. Table 4.1 indicates which type of endpoint will be provided by each of the methods for the calculation of trigger values recommended in this report. Since the choice between degradation values vs. dissipation values will differ depending on context, the decision on how to use these values in the regulatory procedure is left to the user.

Table 4.1. Type of endpoint provided by methods recommended in this report for calculating trigger values

Recommended procedure for: Substance	Compartment	Study type	Results in trigger value of this type:
Parent	Soil	Laboratory	Degradation T50
Parent	Soil	Field	Dissipation T50[1]
Metabolite	Soil	Laboratory	Degradation T50 when feasible[2], otherwise dissipation DT50[3]
Metabolite	Soil	Field	Dissipation T50[1,4]
Parent	Whole water-sediment system	Water-sediment	Degradation T50 (level P-I)
Parent	Water column	Water-sediment	Dissipation T50 (level P-I)
Parent	Sediment	Water-sediment	Dissipation T50 (level P-I) or Degradation T50 (level P-II)
Metabolite	Whole water-sediment system	Water-sediment	Degradation T50 when feasible[2], otherwise dissipation DT50[3] (both Level M-I)
Metabolite	Water column	Water-sediment	Dissipation T50 when feasible, otherwise the System Dissipation T50 or System Degradation T50(all from level M-I)
Metabolite	Sediment	Water-sediment	Dissipation T50 when feasible otherwise System Dissipation T50 or System Degradation T50 (all from Level M-I)

[1] Results from field studies can be used to provide a degradation endpoint when transport and other loss processes are minimal or can be quantified.
[2] DT50 calculated from study on parent and data from all sampling points from the formation of the metabolite are used in the analysis (or DT50 calculated from study on metabolite).
[3] DT50 calculated from study on parent and only data from sampling points of the decline phase are used in the analysis.
[4] The recommended procedure is a kinetic fit to all data for the parent and metabolite and not a fit to the decline phase only. The endpoint, therefore, considers degradation and losses due to e.g. photolysis, leaching and volatilisation. The recommended analysis gives a DT50 that is shorter than the time for a decline of the maximum concentration of the metabolite by 50% due to the ongoing formation from the parent.

4.4 References

A link to Council Directive 91/414/EEC can be found at http://europa.eu.int/comm/food/plant/protection/index_en.htm.

Guidance Document on the Assessment of the Relevance of Metabolites in Groundwater of Substances Regulated under Council Directive 91/414/EEC; Guidance Document on Terrestrial Ecotoxicology; Guidance Document on Aquatic Ecotoxicology (as well as other Guidance Documents generated under the EU work on Plant Protection Products) can be found at http://europa.eu.int/comm/food/plant/protection/evaluation/guidance_en.htm.

FOCUS. 1996. Soil Persistence Models and EU Registration, European Commission Document No. 7617/VI/96. URL: http://europa.eu.int/comm/food/plant/protection/evaluation/focus_en.htm.

FOCUS. 2000. FOCUS Groundwater Scenarios in the EU Review of Active Substances. Report of the FOCUS Groundwater Scenarios Workgroup. EC Document Reference Sanco/321/2000 rev.2, 202 pp. URL: http://europa.eu.int/comm/food/plant/protection/evaluation/focus_en.htm.

FOCUS. 2003. FOCUS Surface Water Scenarios in the EU Evaluation Process under 91/414/EECReview of Active Substances. Report of the FOCUS Working Group on Surface Water Scenarios. EC Document Reference Sanco/4802/2001 rev.2, 245 pp. URL: http://europa.eu.int/comm/food/plant/protection/evaluation/focus_en.htm

5 TYPES OF KINETIC MODELS USED

A large number of kinetic models to describe the change in pesticide concentrations with time are available. Several models have been selected by the FOCUS work group. These are recommended for use as a first step to derive regulatory endpoints for parent compounds and metabolites in soil or water-sediment studies as described in Chapters 7, 8, and 10. This list of core models comprises the single first-order model, a number of models that are able to describe bi-phasic degradation kinetics and two models that are suitable to describe degradation patterns with a lag-phase. An overview of their features is provided in Table 5-1 and the core models are described in detail in this chapter. Alternative models can be used in exceptional cases, but this must be clearly documented and justified.

In the case of pesticide dissipation or degradation in soil or other environmental systems, the above-mentioned models all represent simple and sensible approaches to mathematically describe the experimental data, and do not represent actual chemical reactions. Also, note that the more complex the pathway and the type of kinetics used, the more parameters the model will require, and the more data points are needed for adequate parameter estimation. The simplest model that can provide a sensible description of the proposed pathway and adequate description of the decline curves should always be preferred.

For most kinetics described below, an integrated equation and a differential equation are given. Both can be used in order to derive endpoints for parent compounds in soil. In some cases, a single constituting autonomous differential equation does not exist. This is an equation where the right hand side only contains state variables (variables such as concentration describing the state of the system at some instant of time). Autonomous differential equations must, therefore, not contain dose (initial concentration or application rate) and time. Only autonomous differential equations can be implemented in environmental fate models. However, differential forms can be used for the purpose of deriving estimates of parameters. **The use of these differential forms must be limited to parameter estimation for parent only, and calculation of PEC_s for a single application of parent. These differential forms are not appropriate for parameter estimation of metabolites (unless the metabolite is directly applied to the system, or in the cases where the metabolite decline is being fitted with the initial time set as the time where the peak occurred) or for calculation of PEC_s involving multiple applications.**

For metabolites, the most simple and flexible approach for implementing the conceptual model is to build compartment schemes with software tools that can solve systems of differential equations. In such schemes, the parent substance and the metabolites are defined as compartments and dissipation processes (flows) are postulated between the compartments according to the proposed route of dissipation. Each flow is then described with a differential equation or set of differential equations corresponding to the kinetic model to be applied. For single first-order kinetics, the differential equations given in Box 5-1 should be used to characterise the flow from the parent to the metabolite, corrected for the formation fraction. For the bi-phasic FOMC and DFOP models, a single constituting autonomous differential equation, where the right hand side only contains state variables (variables that change with time), does not exist. The differential forms given in Box 5-2 and 5-4 for these two models both contain time in the right hand side, and therefore are not appropriate for metabolites, which are formed gradually. An alternative formulation of the DFOP model with two sub-compartments and SFO kinetics for each sub-compartment is proposed in Chapter 8, which can be implemented in compartment models with differential equations.

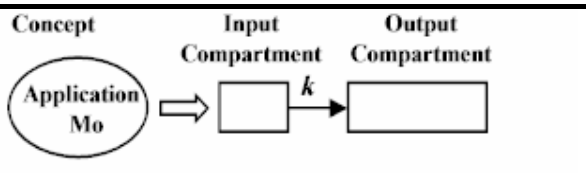
Table 5-1. Features of core models

Name	Other frequently used names	Abbreviation used in this report	No of parameters	Continuous with time	Rate dependent on state variables only[1]	Endpoints from analytical equation or iterative procedure
Single First-Order	Simple First-Order	SFO	2	YES	YES	analytical
Gustafson & Holden	First-Order Multi-Compartment	FOMC	3	YES	NO	analytical
Bi-Exponential	Double First-Order in Parallel First-Order Two Compartment	DFOP FOTC	4	YES	NO[2]	iterative
Hockey-Stick Model	First-Order Sequential Bi-phasic	HS	4	NO	NO	analytical
Modified Hockey-Stick Model (Lag Phase Model)		n/a	3	NO	NO	analytical
Logistic		n/a	4	YES	NO	analytical

[1] State variables are variables such as concentration describing the state of the system at some instant of time (initial amount present or time are **not** state variables).

[2] The DFOP or FOTC model can be expressed with a set of differential equations, where rates only depend on state variables (see Box 8-2).

5.1 Single first-order kinetics



Equation (integrated form)

$$M = M_0 \, e^{-k\,t}$$

Underlying differential equation

$$\frac{dM}{dt} = -k\,M$$

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical present at time $t=0$

k = Rate constant

Parameters to be determined

M_0, k

Endpoints

$$DT_x = \frac{\ln \frac{100}{100 - x}}{k}$$

$$DT_{50} = \frac{\ln 2}{k}$$

$$DT_{90} = \frac{\ln 10}{k}$$

Box 5-1. Single first-order (SFO) kinetics

Single first-order kinetics (SFO) is a simple exponential equation with only two parameters (Box 5-1). It assumes that the number of pesticide molecules is small relative to the number of degrading micro-organisms and their enzymes or number of water molecules in the case of hydrolysis. As a result, the rate of the change in pesticide concentration (dM/dt) is at any time directly proportional to the actual concentration remaining in the system. For SFO kinetics, the time for a decrease in the concentration by a certain percentage is constant throughout the experiment and independent of the initial concentration of the pesticide. For example, the time for a decrease in the concentration from 100% to 50% of the initial amount is identical to the time for a decrease from 50% to 25% of the initial amount. This makes DT50 and DT90 values easy to interpret and SFO kinetics have been the preferred option to derive regulatory degradation endpoints. First-order kinetics have also frequently been used to describe degradation in pesticide fate models.

5.2 Bi-phasic kinetics

Degradation cannot always be described by SFO kinetics. A fast initial decrease in pesticide concentrations is often followed by a slower decline. This is usually referred to as a bi-phasic pattern of pesticide degradation. There are a number of possible reasons for this phenomenon:

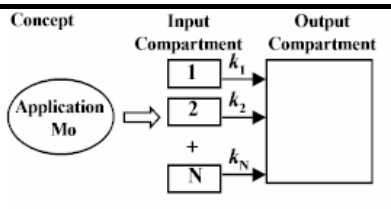
- Scow (1993) hypothesises that only the fraction of the pesticide in soil solution is available for degradation. The available fraction often decreases with time due to slow sorption and diffusion processes (Pignatello, 2000). This may decrease the rate of degradation of the pesticide at later stages of the experiment.
- Non-linear sorption with Freundlich exponents <1 results in a decreasing availability of the pesticide in soil solution with decreasing concentrations. If only the dissolved pesticide is available for degradation, a fast initial decrease in pesticide concentrations will be followed by a slower decline.
- In laboratory degradation studies, the activity of degrading soil microorganisms may decrease with time due to a limited availability of nutrient and carbon sources under laboratory conditions (Anderson, 1987).
- Soil is a spatially variable medium and Gustafson and Holden (1990) hypothesised that the rate of degradation will also be variable throughout the soil. They divided the soil into a large number of unconnected sub-compartments, each with a different first-order degradation rate constant. The distribution of these rate coefficients was described by a gamma-distribution, which results in a relatively simple equation and gives a bi-phasic pattern of pesticide degradation in the soil.
- In field studies, seasonal changes in temperature and/or moisture can affect the degradation rate and cause deviations from first-order kinetics (e.g. degradation rate may decrease in winter due to lower temperatures, degradation rate may decrease in summer due to drier conditions). Such changes may be eliminated by the normalisation process discussed in Chapter 9.

A number of bi-phasic kinetic models exist. Three bi-phasic models have been selected and these are described below. Preference was given to simple models with a small number of parameters. Guidance on how to derive DT50 and DT90 values for bi-phasic degradation kinetics for parent compounds in soil is given in Section 7.1.1 and in water sediment studies in Chapter 10. Guidance for metabolites is provided in Chapter 8.

Degradation rates estimated under laboratory conditions should be representative of field

conditions as far as possible. The use of a bi-phasic degradation model to fit laboratory data is only justified if the underlying mechanisms are expected to influence degradation under field conditions in a similar manner. Efforts to identify experimental artefacts prior to kinetic analysis must be made (Section 6.1.7).

5.2.1 Gustafson and Holden model



Equation (integrated form)

$$M = \frac{M_0}{\left(\frac{t}{\beta}+1\right)^{\alpha}}$$

Differential equation
(to be used only for parameter estimation)

$$\frac{dM}{dt} = -\frac{\alpha}{\beta} M \left(\frac{t}{\beta}+1\right)^{-1}$$

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical applied at time $t=0$

α = Shape parameter determined by coefficient of variation of k values

β = Location parameter

Parameters to be determined

M_0, α, β

Endpoints

$$DT_x = \beta\left[\left(\frac{100}{100-x}\right)^{\left(\frac{1}{\alpha}\right)} - 1\right]$$

$$DT_{50} = \beta\left(2^{\left(\frac{1}{\alpha}\right)} - 1\right)$$

$$DT_{90} = \beta\left(10^{\left(\frac{1}{\alpha}\right)} - 1\right)$$

Note: The proposed equation differs slightly from that in the original Gustafson and Holden (1990) reference. The parameter β corresponds to $1 / \beta$ in the original equation.

Box 5-2. Gustafson and Holden model (FOMC)

The model proposed by Gustafson and Holden (1990) has a mechanistic background.[6] Soil is a heterogeneous medium and it is thus unlikely that degradation occurs at the same rate within individual regions of the soil sample under investigation. This is accounted for in the model by dividing the soil into a large number of sub-compartments each with a different first-order degradation rate constant. If the distribution of these rate coefficients is described by a gamma-distribution then this results in a simple analytical equation with only three parameters (Box 5-2) and a bi-phasic overall pattern of pesticide degradation in soil. This model is also known as First-Order Multi-Compartment model (FOMC). However, the form of the FOMC model in Box 5-2 is not identical with the equation from the original paper (Gustafson and Holden, 1990). The parameter β of the FOMC model of Box 5-2 is the reciprocal value of β from the original equation (the integrated form of the original Gustafson and Holden model therefore reads $M=M_0(1+\beta t)^{-\alpha}$).

Patterns of decline in pesticide concentrations calculated with the Gustafson and Holden model are shown in Figure 5-2 for different values of α and β. Dissipation is faster for larger values of α and for smaller values for β.

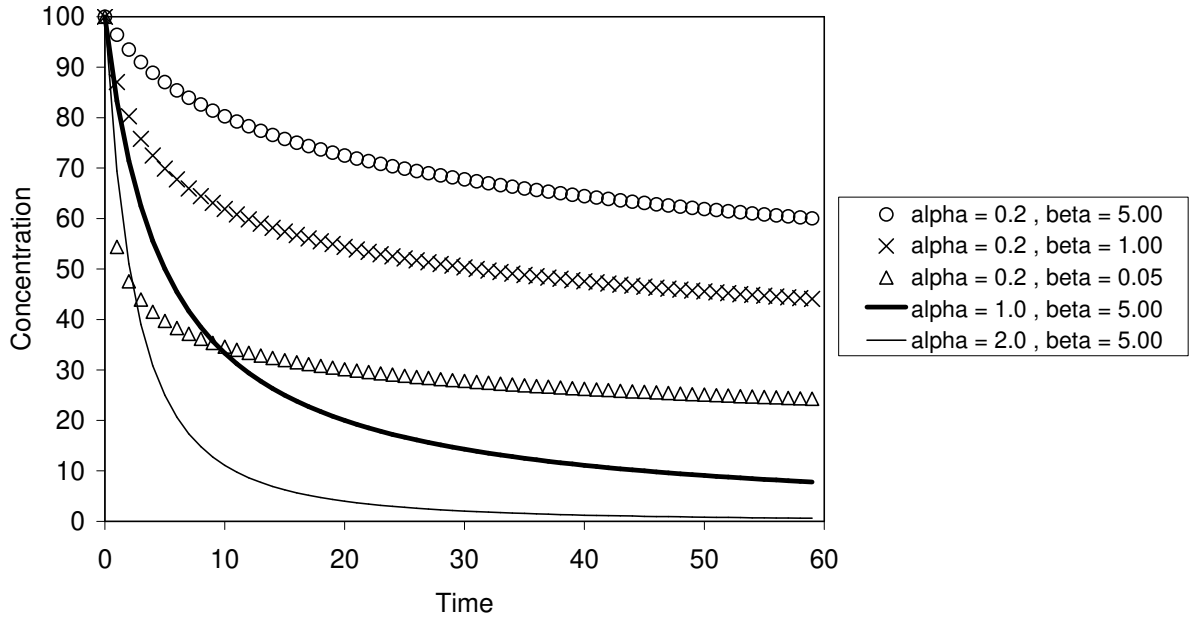
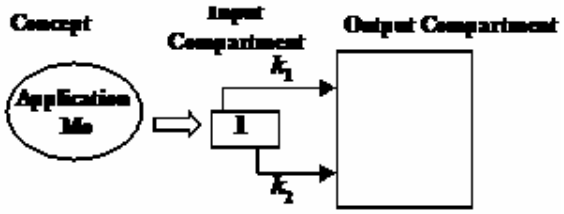


Figure 5-2. Patterns of decline calculated with the Gustafson and Holden model for different values of α and β.

[6] The original model is based on the superposition of single first-order equations with a statistical distribution of the rate constant k. The equation can also be derived from differential equations based on first-order kinetics with fading rate constant. Note that α and β are only defined for values > 0.

A clear advantage of the Gustafson and Holden model compared to other bi-phasic models is the relatively small number of parameters. However, the rate equation includes time on its right hand side (and therefore the degradation rate is time-dependent). As a result, the Gustafson and Holden model is not appropriate for a universally valid implementation in pesticide leaching models. The differential equation presented here should only be used for the purpose of parameter estimation for parent compounds or PEC_s calculations for parent involving only a single application. **The differential equation given in Box 5-2 must not be used for parameter estimation for metabolites.**

5.2.2 Hockey-stick model



Equation (integrated form)		Underlying differential equation	
$M = M_0\ e^{-k_1 t}$	for $t \le t_b$	$\frac{dM}{dt} = -k_1 M$	for $t \le t_b$
$M = M_0\ e^{-k_1 t_b}\ e^{-k_2 (t-t_b)}$	for $t > t_b$	$\frac{dM}{dt} = -k_2 M$	for $t > t_b$

where
M = Total amount of chemical present at time t
M_0 = Total amount of chemical applied at time $t=0$
k_1 = Rate constant until $t=t_b$
k_2 = Rate constant from $t=t_b$
t_b = Breakpoint (time at which rate constant changes)

Parameters to be determined

M_0, k_1, k_2, t_b

Endpoints

$$DT_x = \frac{\ln \frac{100}{100 - x}}{k_1} \qquad \text{if } DT_x \le t_b$$

$$DT_x = t_b + \frac{\left[\ln \frac{100}{100 - x} - k_1 t_b\right]}{k_2} \qquad \text{if } DT_x > t_b$$

Box 5-3. Hockey-stick model (HS)

The hockey-stick model consists of two sequential first-order curves. The pesticide concentration initially declines according to first-order kinetics with a rate constant k_1. At a certain point in time (referred to as the breakpoint), the rate constant changes to a different value k_2. Although the hockey-stick model is continuous with time, the derivative with time of the total amount is not continuous. For typical bi-phasic patterns, the rate constant k_1 is usually larger than k_2. The hockey-stick model has four parameters compared with only three for the Gustafson and Holden model.

By the equations given above the overall decline is calculated. Note that the DT50 value for the overall decline of pesticide concentrations can only be calculated from k_1 if the DT50 is reached before the breakpoint. Otherwise the second equation given in Box 5-3 must be used. The half-life value calculated from k_2 refers to the slow later stage of decline only and will be longer than the DT50.

Patterns of decline in pesticide concentrations calculated with the hockey-stick model for different parameter values are shown in Figure 5-3.

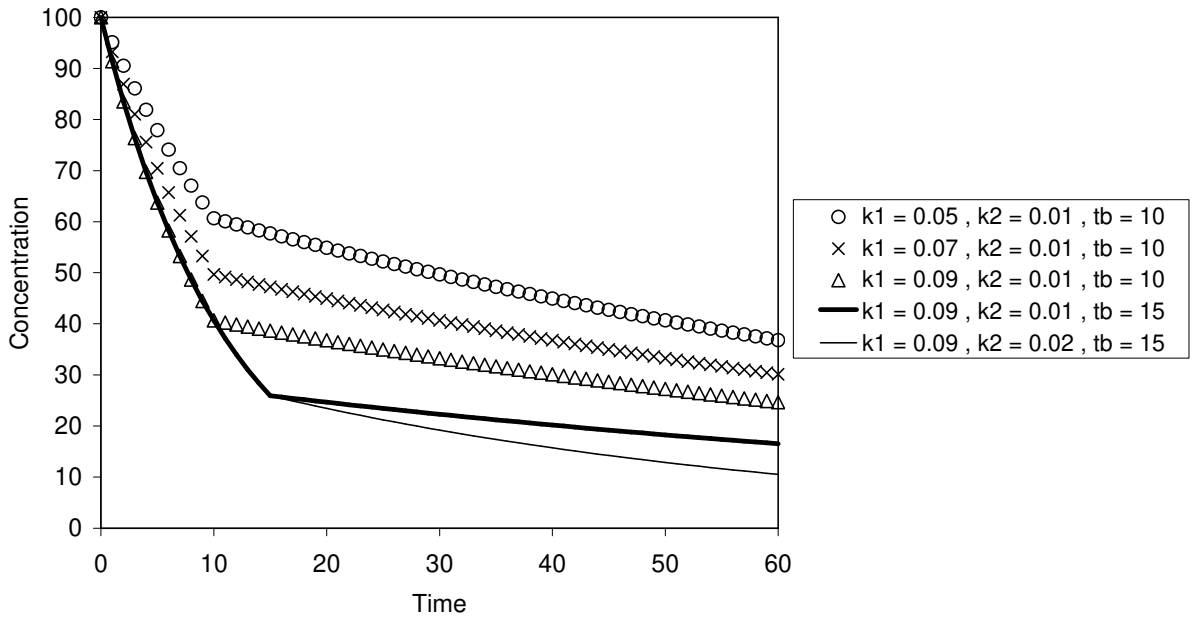


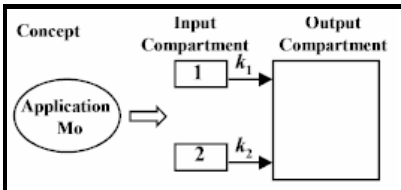
Figure 5-3. Patterns of decline calculated with the hockey-stick model for different values of k_1, k_2 and t_b

The hockey-stick model has no advantage over the other bi-phasic models (Gustafson and Holden model and bi-exponential model) with respect to the description of degradation kinetics for parent compounds in soil. It has, thus, not been included in the list of core recommended bi-phasic models for parent compounds in soil. The hockey-stick model is, however, used to derive tier 1 endpoints needed in fate modelling for the soil compartment if

neither SFO or FOMC can be used for that purpose (for details see Section 7.1.2.1). A special case of the hockey-stick model has been recommended as one of the options to describe decline patterns with a lag-phase (Section 5.3.1).

The hockey-stick model has not been included in the list of core models for simulating the fate of metabolites (Chapter 8). Hockey-stick kinetics are, however, often observed in water-sediment studies and this model has been included in the list of recommended models for this study type (Chapter 10).

5.2.3 Bi-exponential model



Equation (integrated form)

$$M = M_1 e^{-k_1 t} + M_2 e^{-k_2 t}$$

or

$$M = M_0 \left(g e^{-k_1 t} + (1-g) e^{-k_2 t} \right)$$

Differential equation
(to be used only for parameter estimation)

$$\frac{dM}{dt} = -\frac{k_1 g e^{-k_1 t} + k_2 (1-g) e^{-k_2 t}}{g e^{-k_1 t} + (1-g) e^{-k_2 t}} M$$

where

M = Total amount of chemical present at time t
M_1 = Amount of chemical applied to compartment 1 at time $t=0$
M_2 = Amount of chemical applied to compartment 2 at time $t=0$
M_0 = $M_1 + M_2$ = Total amount of chemical applied at time $t=0$
g = fraction of M_0 applied to compartment 1
k_1 = Rate constant in compartment 1
k_2 = Rate constant in compartment 2

Parameters to be determined

M_1, M_2, k_1, k_2 or M_0, g, k_1, k_2

Endpoints

An analytical solution does not exist.
DTx values can only be found by an iterative procedure

Box 5-4. Bi-exponential model (DFOP)

The bi-exponential model is abbreviated as DFOP (Double-First-Order in Parallel model) in this report. There is no analytical equation to calculate degradation endpoints and these must be derived by an iterative procedure. This could, for example, be achieved by using the

goal-seek function in Excel. Alternatively, the DT50 can be taken from a table of calculated concentrations as the time at which the concentration has decreased to ½ the initial fitted value. The DT90 corresponds to the time at which the concentration has decreased to 10% of the initial fitted value. Note: The initial fitted concentration usually deviates somewhat from 100% applied radioactivity. Endpoints for bi-exponential kinetics must not be calculated from the individual rate constants (for example, the overall DT50 is **not** ln(2)/k1). Patterns of decline in pesticide concentrations calculated with the bi-exponential model for different parameter values are shown in Figure 5-4.

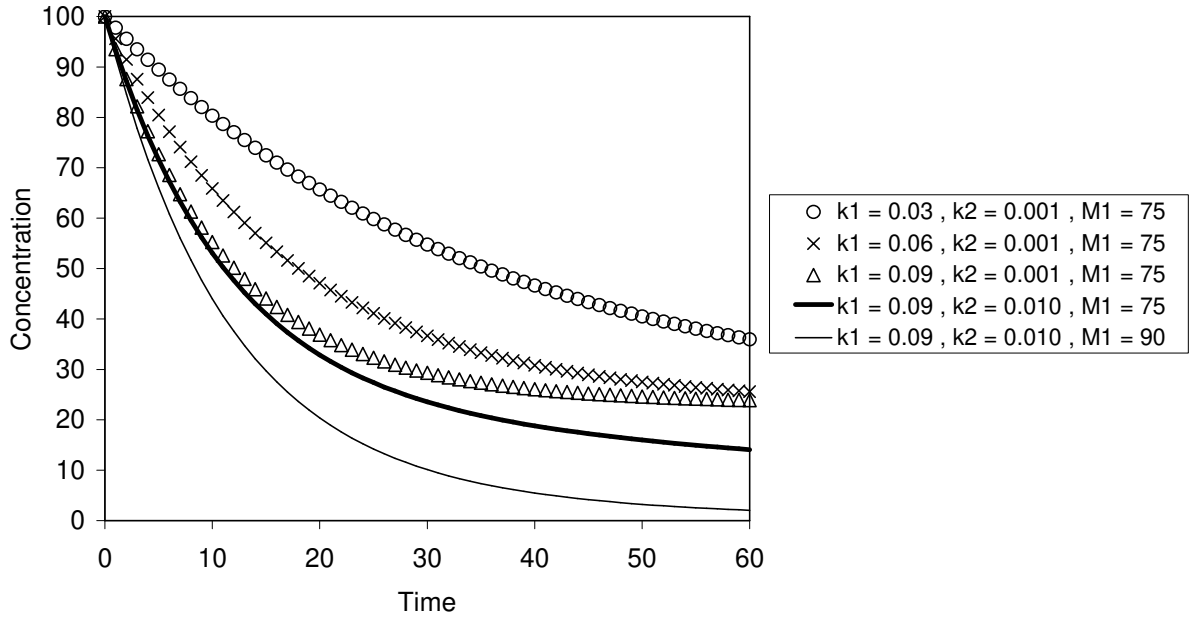


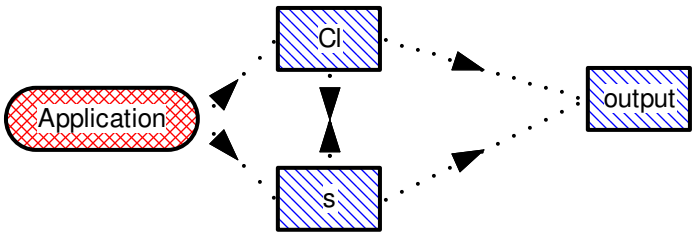
Figure 5-4. Patterns of decline calculated with the bi-exponential model for different values of k_1, k_2 and M_1 (M_2 = 100-M_1)

The integrated form of the bi-exponential model is a sum of two exponential equations with four parameters. Because of its two exponentials, an autonomous constituting differential equation does not exist. The differential equation given in Box 5-4 is not autonomous (it contains time on its right hand side) and must, therefore, only be used for parameter estimation for parent involving only a single application or for PEC_s calculations for parent involving only a single application. **The differential equation given in Box 5-4 must not be used for parameter estimation for metabolites.** However, the DFOP model can be expressed with a set of differential equations where rates only depend on state variables (see Box 8-2).

The integrated form of the bi-exponential model can only be derived from a system based on two ordinary first-order differential equations**.** There are a number of possible model systems that all lead to the bi-exponential model. Two examples are given below:

Example 1

The first example is a combination of first-order degradation in the liquid phase combined with a one-site kinetic sorption model. This model can be represented schematically by



The constituting differential equations are

$$\frac{dC_l}{dt} = -\frac{\lambda\rho}{\theta}(K_d C_l - S) - k_l C_l \qquad \text{(liquid phase concentration)}$$

$$\frac{dS}{dt} = \lambda (K_d C_l - S) - k_s S \qquad \text{(solid phase concentration)}$$

The total amount of chemical is given by

$$M = \theta C_l + \rho S \qquad \text{(total concentration)}$$

The parameters in the integrated equation (macroscopic parameters) are uniquely related to the microscopic parameters of the differential equation.

λ: rate constant for sorption

θ: volumetric water content

ρ : bulk density

k_l: degradation rate in liquid phase

k_s: degradation rate in solid phase

K_d: equilibrium binding constant

In the limiting case of very strong binding the constants k_l and k_s are identical to k_1 and k_2. (Box 5-4)

There is ample evidence in pesticide literature that a one-site kinetic sorption model is not realistic. The above example should therefore be considered as an illustration.

The parameters in the integrated equation are also related to those for a model assuming three phases (liquid phase, fast binding phase and slowly binding phase). For details see Appendix 4.

Example 2 (SFORB model)

The Single First-Order Reversible Binding model is a bi-phasic model consisting of two compartments, an unprotected compartment where application and degradation occurs and a protected compartment. The principle of this model is shown in Figure 5-5. Application is made to the first (unprotected) compartment. The second (protected) compartment interacts with the application compartment via two transfer rates. All transformation as well as transition processes are first order.

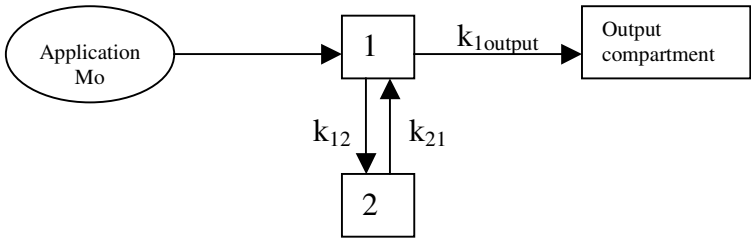


Figure 5-5. Principle of the SFORB model

This model is very similar to the model shown in Example 1. The main differences are:

Example 1	Example 2 (SFORB)
Compartments expressed as liquid and sorbed phase	Compartments consist of degradable and non-degradable compound. Reasons for difference in degradability can be availability or any other mechanism
Applied pesticide split between C and S	Applied pesticide initially all in compartment 1
Degradation in both compartments	Degradation only in compartment 1

In the SFORB model, the applied pesticide is only added to the first compartment. Degradation only occurs from the first (unprotected) compartment whereas the second (protected) compartment is considered as a temporary storage pool where no degradation takes place.

The constituting differential equations of the SFORB model are

$$\frac{dC1}{dt} = -(k_{12} + k_{1output}) \cdot C1 + k_{21} \cdot C2$$

$$\frac{dC2}{dt} = +k_{12} \cdot C1 - k_{21} \cdot C2$$

The integrated form is

$$C1 = C1(0) \cdot \left(\frac{k_{21} - b1}{b2 - b1} \cdot e^{-b1 \cdot t} + \frac{k_{21} - b2}{b1 - b2} \cdot e^{-b2 \cdot t}\right)$$

$$C2 = C1(0) \cdot \left(\frac{k_{12}}{b2 - b1} \cdot e^{-b1 \cdot t} + \frac{k_{12}}{b1 - b2} \cdot e^{-b2 \cdot t}\right)$$

$$C = C1 + C2 = C1(0) \cdot \left(\frac{k_{12} + k_{21} - b1}{b2 - b1} \cdot e^{-b1 \cdot t} + \frac{k_{12} + k_{21} - b2}{b1 - b2} \cdot e^{-b2 \cdot t}\right)$$

where b1 and b2 are given by

$$b_1 = \frac{1}{2}(k_{12} + k_{21} + k_{1output}) + \sqrt{\frac{1}{4}(k_{12} + k_{21} + k_{1output})^2 + k_{12} \cdot k_{21} - (k_{12} + k_{1output}) \cdot k_{21}}$$

$$b_2 = \frac{1}{2}(k_{12} + k_{21} + k_{1output}) - \sqrt{\frac{1}{4}(k_{12} + k_{21} + k_{1output})^2 + k_{12} \cdot k_{21} - (k_{12} + k_{1output}) \cdot k_{21}}$$

The parameters of the SFORB can be directly derived from the parameters of the DFOP model (use the second form of Box 5-4) and vice versa (Richter *et al.*, 1996; Duffy *et al.*, 1993)

$$k_{1output} = g \cdot k_1 + (1 - g) \cdot k_2$$

$$k_{21} = \frac{k_1 \cdot k_2}{g \cdot k_1 + (1 - g) \cdot k_2}$$

$$k_{12} = k_{21} \cdot \frac{g \cdot (1 - g) \cdot (k_1 - k_2)^2}{k_1 \cdot k_2}$$

The exponents b1 and b2 are identical to the parameters k_1 and k_2 of the DFOP model.

The model is conceptually similar to the kinetic desorption model option that is implemented in the pesticide leaching model PEARL. The unprotected compartment refers to the equilibrium phase of the soil including those parts of the liquid and solid phase in the soil that are in instantaneous sorption equilibrium. The protected compartment refers to the non-equilibrium sorbed phase where no degradation occurs. In analogy to the transformations made in Appendix 4 the parameters of the sorption and degradation parameters in PEARL can be easily taken from the SFORB parameters:

$k_t = k_{1output}$

$k_d = k_{21}$

$\Phi = k_{12} / k_{21}$

5.3 Lag-phase models

Pesticide concentrations may be virtually constant for a period of time followed by a first-order or bi-phasic decline in pesticide concentration. The initial phase is referred to as lag-phase. On some occasions, this can be attributed to storage of soil under conditions leading to a decline in active biomass prior to the experiment (e.g. excessively air-dried). This is an experimental artefact which can be avoided by storing the soil under appropriate conditions and by re-establishing the equilibrium of microbial metabolism following the change from

sampling or storage conditions to incubation conditions (OECD guideline 307). The lag-phase must be omitted from kinetic analyses and degradation endpoints derived from the declining part of the curve only if the lag phase is caused by experimental artefacts.

A true lag phase can be caused by slow adaptation of degrading microorganisms. Some pesticides are used as a carbon source by the degrading microflora. Under these conditions, growth of the population and/or the production and release of degrading enzymes is stimulated in the presence of the pesticide. Degradation is delayed until the microbial population has reached a certain density or activity. An alternative explanation is that the pesticide is inhibitory to the degrading microflora at high concentrations. Degradation does not stop completely, but proceeds at very slow rate. Once a critical concentration is reached, the rate of degradation increases. Note that the majority of pesticides are unlikely to exhibit severe inhibitory effects under realistic usage conditions. All data points must be included in the kinetic analysis if a true lag phase exists.

The decision on whether a data set exhibits a lag-phase should be based on a visual assessment. Guidance on how to derive DT50 and DT90 values for degradation kinetics with a lag-phase is given in Section 7.2.

5.3.1 Modified hockey-stick model

Equation (integrated form)		**Underlying differential equation**	
$M = M_0$	for $t \le t_b$	$\frac{dM}{dt} = 0$	for $t \le t_b$
$M = M_0\ e^{-k\,(t-t_b)}$	for $t > t_b$	$\frac{dM}{dt} = -k\,M$	for $t > t_b$

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical applied at time $t=0$

k = Rate constant from $t=t_b$

t_b = Breakpoint (time at which decline starts)

Parameters to be determined

M_0, k, t_b

Endpoints

$$DT_x = \frac{\ln \frac{100}{100-x}}{k} \quad \textbf{or} \quad DT_x = \frac{\ln \frac{100}{100-x}}{k} + t_b$$

Box 5-5. Modified hockey-stick model

The original hockey-stick model (see Section 5.2.2) consists of two sequential exponential curves. The pesticide concentration initially declines according to first-order kinetics with a rate constant k_1. At a certain point in time (referred to as the breakpoint), the rate constant changes to a different value k_2. Concentrations remain constant up to the breakpoint if the first rate constant k_1 is set to zero. This special case of the hockey-stick model (Box 5-5) can be used to describe decline patterns with a lag-phase. Where concentrations are not constant, but decline very slowly up to a breakpoint, the original model (Box 5-3) can be applied with $k_1 << k_2$. In both cases, the lag-phase (t_b) can be included or excluded in the calculation of DT50 and DT90 values (Section 7.2).

5.3.2 Logistic model

Equation (integrated form)

$$M = M_0 \left[\frac{a_{max}}{a_{max} - a_0 + a_0 e^{(r\,t)}}\right]^{\frac{a_{max}}{r}}$$

Differential equation
(to be used only for parameter estimation)

$$a = \frac{a_0\ a_{max}}{a_0 + (a_{max} - a_0) e^{(-r\,t)}}$$

where

M = Total amount of chemical present at time t

M_0 = Total amount of chemical applied at time $t = 0$

a_{max} = Maximum value of degradation constant (reflecting microbial activity)

a_0 = Initial value of degradation constant

r = Microbial growth rate

Note:

For $a_0 = a_{max}$ (i.e. activity of degrading microorganisms is already at its maximum at the start of the experiment) the model reduces to SFO kinetics with rate constant a_{max}

Parameters to be determined:

M_0, a_{max}, a_0, r

Endpoints

$$DT_x = \frac{1}{r} \ln \left[1 - \frac{a_{max}}{a_0}\left(1 - \frac{100}{100 - x}^{\,r/a_{max}}\right)\right]$$

$$DT_{50} = \frac{1}{r} \ln \left[1 - \frac{a_{max}}{a_0}\left(1 - 2^{r/a_{max}}\right)\right]$$

$$DT_{90} = \frac{1}{r} \ln \left[1 - \frac{a_{max}}{a_0}\left(1 - 10^{r/a_{max}}\right)\right]$$

Box 5-6. Logistic model

The logistic model assumes that the degradation rate constant increases after application of the compound up to a maximum value. This could be due to an increase in the number (or activity) of degrading micro-organisms. The model can be used to describe the pattern of decline of the total amount of pesticide residues in soil, M, when a true lag phase with no clear break point exists.

Patterns of decline in pesticide concentrations calculated with the logistic model for different parameter values are shown in Figure 5-6. The kinetics approach first order once the degradation rate constant has reached its maximum value. The maximum is reached faster

(i.e., the lag phase is shorter) for larger values of the growth rate r and for larger values of the initial rate constant a_0. The rate of degradation after the lag phase is determined by a_{max} (the larger the value the faster). The model reduces to first-order kinetics for $a_0 = a_{max}$ (for $a_0 = a_{max} = 0.08$ in the graph shown below). The differential equation given in Box 5-6 is not autonomous (it contains time on its right hand side) and must, therefore, only be used for parameter estimation or PEC_s calculations for parent involving a single application only.

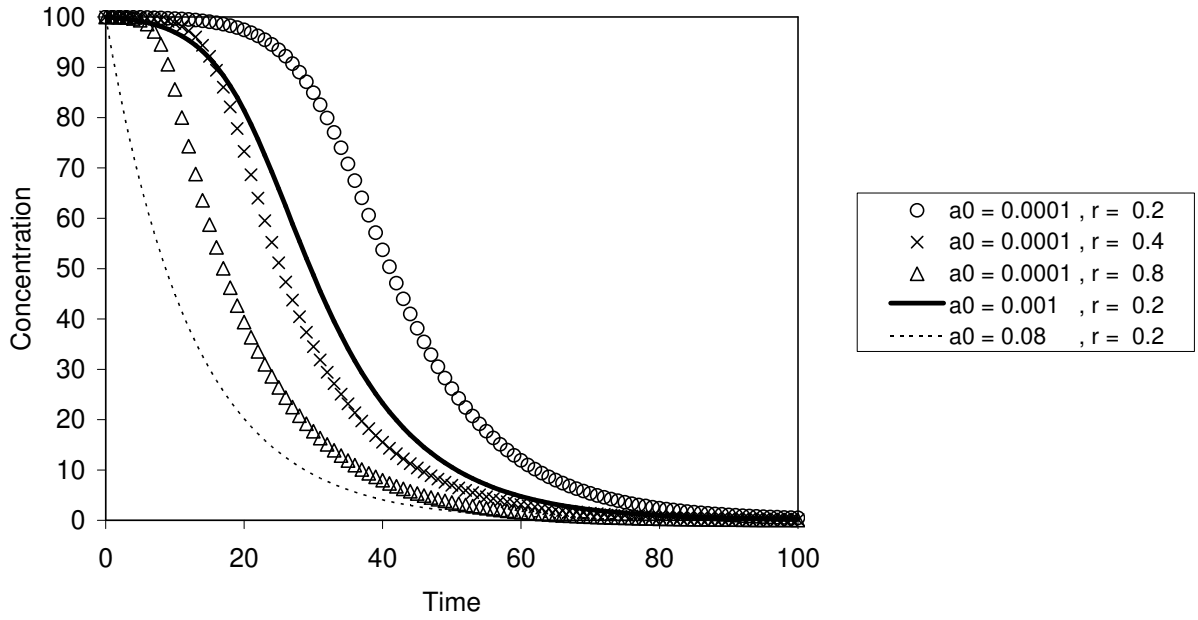


Figure 5-6. Patterns of decline calculated with the logistic model for different values of a_0 and r ($M_0 = 100$, $a_{max} = 0.08$)

5.4 Alternative models

A number of alternative models exist and these can be used to estimate degradation endpoints for use as trigger values provided the approach is justified in the report (information on one of these alternatives, Michaelis-Menten kinetics is provided in Appendix 2). The selected model must be described and its features summarised in line with Table 5-1. Models that result in time-dependent or concentration-dependent endpoints or models that contain a large number of parameters in relation to the number of measurements should be avoided, when possible. Preferably, the model should not include a description of microbial population dynamics in order to limit its complexity.

The optimisation tool by Timme *et al.* (1986) includes a number of empirical equations. Most of the proposed equations are purely empirical and the derived parameters are influenced by the initial concentration of the chemical. The equations are converted to their linear form and

a straight line fitted to the data. This assigns a larger weight to some data points and can lead to inadequate fits. The tool has, thus, serious drawbacks and its use is no longer recommended. This recommendation is in line with the opinion by the Scientific Committee on Plants on the Draft Guidance Document on Persistence in Soil (DG VI - 9188/VI/97-Rev.5 of 20.12.1998*)* expressed on 24 September 1999 which stated with reference to the draft guidance document on persistence in soil: "A number of curve fitting procedures are now available e.g. […] and it is generally agreed that the work of Timme *et al.* (1986) has now been superseded and should not be cited."

Current versions of most tools to calculate predicted environmental concentrations of pesticides in groundwater, surface water and sediment within the regulatory framework (PEARL, PELMO, PRZM, MACRO, TOXSWA) are based on first-order degradation kinetics (a description of these models may be found at the FOCUS website: http://viso.ei.jrc.it/focus/.) Overcoming the limitation of first order kinetics is desirable and the work group attempted without success to identify a bi-phasic kinetics that could be implemented into existing tools. A suitable model must meet the following requirements:

- The model must be suitable to describe a bi-phasic pattern of degradation;
- The right hand side of the equation must only contain state variables (variables that change with time). It must, therefore, not contain dose (initial concentration or application rate) and time.

To date no model has been identified which meets all criteria. The implementation of the Gustafson and Holden model, bi-exponential model and hockey-stick model into pesticide fate models is not universally valid.

Pragmatic approaches to implementing bi-phasic kinetics into pesticide fate models are presented in Chapter 7. One of the models used for this purpose is the FOTC model (first-order, two compartment).

In the FOTC model, the soil is assumed to consist of a rapidly and slowly degrading compartment. All of the compound is initially applied to the rapidly degrading compartment and is transferred to the slowly degrading compartment by a first-order process with the rate k_2. This is the main difference to the bi-exponential model where the applied compound is instantaneously split between the two compartments and not transferred between them. Degradation of the compound to a metabolite or sink takes place in the rapidly degrading compartment with the first-order rate constant k_1. Transformation in the slowly degrading compartment occurs with a rate constant k_3.

The most convenient way of fitting the FOTC model to experimental data is to use differential equations for each compartment:

$$\frac{dRapid}{dt} = -k_1\ Rapid - k_2\ Rapid$$

$$\frac{dSlow}{dt} = +k_2\ Rapid - k_3\ Slow$$

The sum of these two compartments can be fitted against the measured concentration of the pesticide. All pesticide is initially in the rapid compartment, the initial concentration in the slow compartment is zero.

5.5 References

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6 GENERAL RECOMMENDATIONS

6.1 Data issues

In this chapter, general recommendations are given on data quality and data handling issues that are relevant to kinetic analysis. The main recommendations are summarised below:

Data quality	The data set must be of sufficient quality to clearly establish the dissipation pattern and - for metabolites - the formation, plateau and decline phase. The number of data points must be appreciably larger than the number of parameters.
Replicates	Use true replicates individually in the optimisation. Average analytical replicates prior to curve fitting. Average all replicates prior to calculating χ^2 statistics.
Weighting	Carry out unweighted fits initially.
Values < LOQ and <LOD	Parent: Set sample <LOD just after detectable amount to ½ LOD Omit all subsequent samples < LOD (unless later samples > LOQ, see text) Set samples between LOD and LOQ to measured value or 0.5 (LOQ + LOD) Metabolites[1]: Set time zero samples < LOD to 0 Set sample <LOD just before & after detectable amount to ½ LOD Omit all other samples < LOD (for exceptions see text) Set samples between LOD and LOQ to measured value or 0.5 (LOQ + LOD)
Outliers	Include all data points initially.
Time zero concentration	Include in optimisation initially.
Experimental artefacts	Check for experimental artefacts (e.g. declining microbial activity), see text for details.

LOD = limit of detection
LOQ = limit of quantification
[1]Details are included in section 8.3.1.3.

6.1.1 Minimum number of data points

Experimental studies must provide sufficient and adequate sampling points to ensure a robust estimation of parameters. OECD guideline 307 states that a minimum of six samples should be taken over the incubation period from laboratory degradation studies. A minimum of eight samples must be taken according to SETAC (1995). The number of data points available for parameter estimation for parent compounds may be smaller following elimination of outliers, non-detects or a lag phase. Estimation of DT50 and DT90 values is less reliable if the pattern of decline is not clearly established. The report should indicate if the DT50 and/or DT90 was extrapolated beyond the experimental period. Ideally, the number of data points remaining after the elimination of a lag phase, non-detects or outliers should not be smaller than five in accordance with the EC Guidance Document on Persistence in Soil (DG VI - 9188/VI/97 - Rev 8 of 12.07.2000). However, in cases where

degradation of the parent is very rapid (e.g. due to hydrolysis of an ester), obtaining five data points before the parent is completely degraded may not be practical. If with the available data an acceptable fit can be achieved according to criteria outlined in Chapter 7, the endpoints should be considered acceptable.

For metabolites, the formation phase, plateau or maximum concentration, and decline phase should be clearly established. Parameter estimation for metabolites may be very uncertain if the majority of samples show concentrations below the limit of detection or quantification or there is no clear decline within the experimental period (see Section 8.5.1). However, even a highly conservative estimate of the degradation rate may be adequate if modelling results show no concern.

Guidance for water sediment studies is, for example, provided by OECD (in Test Guideline No. 308) and SETAC (1995). Both state that a minimum of 6 sampling points should be included. However, the fact that the test system comprises two compartments (water column and sediment) may necessitate further consideration of number and timing of sampling intervals. For instance, the OECD guideline states that additional sampling points during the initial period of the study may be needed for hydrophobic substances in order to determine the rate of distribution between water column and sediment compartments. This should be particularly important in case DT50/DT90 values need to be determined for both the water column and the sediment compartment.

6.1.2 Replicates

Laboratory degradation, water-sediment and field dissipation studies can be carried out with either single or replicate sampling at each time point. When replicate samples are collected, the procedure for their use in kinetic analyses depends on the nature of the replication.

There are two general procedures for laboratory studies:

- Substrate is treated with the pesticide, mixed and sub-samples are filled into individual flasks for incubation;
- Smaller amounts of substrate are treated individually with the pesticide and incubated in different flasks.

Both procedures are able to generate true, independent replicates and it is recommended that replicate values are used individually for each sampling interval. The degradation model is then fitted to all individual data points at the same time. Replicate analytical results from a single sample are, however, not true, independent replicates and should be averaged and

treated as one sample during parameter optimisation.

In field studies a number of soil cores is generally taken from a test plot. The cores are combined, homogenised and sub-sampled. In some study designs, replicate samples are collected from the different locations within the plot. One core is taken from each location or several cores are homogenised. In other designs, replicates are taken for residue analysis from the same sub-sample. As stated previously, replicate analytical results from a single sub-sample should be averaged and treated as a single sample during curve-fitting. Samples collected from different locations within the same field are considered true replicates and are used individually in the kinetic analysis.

6.1.3 Log transformation and other methods of weighting

Several methods are available for weighting the fits of kinetic models to measured pesticide data. In the simplest case, the fits to the individual untransformed data points are each given the same weight, irrespective of the precision or uncertainty associated with each measurement. However, assigning different weights to fits to different data points is sometimes desirable. The most common method is to perform a logarithmic transformation of the measured concentrations and then fit the logarithmic transformation of the kinetics to the data. In the special case of SFO kinetics, the fitting becomes easier because the log transform of SFO kinetics is the equation of a straight line, which is easy to fit using linear least squares. However, this method is the same as fitting kinetics to untransformed data by $1/(\text{fitted value})^2$, which gives increasing weight to decreasing fitted values, and is based on the assumption that the precision of the data are proportion to the magnitude of the data. If the precision of data does not increase in this way, e.g. when precision decreases close to and below the LOQ, then such a transformation is not appropriate.

Other methods include weighting untransformed measured data at each sampling point by $1/(\text{measured value})^2$, 1/(measured value) or 1/(measured variance). The first option is similar to logarithmic weighting; the second option is somewhere between unweighted and logarithmic fitting; and the third option takes account of the actual measured precision at each time point in the experiments.

Ideally, the method of weighting fits to data should represent the measurement precision or uncertainty of the experimental data. More weight should be given to fitting to data that are measured to greater precision or with less uncertainty.

Statistical criteria to evaluate the goodness of fit should account for any weighting of fits to

the data. Weighting should be used as a tool to reflect the uncertainty associated with each data point. However, weighting should not be performed merely to change the fit, when the weighting is not reflected in the uncertainty of the data.

Laboratory studies

Unweighted fitting to data often results in a better overall fit of SFO kinetics due to lower sensitivity to deviations of calculated from observed data in the later stages of dissipation. In laboratory experiments, these deviations may be due to the influence of increasing sorption and/or decreasing microbial activity. Hence, a good fit to the majority of the dissipation curve represents the actual degradation rate better and unweighted fits are recommended as a first step, particularly if the precision of the later data are no longer proportional to the magnitude of the data, e.g. if close to or below the LOQ.

First-order degradation parameters can be estimated by fitting the exponential equation without weighting to untransformed measured concentrations. However, this requires the use of iterative, non-linear fitting routines that have not been readily available to non-experts until relatively recently. Prior to this, the equation has often been logarithmically transformed. This yields a straight line, which can then be fitted to logarithmically transformed concentrations using linear regression methods. However, logarithmic transformation effectively weights the fits to the data by $1/(\text{fitted value})^2$ which implies an increase in the measurement precision of data as residue levels decrease. When degradation is initially fast followed by a slower decline, logarithmic transformation usually results in underestimating the initial concentrations and a longer first-order DT50 value than a direct fit of the exponential equation to untransformed data. This is illustrated below for a hypothetical data set.

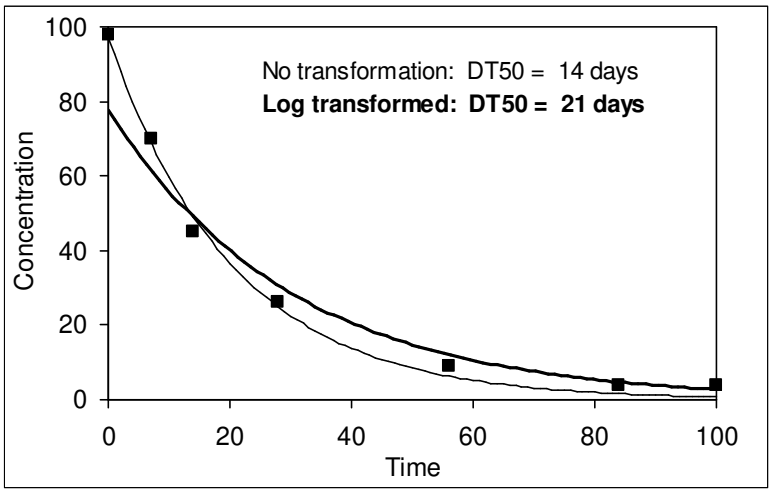


Figure 6-1. Hypothetical example to illustrate the possible influence of data transformation on the derived DT50 value

Note that concentrations are automatically transformed when an exponential trendline is fitted with Microsoft Excel. Logarithmic transformation may be justified if there is experimental evidence that smaller concentrations can be determined with greater precision than larger values. Otherwise, unweighted fitting to untransformed data is recommended.

Alternative methods of weighting the fits to data can be adopted if the use of unweighted fits to untransformed data or logarithmic weighting fails to give a satisfactory representation of the overall decline pattern.

Field studies

Under field conditions, pesticide degradation is influenced by a large number of environmental factors, which are spatially variable at a small scale. It is not clear whether differences between individual points within the field increase or decrease as time progresses. Therefore, establishing general rules for weighting fits of kinetics to field data is difficult so the use of unweighted fits to untransformed data is recommended as a first step. Alternative methods can be applied if a satisfactory fit cannot be achieved.

6.1.4 Values below the quantification and detection limit

Experimental results often include measurements below the limit of quantification (LOQ) or the limit of detection (LOD). The handling of these data may influence the estimated degradation endpoints. The following standard procedure is recommended for parent compounds in soil and water-sediment systems (the procedure for metabolites is discussed in Section 8.3.1.3):

- All values between LOD and LOQ are set to the actual measured value. If the actual measured concentration has not been reported, use 0.5 x (LOQ + LOD).
- All samples < LOD are set to ½ LOD.
- The curve should be cut off after the pesticide has largely dissipated. All samples after the first non-detect (< LOD) should be omitted unless positive detections above LOQ are made later in the experiment. In that case, samples are included up to the first non-detect (<LOD) which is NOT followed by later positive samples above LOQ.

The approach is illustrated for three examples in Table 6-1.

Table 6-1. Three examples to illustrate the handling of concentrations below the limit of detection and quantification for parent compounds (LOQ = 0.05, LOD = 0.02)

Parent 1		Parent 2		Parent 3	
Measured	Set to	Measured	Set to	Measured	Set to
0.12	0.12	0.12	0.12	0.12	0.12
0.09	0.09	0.09	0.09	0.09	0.09
0.05	0.05	0.05	0.05	0.05	0.05
0.03	0.03	0.03	0.03	0.03	0.03
< LOD	0.01	< LOD	0.01	< LOD	0.01
< LOD	-	< LOD	-	< LOD	0.01
< LOD	-	0.03	-	0.06	0.06
< LOD	-	< LOD	-	< LOD	0.01
< LOD	-	< LOD	-	< LOD	-
< LOD	-	< LOD	-	< LOD	-

Note that when transformed data are used in the kinetic analysis, the results are more sensitive to values used for compounds below the LOQ. Therefore, if log-transformed data are used, deviations from this proposal may be justified if an unrealistic result is obtained.

6.1.5 Outliers

Outliers in laboratory studies can be individual (or several) replicates or sampling dates. Ideally, clear outliers should be eliminated before curve fitting, because these may influence the decision on the most appropriate kinetic model. However, statistical tests for identifying outliers in an objective manner may not be appropriate given the limited number of data available. Therefore, all measurements should initially be included in the optimisation. Samples that clearly differ from others can then be eliminated based on expert judgement

and the fitting procedure repeated. The removal of any data points as outliers must be clearly documented and justified in the report. Experimental errors should be identified wherever possible. Another approach to removing outliers is to weight data points by variance, thus assigning low weight to highly variable data.

In field studies, individual sampling dates are occasionally not in line with earlier or later samples. Experimental problems (e.g. sampling error, failure of analytical equipment) or the natural variability of experimental data can result in these outliers. An increase in the concentration from the first to the second sample taken in field studies is a common phenomenon. All data should be included in the curve-fitting procedure as a first step and results should be reported and graphically presented. Outliers can then be omitted from the analysis. Field information on initial concentrations (e.g. from filter paper analyses) can provide useful information and help to identify outliers at early time points. If an outlier is rejected based on expert judgement, this must be clearly indicated in the report and, where possible, supported by statistical analysis.

Identifying an outlier in formation-decline curves of metabolites and in water-sediment studies is more difficult. Again, all data should initially be included and subsequent elimination of outliers must be clearly documented in the report.

6.1.6 Time zero samples

The initial pesticide concentration is usually relatively well known in laboratory samples where a defined amount of the pesticide is added to the system. All experimental and analytical procedures are, however, subject to potential error and the initial concentration is uncertain. The best estimate of the amount of material dosed into the system may, therefore, be derived from the dose checks and chemical purity of the test item. As a first step the initial concentration should be included in the parameter optimisation procedure. The estimated initial concentration is expected to be close to the measured value if the decline in pesticide residues is fitted well by the selected model, rapid degradation/binding of the compound has not occurred, and the applied chemical extraction method has a recovery close to 100 percent. If this is not the case, the underlying reasons should be established. Using a different model or fixing the initial concentration to the applied dose may be necessary.

In the case when first-order kinetics are preferred (such as for PEC_{GW} and PEC_{SW} calculations), the procedure can be modified. The first step is the same as before in which the initial concentration is included in the fitting procedure as a first step. If an acceptable fit

is not obtained, a second optimisation can be carried out where the initial concentration is fixed to the measured value (after correction for any formation of metabolites or bound residues found in the time zero sample analysis, see information later in this section) and the optimisation procedure is limited to the degradation rate constant. The most appropriate method should be selected by expert judgement on a case-by-case basis following a statistical and visual assessment.

The initial pesticide concentration in field studies is more uncertain than that in closed laboratory systems and subject to variability. The initial value should be estimated using curve-fitting procedures, rather than being set to the measured day 0 value. If a satisfactory fit is not obtained using unconstrained fitting, the initial concentration may be constrained. Correction of parent concentrations to account for non-zero residues of metabolites in time zero samples should be considered (important for compounds that degrade rapidly). Where appreciable variation in time zero residues are found in the field, calibrated spray application rates or those derived from filter paper analyses can help to define the initial concentration. The most appropriate method should be selected by expert judgement on a case-by-case basis following statistical and visual assessment.

Initial concentrations for metabolites and bound residues (in laboratory studies) are expected to be zero. However, the time from application of the parent compound to taking the first soil sample is usually in the range of 0.5-2 hours. Rapidly formed metabolites and bound residues may thus be detectable in the first sample. In this situation, the amount of the parent compound present in soil at time zero should be adjusted during kinetic analysis (i.e., the mass of the metabolite found in the initial sample is converted to mass parent and added to the value measured for the parent compound). The mass of the metabolite should be set to zero. Where possible, this procedure should also be followed if unidentified metabolites or bound residues (sink compartment) are present in the first sample due to rapid formation.

Another approach sometimes used to adjust for degradation in initial samples is to consider the time between sampling and when degradation stops due to cooling of the samples (often 0.5-2 hours). Disadvantages of this approach are the difficulty in determining this time and the often considerable difference in the time between the collection of the first and last replicate samples, especially in field studies.

Initial concentrations of a metabolite larger than zero may also be due to impurities in the application solution. In this case, during kinetic analysis the initial metabolite concentration should be set to the measured value.

A formation of the metabolite during preparation of the first soil sample for analysis in the laboratory is also possible. This is an experimental artefact and a decision on how to proceed in this situation should be made on a case-by-case basis.

6.1.7 Experimental artefacts

In the laboratory, faster degradation is often followed by a slower decline in pesticide residues. This phenomenon may also occur under field conditions. In some cases, the bi-phasic behaviour can, however, be attributed to experimental artefacts. Other experimental artefacts can result in a very slow decline in concentrations for a certain period after treatment (lag-phase). Degradation endpoints can strongly depend on the model used to fit the data and an assessment should be made whether the degradation pattern observed in the laboratory is representative of field conditions prior to kinetic analysis. Efforts to identify experimental artefacts in laboratory studies must be made. Artefacts that can influence the pattern of concentrations in a laboratory study, information than can be used to identify an artefact and recommendations on how to proceed in such cases are summarised in Table 6-2.

Table 6-2. Typical experimental artefacts

Artefact	Source of information	Recommendation
Lag-phase because of inadequate soil storage or excessive drying of soil before incubation	Study records about soil storage/handling	Discard lag-phase for DT50 and DT90 calculation
Soil was not viable throughout study duration, 'dying-off' towards study end	Measurements of soil microbial number/activity[1]	Discard later sampling dates for kinetic analysis
Residue data were determined by different extraction methods at different sampling dates	Study records	Evaluate effect on total extractable residue, if not negligible, case-by-case decision
Soil pH shift within study period	Measurements	Evaluate effect of changing pH, case-by-case decision

[1] OECD (1995) states that in soil experiments studying the transformation route and rate, the microbial biomass should constitute more than 1% of the total organic carbon. (Final report of the OECD workshop on selection of soils/sediments, TG95.25, Belgirate, Italy, 18-20 January 1995)

Degradation rate studies under field conditions also include loss processes such as photolysis on the soil surface, losses through volatilisation and/or leaching to deeper soil layers. Under field conditions transient soil moisture and temperature conditions prevail compared to the static conditions in laboratory studies. Recommendations on how to account for these processes in the kinetic analysis are given in Chapter 9.

6.2 General recommendations on kinetic analysis

The estimation of parameter values from degradation studies consists of several steps:

1. Entering the measured data for each sampling time.
2. Selection of the kinetic model.
3. Making an initial guess for each parameter value of the selected model (referred to as "starting value" in the following text).
4. Calculation of the concentrations at each time point with the selected kinetic model.
5. Comparison between the calculated and measured concentrations.
6. Adjustment of the parameter values until the discrepancy between the calculated and measured concentrations is as small as possible ("best fit").

Usually, steps 4-6 are carried out automatically using software tools. These packages start from the initial guess made by the modeller and repeatedly change the parameter values in order to find the best-fit combination. In order to use such an automated procedure, "best fit" must be defined in the form of a mathematical expression. Often, the sum of the squared differences between the calculated and observed data (residual sum of squares = RSS) is used. The software package aims at finding the combination of parameters that gives the smallest RSS. This method is referred to as Least Squares method.

There may be a single combination of parameters that results in the smallest possible value for the residual sums of squares ("global minimum"). However, often there are several additional combinations that also result in small RSS ("local minima"). In this case, the software may stop the optimisation procedure before the global minimum is found. The ability to reach the global minimum depends on the initial guess (the closer the initial guess to the best possible value, the better), the nature of the specific optimisation problem and the settings within the software package. Different endpoints may be obtained by different software packages and the derived combination of parameters does not necessarily provide the best possible fit to the measured data.

To minimise these problems, some general guidance on parameter optimisation should be followed. For details specific to the selected software tools please refer to the respective user manual.

Always evaluate the visual fit

As a first step, the measured data should be plotted against time to help identifying the appropriate type of model. For example, kinetics for decline patterns without a lag phase must not be fitted where a true lag phase exists.

A decline curve should always be calculated using “optimised” parameters (i.e. those returned by the software tool after certain criteria are met) and plotted against measured data. If the calculated curve differs strongly from the measured concentrations, the optimisation tool may be able to improve the fit if better starting values are provided. The calculated endpoints for parent compounds should also be compared with the value obtained by interpolation of the measured values.

Please note that using the best combination of parameters does not guarantee a good fit. If the selected model is not appropriate to describe measured behaviour, even the best possible parameter combination for that model won’t give an adequate fit to the data. Always evaluate the visual fit to decide if a model is acceptable.

Avoid over-parameterisation

A robust optimisation of parameters is only possible if the number of observations is appreciably larger than the number of model parameters. A kinetic analysis should not be performed if the number of data points is too small following elimination of outliers or non-detects. The appropriate number of data points is different for each actual optimisation problem and universally valid recommendations cannot be made.

Use realistic starting values

Different optimised values may be returned by the software for different combinations of initial guesses for the parameters provided by the modeller (starting values). The nature of the particular optimisation problem and characteristics and settings of the software package determine whether or not starting values influence the outcome. For example, many software packages run the optimisation procedure up to a maximum number of times specified by the user. The package stops after the last step, irrespective of whether the best possible fit was reached.

In general, the closer the initial guess for the parameter value to the optimum value, the better the chance to find the global minimum (i.e. the true best-fit value). The optimisation

should be repeated with a number of different initial combinations of parameter values. If the answer is different each time, then finding good starting values is very important for the situation at hand.

Finding appropriate starting values is easier for some parameters than others. For example, it is easy to select a starting value for the parameter M0 in the SFO and FOMC model as this is expected to be close to the measured initial concentration. The initial value for the degradation rate constant of a parent compound in soil can be set to ln(2) divided by the measured (interpolated) time until 50% of the initial concentration has disappeared. Initial guesses for the degradation rate constant and the formation fraction of a metabolite can be calculated from the degradation rate for the parent, the time to maximum formation of the metabolite and the maximum amount formed in the degradation study (Gurney, 2004).

Plotting the measured concentrations and the calculated curve is a useful technique for deriving good starting values. The parameter values that produce a curve that is reasonably close to the measured data can be used as starting points in a software package.

Plotting several calculated curves for different combinations of parameter values helps understanding the effect of individual parameters on the shape and steepness of the curve. For example, dissipation calculated by the FOMC model is faster for larger values of α and for smaller values for β (see Figure 5-2).

In some cases, a number of different parameter combinations give nearly identical curves. When this occurs, the optimisation is unlikely to give a unique answer (i.e. the result will depend on the starting values). For example, this may occur if bi-phasic models are fitted to decline curves that are well described by first-order kinetics. In this case, the visual plot should be evaluated and the endpoints calculated for the different optimised parameter combinations. If the visual fit and endpoints are similar for different parameter combinations, then the results are acceptable. However, in this situation, results from SFO kinetics are often equally valid and the use of a bi-phasic model may not be warranted.

Finding appropriate starting values is particularly important where a large number of parameters are optimised at the same time. Different combinations of initial guesses must be tested to investigate the influence of the starting values on the result.

Constrain parameter ranges and carry out plausibility checks

For some parameters, realistic ranges can be identified and these can be used to constrain the fitting procedure (i.e. the parameter will only be varied within these limits during optimisation) or to evaluate the plausibility of an optimised parameter value. For example, the formation fraction of a metabolite should, in theory, not be larger than 1 (note that both the parent and metabolite must be expressed as a percentage of applied radioactivity or in moles). The sum of formation fractions of several metabolites formed from the same radiolabelled molecule of parent should also not exceed 1. However, in some cases two molecules of a metabolite are formed from one molecule of the parent. In this situation, the formation fraction can be larger than 1 when the data are expressed in moles. Rate constants should always be positive.

Optimised parameters from simpler models can sometimes be used to constrain the fitting at higher levels of complexity.

Carry out stepwise fitting where necessary

Sometimes, temporarily fixing those values for which a good initial guess is available and optimising only the other variables is helpful. Then all values should be released and optimised simultaneously. This can be useful for deriving parameters for metabolites (See Section 8.4.1).

6.3 Assessment of goodness of fit and model comparison

A number of methods to assess the goodness of fit of an individual model and to compare different kinetics have been reviewed by this FOCUS work group. For further details and additional methods, the reader is referred to statistical handbooks (Bates and Watts, 1988; Draper and Smith, 1998; Gallant, 1987; Seber and Wild, 2003; Snedecor and Cochran, 1967).

Criteria that are recommended for a standard assessment are given in Section 6.3.1. Optional methods are described under Section 6.3.2. These can be used to give additional information, provided their limitations are taken into account.

6.3.1 Recommended methods

The FOCUS group aimed at identifying a statistical method that provides an objective framework for evaluating the goodness of fit of an individual model and to compare two different models. The aim was to propose a test that is universally valid for all kinetics and matches the decision made by experienced users on the basis of visual assessment. Unfortunately, no such test was found and visual assessment will continue to play a major role in evaluating the goodness of fit. This should be used in combination with a χ^2-test to compare the goodness of fit of two different kinetics and a t-test to evaluate the confidence in the parameter estimates.

6.3.1.1 Visual assessment

Visual assessments have long been used to compare predicted and observed data for a large number of applications and a variety of approaches are presented in standard textbooks. Massey *et al.* (2003) describes the application of these techniques to soil residue studies. This section describes the procedure recommended by the FOCUS work group.

In addition to the calculation of statistical indices (see below), measured and fitted data must always be presented graphically and a visual assessment of the goodness of fit must be made. Measured concentrations and the calculated curve should be plotted versus time. A second plot should be made of calculated minus measured data (residuals). This is useful for revealing patterns of over- or under-predictions. For an exact fit, all residuals are zero. Systematic deviations occur if negative and positive residuals are not randomly scattered around the zero line. Note, if the concentrations are log-transformed prior to curve-fitting, the differences between the transformed data (log calculated minus log measured) must be shown in the residual plot.

Modellers may also wish to evaluate a plot of measured versus calculated values. All points should be randomly scattered around the 1:1 line and ideally fall exactly on the line. This plot is optional and not required for a standard assessment.

The two standard plots are shown below for three hypothetical examples.

EXCELLENT FIT

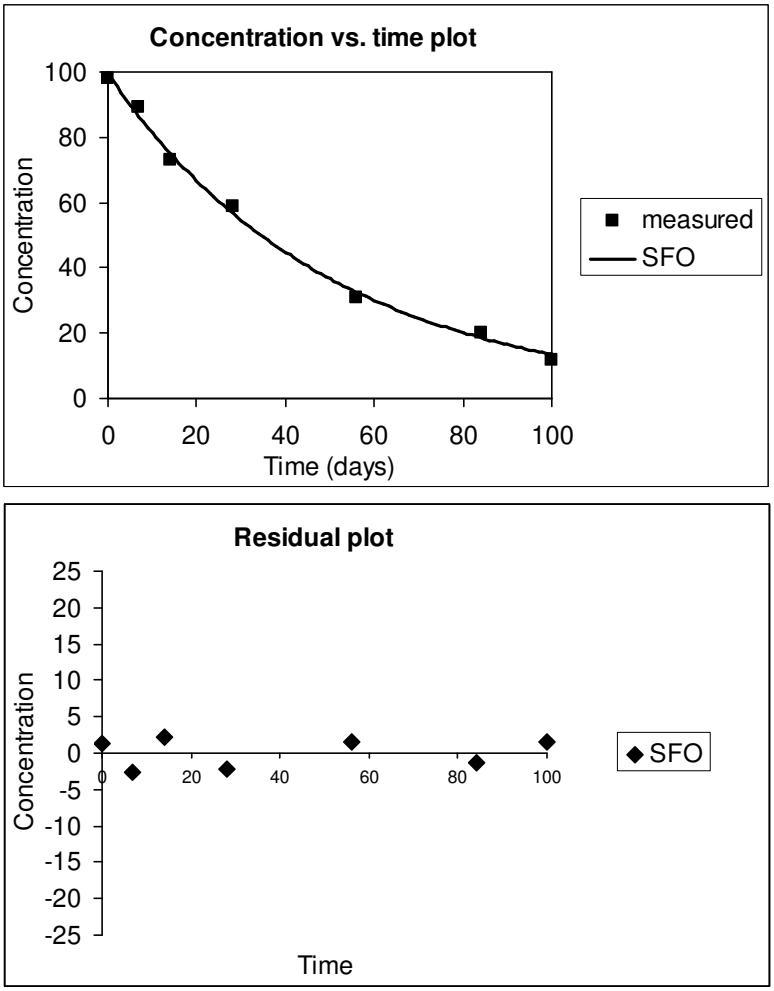


Figure 6-2. Plots for visual assessment –example of an excellent fit

In Figure 6-2, SFO kinetics give an excellent fit. The calculated curve matches the observed behaviour very well. The residuals are small and randomly scattered around the zero line.

POOR SFO FIT

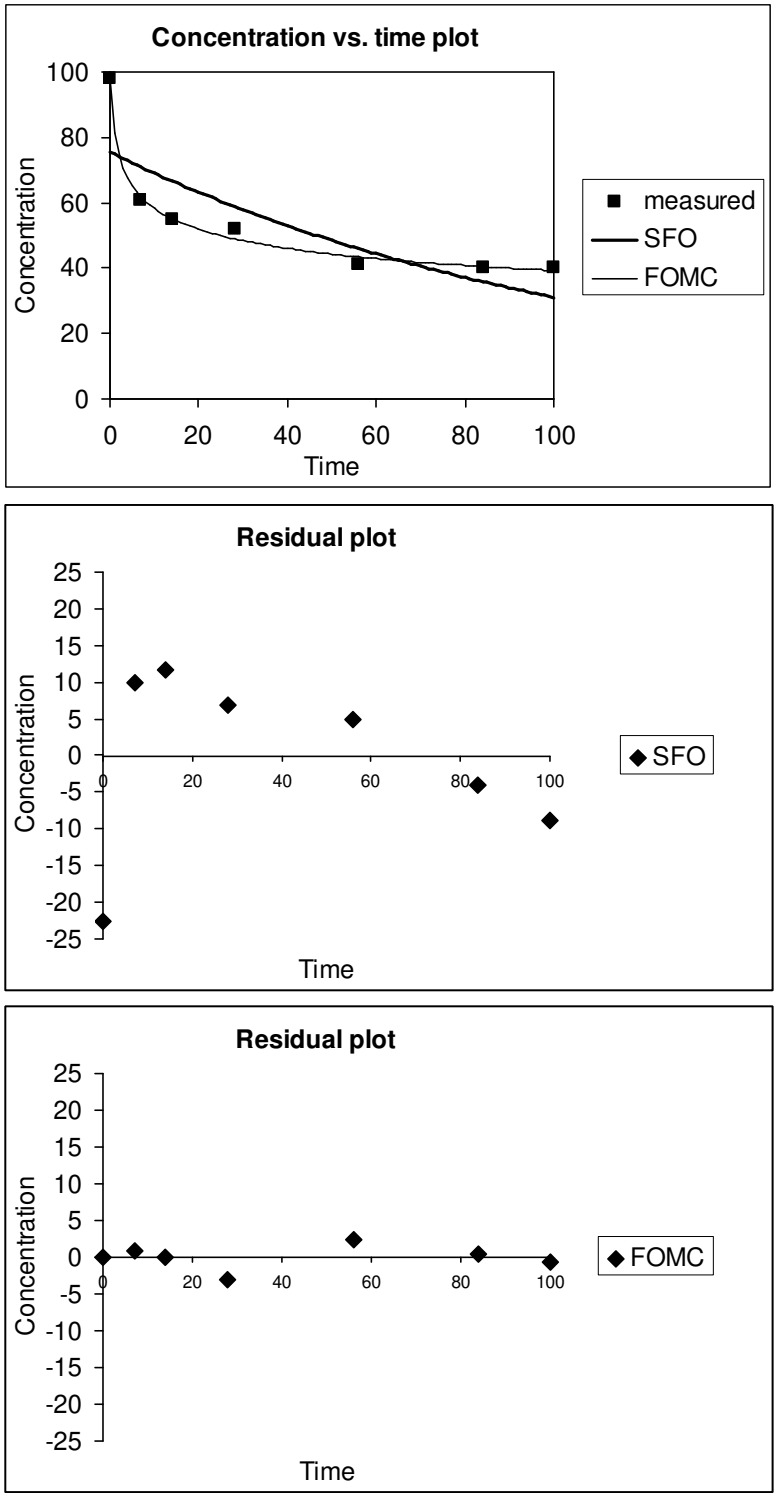


Figure 6-3. Plots for visual assessment –example of a poor fit for SFO kinetics

Figure 6-3 is an example where SFO kinetics provide a poor fit to the data. This is obvious from both visual plots. The calculated curve does not match the observed pattern. The initial concentration is markedly under-estimated by the SFO model. The residuals are large and show systematic deviations (four consecutive positive residuals). The bi-phasic Gustafson

and Holden model fits the data much better than SFO kinetics. In this situation, the SFO fit must be rejected. This example is for a hypothetical data set created for illustration purposes. Most real data sets are likely to be described well by first-order kinetics or fall into an intermediate category where the SFO model and the bi-phasic fit deviate to a lesser extent than shown above.

INTERMEDIATE SFO FIT

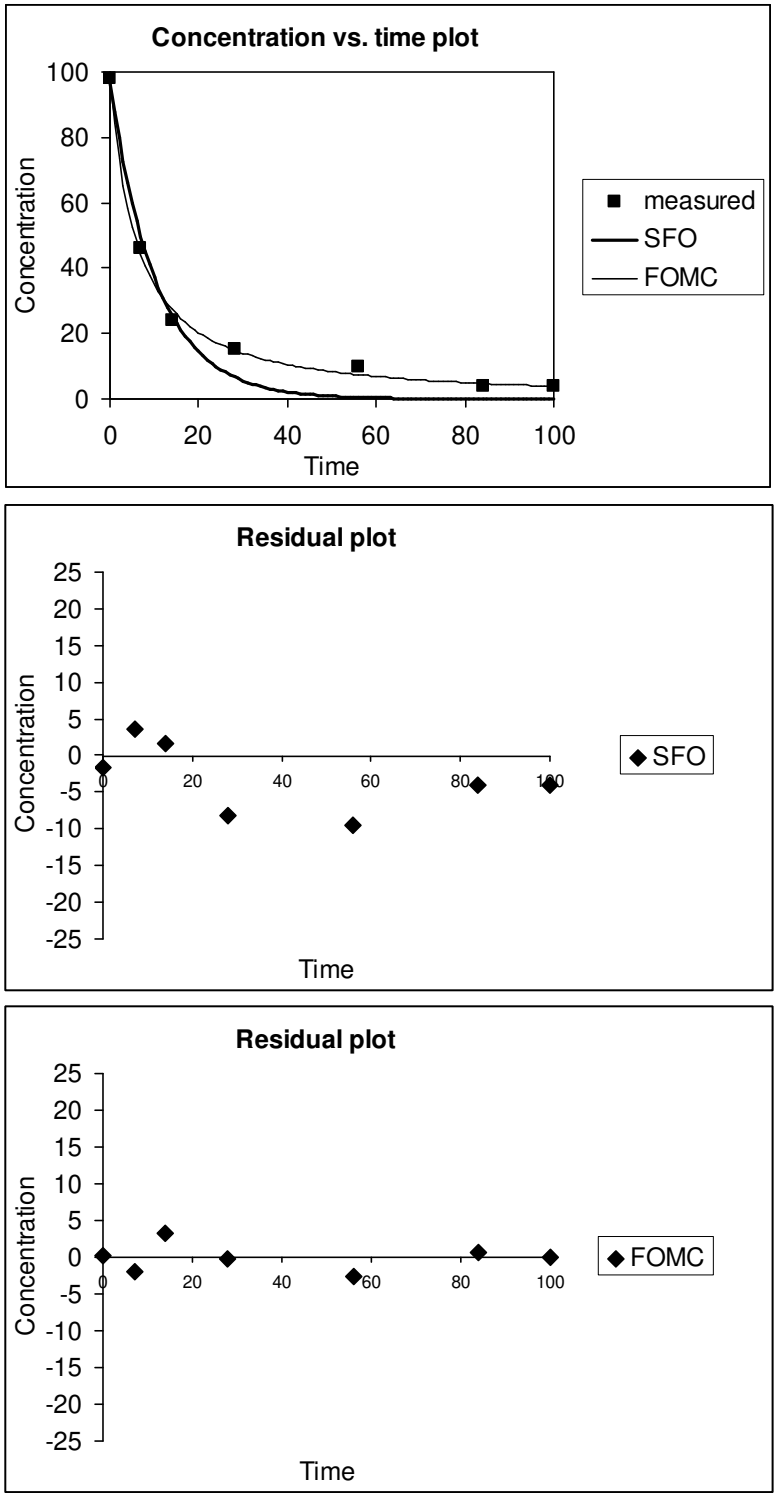


Figure 6-4. Plots for visual assessment –example of an intermediate fit

The data shown in Figure 6-4 are adequately described by SFO kinetics up to day 14. Concentrations measured at later dates are under-estimated by SFO kinetics. The residual plot shows systematic deviations (two positive residuals followed by four negative residuals). The Gustafson and Holden model provides a better fit to the data. However, the difference

between calculated and observed values for the SFO fit and the difference between the two models is much smaller than for the previous example.

In intermediate cases, the decision on the acceptance of the SFO fit depends on the intended use of the endpoint (trigger value or modelling input) and should be based on statistical indices and expert judgement. This will include a consideration of the match of observed data around the DT50 and DT90. In this example, the observed DT50 value is matched well by SFO kinetics and similar values are obtained for both models (7.3 days for SFO and 5.7 days for FOMC). The measured data declined to less than 10% of the initial amount within the experimental period. In this situation, the measured DT90 can be compared with the calculated value. The DT90 value for the Gustafson and Holden model (42.6 days) is longer than that for SFO kinetics (24.3 days) and describes the measured value better. However, both calculated values are clearly shorter than the shortest laboratory DT90 trigger value of 100 days. Thus, the acceptance or rejection of SFO kinetics would not influence the decision on whether additional higher-tier studies are required. The decision is often more difficult where 10% of the initial concentration was not reached within the experimental period. In this case, the DT90 will be longer than the duration of the study (typically around 100 days), but its real value is difficult to establish. The use of the DT90 calculated from SFO kinetics as a trigger for additional work may under-estimate persistence whereas the extrapolation of bi-phasic models often results in unrealistically long DT90 values. A case-by-case decision should be made and the result should be seen in the light of data from other studies with the same compound. Endpoints for use as trigger values can be determined using non-SFO kinetics where necessary. In contrast, first-order DT50 values are required for use in pesticide fate models and the requirements for accepting intermediate SFO fits can potentially be relaxed.

For details on the recommended decision process and its application to real data sets see Chapter 7.

6.3.1.2 Chi-square (χ^2) test

The χ^2-test and its use as a tool to compare the goodness of fit of two or more models are described in detail in the following sections. The following is a summary of this information:

True replicates	Use individually in curve fitting, but average before calculating chi-square
Chi-square statistics	$\chi^2 = \sum \frac{(C-O)^2}{(err/100 \times \overline{O})^2}$ where C = calculated value O = observed value $\overline{O}$ = mean of all observed values (element of scale) err = measurement error percentage (element of proportionality) If χ^2 > tabulated $\chi^2_{m,\alpha}$, then the model is not appropriate at the chosen level of significance where m = degrees of freedom α = probability that one may obtain the given or higher χ^2 by chance
Tabulated $\chi^2_{m,\alpha}$	See Table 6-5 or use CHIINV(α, m) function in Excel
Degrees of freedom m	Number of measurements (after averaging of replicates) minus number of parameters. Do not take into account any parameters or data points excluded from the optimisation
Probability α	Usually 5%
Model error	Calculate model error at which test is passed explicitly by solving Equation 6-1 for *err*: $err = 100 \cdot \sqrt{\frac{1}{\chi^2_{tabulated}} \cdot \sum \frac{(C-O)^2}{\overline{O^2}}}$
Model comparison	Compare error levels: The model which passes at the smaller level describes the data better

6.3.1.2.1 Chi-square (χ^2) statistics

The chi-square test considers the deviations between observed and calculated values relative to the uncertainty of the measurements.

$$\chi^2 = \sum \frac{(C-O)^2}{(err/100 \times \overline{O})^2} \qquad (6\text{-}1)$$

where

C = calculated value

O = observed value

$\overline{O}$ = mean of all observed values (element of scale in error term)

err = measurement error percentage (element of proportionality in error term, see Section 6.3.1.2.2)

The calculated χ^2 for a specific fit may be compared to tabulated $\chi^2_{m,\alpha}$

where

m = degrees of freedom = number of measurements (after averaging of replicates) minus number of model parameters
α = probability that one may obtain the given or higher χ^2 by chance.

Tabulated values are given in Table 6-5. Alternatively, they can be calculated in Excel using the CHIINV(α,m) function.

When calculating the degrees of freedom, no parameter that is fixed during a fit should be included in the number of measurements. When fitting parent and metabolites in a stepwise approach, this principle should be followed, i.e. do not account for fixed parent kinetic parameters while fitting the metabolite. Any data that were fixed during the optimisation (e.g. initial mass) should be excluded in the calculation of χ^2. The number of model parameters for selected model fits is given in Table 6-3.

Table 6-3. Number of model parameters for selected kinetic model fits considering one compartment, e.g. parent only

Kinetic model	Number of model parameters	Fitted parameters
SFO	1	k, (M0 fixed)
SFO	2	k, M0
FOMC	2	α, β (M0 fixed)
FOMC	3	α, β, M0
HS	3	k1, k2, tb (M0 fixed)
HS	4	k1, k2, tb, M0
DFOP	4	M1, M2, k1, k2 or M0, k1, k2 g

The χ^2 significance test indicates whether the model is probably not appropriate, i.e. demonstrating that the differences between calculated and observed are unlikely due to chance. Often α = 0.05 is used, that is a χ^2 greater than $\chi^2_{m,0.05}$ indicates that the probability that the model is not appropriate is greater than 95 %. In this report, the χ^2 test is recommended as a tool for model comparison (see below), and as a supplementary tool for assessing the goodness of fit of an individual model (the visual assessment is the main tool for assessing goodness of fit).

6.3.1.2.2 Chi-square (χ^2): Accounting for measurement error

The χ^2-test considers the deviations between observed and predicted values for each separate model relative to the uncertainty of the measurements (see denominator In Equation 6-1). Ideally, the measurement variation at each time point could be determined from numerous replicate values. However, such replicate values are rarely available. Therefore, a pragmatic approach to simply define the measurement variation is proposed. The measurement uncertainty is expressed with a common error model that consists of two elements. A percent error value is scaled with the mean of all the observed values. Thus, this error term is constant throughout the measurement period. The relative overall error is lower for early time points, equal to the error term at the mean observed and increases for later time points, thus being consistent with the recommendation of unweighted fitting (see Section 6.1.3). Note that there is no inherent value for the percent error for any given test system. The selection of an acceptable value is purely pragmatic (see 6.3.1.2.4).

6.3.1.2.3 Chi-square (χ^2): Dealing with replicate measurements

The pragmatic solution to address the uncertainty of the measurements permits restricting the computation of χ^2 to using the calculated mean and observed mean values. In this way the test evaluates the goodness of the model fit and not the variation in replicate values. However, true replicate values should be used for the kinetic fit (see Section 6.1.2).

6.3.1.2.4 Chi-square (χ^2): Differentiating between kinetic models

The χ^2 test can be used to test the quality of the measured data and the agreement between calculated and observed for a given fit. A suitable model should pass the test at a significance level of 5%. However, this is only possible if the percent error is known. This is often not the case.

- The minimum error-% of the error term (error-% / 100 * mean observed) at which the test is passed can be directly derived from Equation 6-2. This is the case if the calculated value of χ^2 is equal to or smaller than the standard tabulated value at the 5% significance level and the given degrees of freedom.

$$err = 100 \cdot \sqrt{\frac{1}{X^2_{tabulated}} \cdot \sum \frac{(C-O)^2}{\overline{O^2}}} \qquad (6\text{-}2)$$

where

C = calculated value

O = observed value

$\overline{O}$ = mean of all observed values

- err = measurement error percentage (see Section 6.3.1.2.2)
  The model with the smallest error percentage is defined as most appropriate, because it describes the measured data in the most robust way.
- Field data will be inherently more variable than laboratory data generated under controlled conditions. Therefore, for field studies, the error percentages at which χ^2-passes will generally be larger than for laboratory studies.

In the example presented in Table 6-4 the minimum error % value to pass the test can be calculated explicitly with Equation 6-2 using the appropriate χ^2_{tab} values, as well as the observed and predicted values.

Table 6-4. Example: Determination of appropriate χ^2_{tab} value to calculate minimum err-% to pass test at a significance level $\alpha = 0.05$

Model	Parameters	n	m	χ^2_{tab}
SFO	2	9	7	14.067
FOMC	3	9	6	12.592

n: Number of measurements

m: Degrees of freedom = number of measurements minus number of model parameters

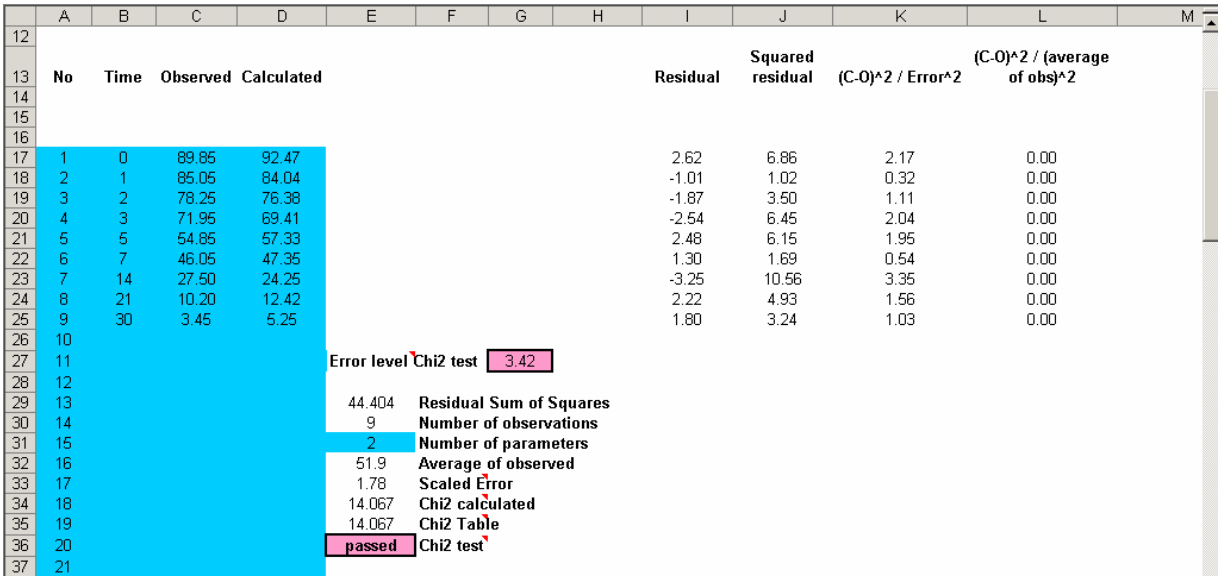
χ^2_{tab} Taken from Table 6-5 for appropriate m, $\alpha = 0.05$.

Table 6-5. Tabulated $\chi^2_{m,\alpha}$ values

	Probabilities α				
m	**0.10**	**0.05**	**0.025**	**0.01**	**0.005**
1	2.706	3.841	5.024	6.635	7.879
2	4.605	5.991	7.378	9.210	10.597
3	6.251	7.815	9.348	11.345	12.838
4	7.779	9.488	11.143	13.277	14.860
5	9.236	11.070	12.833	15.086	16.750
6	10.645	**12.592**	14.449	16.812	18.548
7	12.017	**14.067**	16.013	18.475	20.278
8	13.362	15.507	17.535	20.090	21.955
9	14.684	16.919	19.023	21.666	23.589
10	15.987	18.307	20.483	23.209	25.188
11	17.275	19.675	21.920	24.725	26.757
12	18.549	21.026	23.337	26.217	28.300
13	19.812	22.362	24.736	27.688	29.819
14	21.064	23.685	26.119	29.141	31.319
15	22.307	24.996	27.488	30.578	32.801
16	23.542	26.296	28.845	32.000	34.267
17	24.769	27.587	30.191	33.409	35.718
18	25.989	28.869	31.526	34.805	37.156
19	27.204	30.144	32.852	36.191	38.582
20	28.412	31.410	34.170	37.566	39.997
21	29.615	32.671	35.479	38.932	41.401
22	30.813	33.924	36.781	40.289	42.796
23	32.007	35.172	38.076	41.638	44.181
24	33.196	36.415	39.364	42.980	45.559
25	34.382	37.652	40.646	44.314	46.928
26	35.563	38.885	41.923	45.642	48.290
27	36.741	40.113	43.195	46.963	49.645
28	37.916	41.337	44.461	48.278	50.993
29	39.087	42.557	45.722	49.588	52.336
30	40.256	43.773	46.979	50.892	53.672
40	51.805	55.758	59.342	63.691	66.766
50	63.167	67.505	71.420	76.154	79.490
60	74.397	79.082	83.298	88.379	91.952
70	85.527	90.531	95.023	100.425	104.215
80	96.578	101.879	106.629	112.329	116.321
90	107.565	113.145	118.136	124.116	128.299
100	118.498	124.342	129.561	135.807	140.169

Alternatively, the CHIINV(α,m) function in Excel can be used to obtain the appropriate χ^2_{tab}-value.

An Excel spreadsheet is provided on the FOCUS website to assist in these calculations:



No	Time	Observed	Calculated	Residual	Squared residual	(C-O)^2 / Error^2	(C-O)^2 / (average of obs)^2
1	0	89.85	92.47	2.62	6.86	2.17	0.00
2	1	85.05	84.04	-1.01	1.02	0.32	0.00
3	2	78.25	76.38	-1.87	3.50	1.11	0.00
4	3	71.95	69.41	-2.54	6.45	2.04	0.00
5	5	54.85	57.33	2.48	6.15	1.95	0.00
6	7	46.05	47.35	1.30	1.69	0.54	0.00
7	14	27.50	24.25	-3.25	10.56	3.35	0.00
8	21	10.20	12.42	2.22	4.93	1.56	0.00
9	30	3.45	5.25	1.80	3.24	1.03	0.00
10							
11							
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Error level Chi2 test	3.42
44.404	Residual Sum of Squares
9	Number of observations
2	Number of parameters
51.9	Average of observed
1.78	Scaled Error
14.067	Chi2 calculated
14.067	Chi2 Table
passed	Chi2 test

The χ^2-test was applied to a number of real data sets as shown in Appendix 3.

The χ^2-test has the following advantages over the calculation of the Scaled Root Mean Squared Error (SRMSE) and the Scaled Total Error (STE) described in Sections 6.3.2.2 and 6.3.2.3:

- For each kinetic model, the appropriate degrees of freedom (number of observed data points – number of model parameters) are taken into account.
- The defined underlying χ^2-distribution allows a test of significance at a desired level (e.g. $\alpha = 0.05$, 5%).
- The calculated value of χ^2 may be compared with standard values of χ^2.

As with the SRMSE and STE, the χ^2-statistic is sensitive to the shape and vertical location of the data. Data sets that are fit equally well by a given kinetic model can have different χ^2-values simply based on the shape of the curve. However, this effect is less pronounced compared to the SRMSE and STE (see Section 6.3.2.2).

Some software packages perform a χ^2-test as a default option. Since there are several different commonly used forms of the χ^2-test, the χ^2-test in a specific software package may differ from the test recommended in this report. In this case, results on the χ^2-test performed by the software must not be reported to avoid confusion. If in doubt, compare the equations given in the software's user manual with those in this report.

6.3.1.3 t-test and confidence intervals

The confidence that can be assigned to a parameter value returned after the optimisation must be assessed. If a parameter is not significantly different from zero, then the parameter is either very uncertain due to variability in the data or the model is not adequate with respect to the data. Three examples are given below:

- Parameter estimates for a parent compound may have low confidence if e.g. the degradation rate constant approaches zero in the second phase of the hockey-stick model or bi-exponential curve.
- Knowing if a degradation rate constant of a metabolite is greater than zero is important, particularly if the amount of the metabolite declines very slowly or does not appear to decline.
- If the fraction of formation of a metabolite from one of two possible predecessors is zero, then this route of degradation is likely to be unimportant and the model can be simplified.

A t-test can be carried out to assess whether a parameter differs from zero at the chosen significance level. Alternatively, the confidence interval can be reported. If zero is not included in the confidence interval, the parameter is significantly different from zero. Both, the t-test and the confidence interval give the same answer, provided the underlying assumptions are identical (distribution of the parameter and level of probability). The user must choose the t-test or the confidence interval. In practice, this decision will depend on the output provided by the software package.

t-test

If the parameters are normally distributed, then the statistics

$$t = \frac{\hat{a}_i}{\sigma_i} \qquad (6\text{-}3)$$

is t-distributed.

$\hat{a}_i$ = estimate of parameter i
σ_i = standard error of parameter i

The probability (p-value) corresponding to the calculated t-value is read from statistical tables or calculated with Excel (TDIST) or statistical packages (single-sided; degrees of freedom equals the number of observations minus the total number of estimated model parameters).

Unlike the χ^2-test, the number of observations includes replicates. If the kinetic model is for parent, only parent observations are used. If the kinetic model is for parent and metabolites, the data for parent and the included metabolites are used. Like the χ^2-test, values which are fixed are not included as an observation. For example, the number of observations would be ten for a kinetic model of a study in which there were five sampling points at which two replicate samples were collected. For a kinetic model for the same study design including parent and two metabolites (with measurable concentrations at each sampling point), the number of observations would be 30. If in the regression the amount was set to zero for the two metabolites in the first sampling point, the number of observations would be 26. If in addition the value for parent was fixed, then the number of observations would be 24.

The parameter is considered significantly different from zero if the probability is smaller than 0.05, i.e. considering a 5 percent significance level (or 10 percent for water-sediment studies). In cases where the probability is between 0.05 to 0.1, the parameter may still be considered acceptable; however further discussion and justification based on the fit as well as on weight of evidence from other available data for the substance is then necessary. Significance levels above 10 percent are not considered acceptable.

Often, the t-test or the standard error required to calculate the t-statistics will be provided by the software tool used for the kinetic analysis. For example, ModelMaker provides the parameter value $\pm$ its standard error at the end of the optimisation.

Note that this application of the t-test, while applicable for rate constants, may not be appropriate for all model parameters. For example, with FOMC smaller values of beta indicate more rapid degradation, and alpha only indicates the shape of the curve and has nothing to do with the rate of degradation.

Confidence intervals

A confidence interval is an estimate of the uncertainty in a model parameter. The underlying assumption is: If the experiment and the estimation procedure are repeated infinitely often, then the true value of the parameter lies within the confidence interval with the chosen probability. The calculation of the confidence interval is not straightforward. Fortunately, many software packages provide confidence limits for the parameters. Usually, a 95% confidence interval is provided. The narrower the confidence interval the greater the precision with which the parameter can be estimated.

Clear cut-off criteria cannot be provided for acceptable confidence intervals (e.g. parameter y is considered acceptable if the confidence interval is not larger than x times the parameter value). The confidence interval can, however, be used to assess whether a parameter value is significantly different from zero. This is the case if zero is NOT included in the confidence interval.

Note that the confidence interval for a parameter cannot be easily converted to the confidence interval for the degradation endpoints (e.g. DT50 or DT90 values). This is because the confidence interval for a parameter is based on the assumption that this parameter is normally distributed. For example, if the degradation rate constant k of the SFO model is normally distributed, then the DT50 value is certainly not normally distributed, because there is a reciprocal relationship between k and DT50. The situation is even more complicated if more than one parameter is required to calculate the endpoints (e.g. α and β from the FOMC model).

Confidence intervals around the calculated curve based on the uncertainty of all model parameters can sometimes be calculated by the optimisation software tool. For example, the dashed curves shown below were generated with ModelMaker and represent the upper and lower confidence limits arising from the uncertainty in the parameters M_0, α, β of the FOMC model.

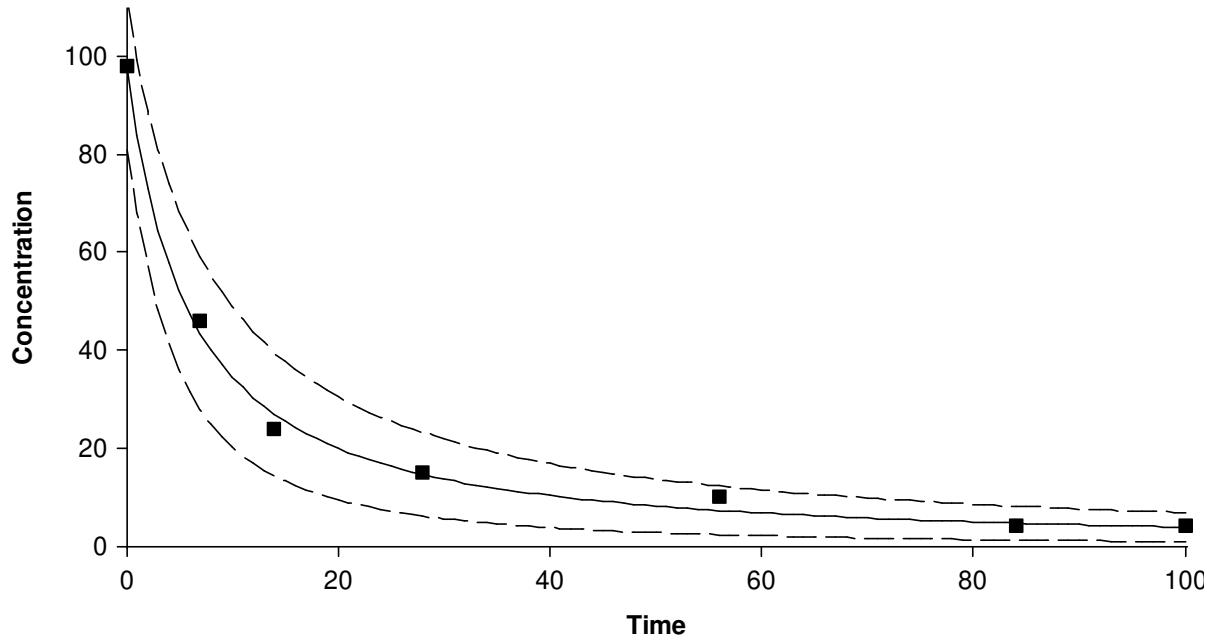

Figure 6-5. Confidence interval for the FOMC model fitted to an example data set

Confidence in a parameter is particularly critical where a parameter influences the value of other parameters. For example, formation fractions and degradation rate constants for metabolites are influenced by the degradation rate constant of the parent. The uncertainty of the parameters for the parent should, thus, be evaluated before proceeding with the analysis for the metabolite.

6.3.2 Optional methods

The following methods are not recommended for a standard kinetic assessment for reasons given below, but they may be used to provide additional information.

6.3.2.1 Coefficient of determination (r^2 value) and model efficiency (EF)

The coefficient of determination is the square of Pearson's correlation coefficient (r). The correlation coefficient determines the extent to which values of two variables are "proportional" to each other. Proportional means linearly related; that is, the correlation is high if it can be approximated by a straight line (sloped upwards or downwards).

The general form of the equation for the coefficient of determination (r^2) is:

$$r^2 = \left\{ \frac{\sum_{i=1}^{n} \left(X_i - \overline{X}\right)\left(Y_i - \overline{Y}\right)}{\sqrt{\sum_{i=1}^{n} \left(X_i - \overline{X}\right)^2 \sum_{i=1}^{n} \left(Y_i - \overline{Y}\right)^2}} \right\}^2 \qquad (6\text{-}4)$$

n = total number of paired observations (X, Y)
X_i = i^{th} value of variable 1 (with i = 1,2,…,n)
Y_i = i^{th} value of variable 2 (with i = 1,2,…,n)
$\overline{X}$ = mean of all values for variable 1
$\overline{Y}$ = mean of all values for variable 2

The r^2 value ranges from 0 to 1 with higher values indicating a stronger relationship. It can be interpreted as the fraction of the change in one variable that can be explained by the change in the other variable.

Until recently, pesticide concentrations were often transformed logarithmically and plotted against time. If degradation follows first-order kinetics, this plot should yield a straight line and the r^2 value can be used as an indication of the goodness of fit.

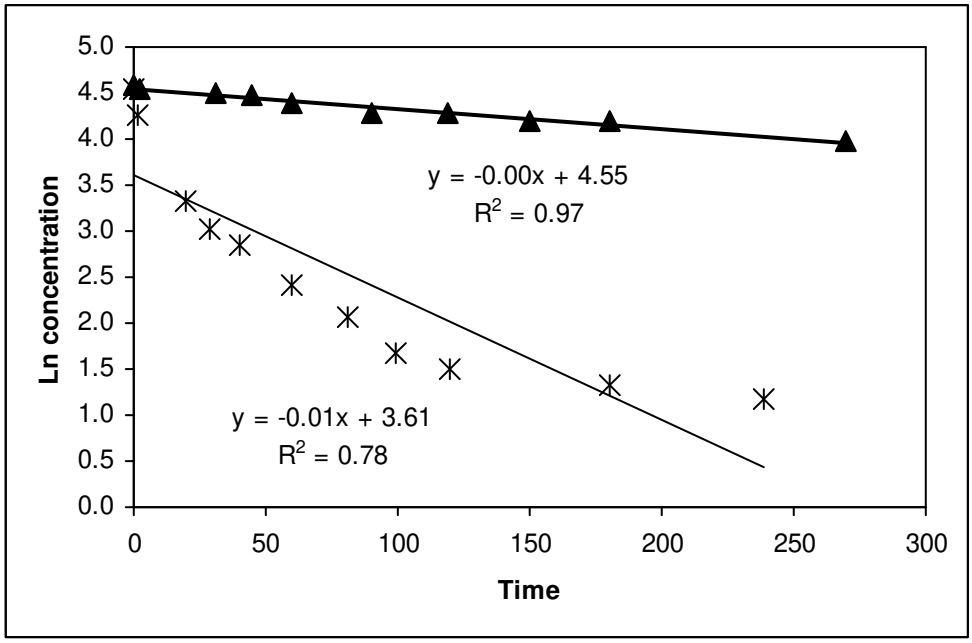

Figure 6-6. Fit of straight line to logarithmically transformed concentrations

The following recommendation was made in the EC Guidance Document on Persistence Soil (9188/VI/97 rev. 8, 12.07.2000):

> The determination coefficient r2 should be in a range between 0.85 and 1.0. In a practice there will be many cases where r2 will be lower than 0.85. In such situations it is advisable to distinguish if a DT50 is needed for modelling purposes or as a trigger value for further (field) studies. Since most models can handle only 1st order kinetics, for pragmatic reasons the determination coefficient r2 ≥ 0.7 can still be accepted. In order to trigger further studies a DT50 value can be calculated according to the best fit. If the use of first order kinetics to calculate degradation rates results in a determination coefficient of r2 < 0.7, then other methods can be tested and used.

However, log-transformation of the measured concentrations is no longer recommended and non-linear curve fitting to untransformed data should always be carried out as a first step. In this case, the coefficient of determination in its strictest sense is no longer valid, because it only applies to linear relationships between two variables.

Some modellers try to overcome this problem by using the coefficient of determination to characterise the relationship between calculated and observed values. Ideally, the plot of calculated versus observed should yield a straight line with an intercept of zero and a slope of 1. The coefficient of determination is then calculated as:

$$r^2 = \left\{ \frac{\sum_{i=1}^{n} \left(O_i - \overline{O}\right)\left(C_i - \overline{C}\right)}{\sqrt{\sum_{i=1}^{n} \left(O_i - \overline{O}\right)^2 \sum_{i=1}^{n} \left(C_i - \overline{C}\right)^2}} \right\}^2 \qquad (6\text{-}5)$$

n = total number of observations
O_i = i^{th} observed value (with i = 1,2,…,n)
C_i = i^{th} value calculated with selected model (with i = 1,2,…,n)
$\overline{O}$ = mean of all observed values
$\overline{C}$ = mean of all calculated values

Expressed in this way, the r^2 value can be interpreted as the fraction of the variance of the observed data that is explained by the model. An r^2 value close to 1 indicates a linear relationship between the two variables, but it does not give an indication of the intercept or slope. For example, a large value of 1 for r^2 would also be obtained if the calculated values exceeded the observed by a factor of 2.

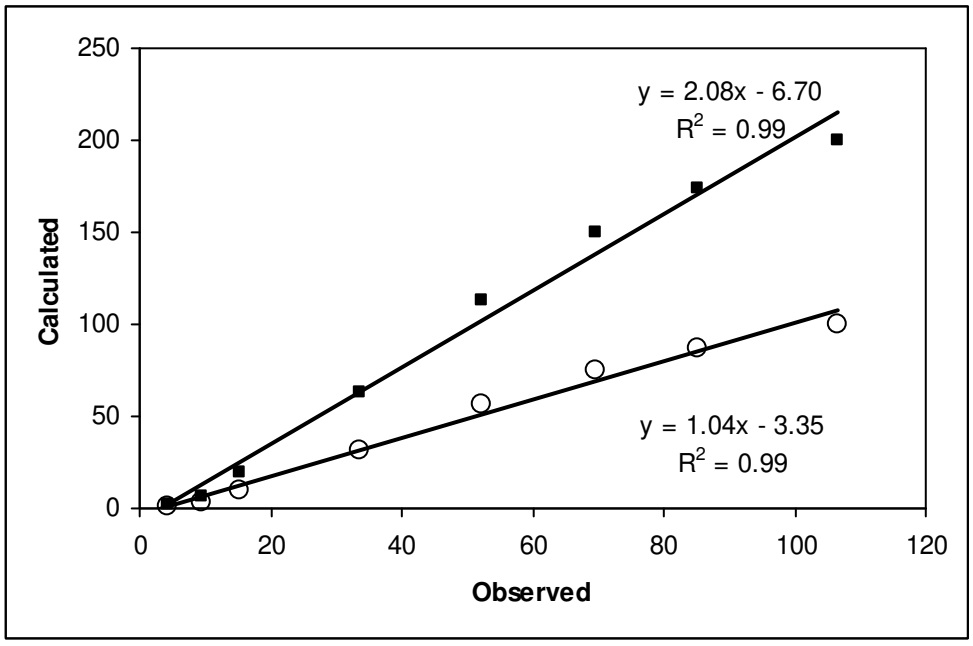

Figure 6-7. Plot of calculated *vs.* observed concentrations for a good fit (slope close to 1) and a hypothetical poor fit (slope = 2)

Therefore, the r^2 value for the plot of calculated *vs.* observed data is not a valid indication of a good fit if considered on its own. It must be combined with a statistical method that tests if the intercept is significantly different from zero and if the slope is significantly different from 1. Such a method was evaluated by the FOCUS group, but the method was not able to discriminate with sufficient power between a visually good and poor fit in the examples tested and is, therefore, not recommended as a standard method.

As mentioned above, the r^2 value is limited to linear relationships. An alternative statistical criterion, which is also applicable to non-linear models, is model efficiency (EF):

$$EF = 1 - \frac{\sum_{i=1}^{n}(C_i - O_i)^2}{\sum_{i=1}^{n}(O_i - \overline{O})^2} \text{ which can also be expressed as } EF = 1 - \frac{RSS}{TSS} \qquad (6\text{-}6)$$

n = total number of observations
O_i = i^{th} observed value (with i = 1,2,…,n)
C_i = i^{th} value calculated with selected model (with i = 1,2,…,n)
$\overline{O}$ = mean of all observed values
RSS = Residual sum of squares
TSS = Total sum of Squares

EF ranges from minus infinity to +1 with larger values indicating better agreement. EF compares the sum of squared differences between calculated and observed data (RSS) with the variability in the observed data. For EF < 0, the mean of the observed data is a better predictor of the observed values than the model. For EF > 0, the value gives an indication of the fraction of the total variance of the data set that can be explained by the model. This interpretation is similar to that for the r^2 value (see above), which leads to some confusion in the terminology. The term r^2 value is often used for non-linear models where it refers to the fraction of the variance explained calculated from 1- RSS/TSS. r^2 values given by software packages for non-linear curve fitting are usually calculated in this way.

A disadvantage of model efficiency is its dependency on the slope of the curve. The overall variance (and thus the denominator in the above equation) is small where concentrations decline relatively slowly. EF will thus always be relatively small for relatively flat decline patterns, irrespective of the scatter of measured data around the calculated curve. The data shown in Figure 6-8 are scattered around the curve for the first sampling points, but overall the fit is acceptable. The model efficiency for this fit was calculated to be 0.61.

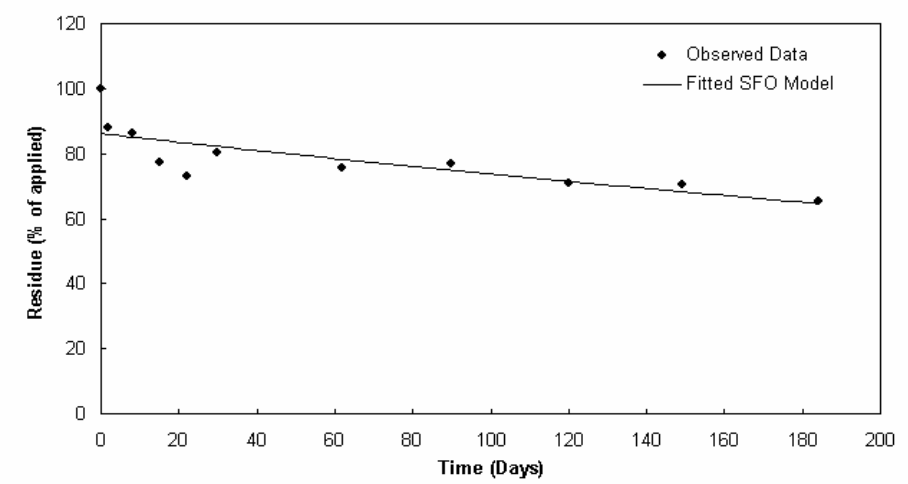

Figure 6-8. Fit of first-order kinetics to data for a slowly degrading compound resulting in EF= 0.61

On the other hand, large EF values do not guarantee a good agreement of calculated and measured data. The fit shown in Figure 6-9 resulted in an EF value of 0.94 although the pattern of degradation clearly deviates from first-order kinetics at the later sampling times.

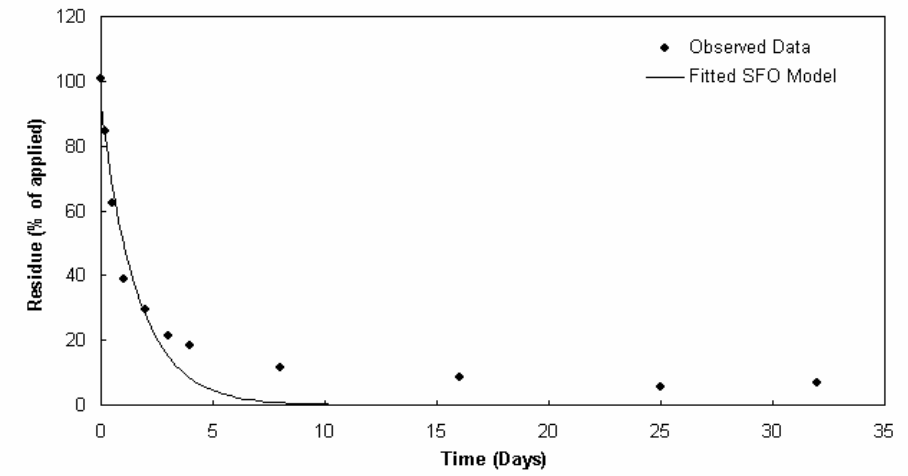

Figure 6-9. Fit of first-order kinetics to data for a rapidly degrading compound resulting in EF =0.94

Due to these shortcomings of model efficiency, the FOCUS work group omitted this criterion from the core list of recommended statistical parameters.

6.3.2.2 Scaled Root Mean Squared Error

The Scaled Root Mean Squared Error (SRMSE) gives an indication of the deviation from the ideal case where $P_i = O_i$. The error is scaled in relation to the mean of all observed values. Walker *et al.* (1995) used it to assess the goodness of fit of calculated soil residue profiles, but it can also be applied in other areas, such as in engineering applications.

$$SRMSE = \frac{1}{\overline{O}} \sqrt{\frac{\sum_{i=1}^{n} (C_i - O_i)^2}{n}} \qquad (6\text{-}7)$$

where

C = calculated values

O = observed values

$\overline{O}$ = mean of all observed values

n = number of values

The SRMSE is always larger than zero. The smaller the value, the better the fit. However, there are some issues associated with use of SRMSE to define “best fit”. Fundamentally, the underlying distribution of SRMSE is not well documented (difficult to mathematically develop). Therefore, deriving a meaningful value that identifies an acceptable fit is difficult.

Further, the measure does not distinguish between 1) the variability of the data at a given time point about the mean for that time point and 2) the variability of those means about the fitted curve (calculated means). A kinetic model could perfectly fit the mean of the observed data at each time point, but high variability in the data could still lead to large values of SRMSE. A “significant” test may simply indicate variable data, not a problem with the curve fitting.

Data sets fit equally well by a given kinetic model can have different SRMSE values simply based on the shape of the curve. This characteristic puts more emphasis on the peak measured values that are generally determined with larger confidence. This is illustrated for two examples in Figure 6-10 and 6-11. The data set with the larger absolute data gives a smaller (better) SRMSE for the metabolite, although both data sets are fitted equally well by the model. These examples demonstrate that the SRMSE is sensitive to the shape and vertical location of the data.

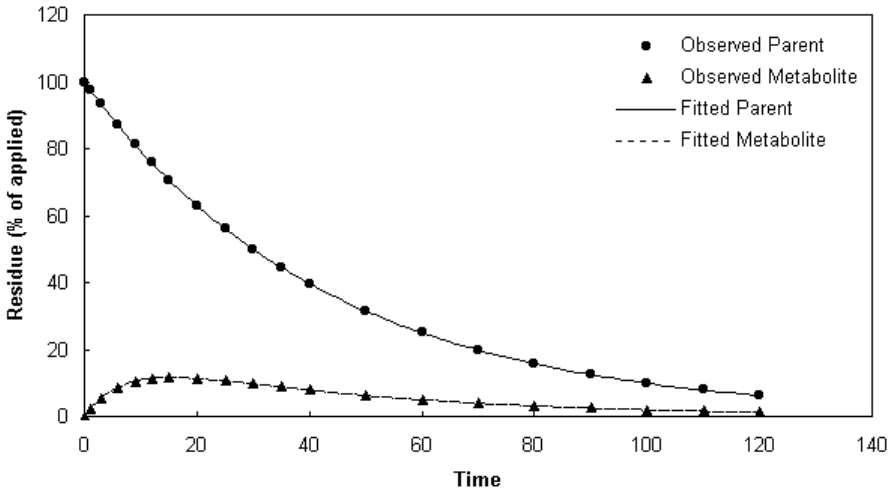

Figure 6-10. Fit of sequential first-order kinetics to parent degrading to one metabolite (peak of 11.6 % AR) resulting in a value of 0.0049 for the SRMSE of the metabolite

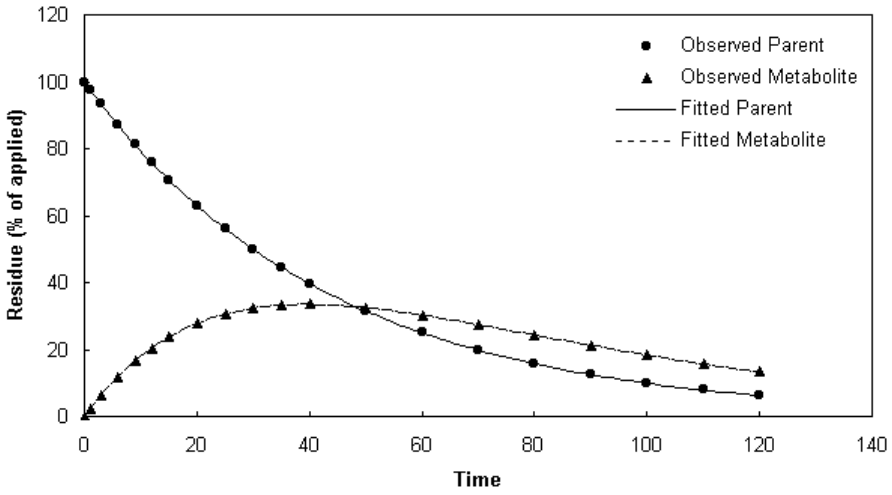

Figure 6-11. Fit of sequential first-order kinetics to parent degrading to one metabolite (peak of 33.5 % AR) resulting in a value of 0.0011 for the SRMSE of the metabolite

Note that the χ^2-statistics, which were recommended as a standard method for assessing the goodness of fit (Section 6.3.1.2), is also sensitive to the shape and vertical location of the data. Data sets fit equally well by a given kinetic model can have different calculated χ^2-values for an identical error percent value, based on the shape of the curve. As a consequence, similar metabolite fits pass the χ^2-test at $\alpha = 0.05$ at different error percent values, namely at 0.41 percent (Figure 6-10) and 0.11 percent (Figure 6-11), respectively.

6.3.2.3 Scaled Total Error

The scaled total error is the average absolute error standardised to the mean of all observed values. The STE is similar to the SRMSE and the same issues apply, namely a poorly defined underlying distribution and sensitivity to the shape and vertical location of the data.

$$STE = \frac{\sum|C-O|}{\sum O} = \frac{1}{\overline{O}}\frac{\sum|C-O|}{n} \tag{6-8}$$

where

C = calculated values

O = observed values

$\overline{O}$ = mean of all observed values

n = number of values

6.3.2.4 F-test and Generalised Likelihood Ratio test for model comparison

F-test

A classical method to compare the goodness of fit of two models is the F-test, for example, a simpler model (e.g. SFO) with a more complex model (e.g. DFOP). If both models are also linear with respect to the parameters, then the statistic

$$F = \frac{(n - m_{complex} - 1)\ (RSS(\hat{f}_{basic}) - RSS(\hat{f}_{complex}))}{(m_{complex} - m_{basic})\, RSS(\hat{f}_{complex})} \tag{6-9}$$

where

$RSS(f_{basic})$ = Sum of squared residuals for the basic model 1 (the simpler model): $\sum_{i=1}^{n}(C_i - O_i)^2$

$RSS(f_{complex})$ = Sum of squared residuals for model 2 (the more complex model)

n = Total number of observations

m_{basic} = Number of parameters for model 1

$m_{complex}$ = Number of parameters for model 2

is exactly F-distributed. The test starts from the hypothesis that the simpler model (model 1) is better than the more complex model (model 2). The probability (p-value) corresponding to the calculated F-value is read from statistical tables or calculated with Excel (FDIST function) or statistical packages for $df_1 = m_{complex} - m_{basic}$ and $df_2 = n - m_{complex} - 1$ degrees of freedom.

If the probability is smaller than the selected significance level (e.g. 0.05), model 2 is statistically better than model 1. An example is provided below.

Baird (1974) claims that the F-test also applies for nonlinear models, if the Taylor expansion is essentially linear in the vicinity of the estimate.

Generalised Likelihood Ratio Test

To compare a simpler model (e.g. SFO) with a more complex model (e.g. DFOP), the likelihood ratio statistic

$$\lambda = \left(\frac{RSS(\hat{f}_{complex})}{RSS(\hat{f}_{basic})} \right)^{\frac{n}{2}} \qquad (6\text{-}10)$$

$RSS(f_{basic})$ = Sum of squared residuals for the basic model 1 (the simpler model):

$$\sum_{i=1}^{n} (C_i - O_i)^2$$

$RSS(f_{complex})$ = Sum of squared residuals for model 2 (the more complex model)

n = Total number of observations

can be used to test the hypothesis that the most basic model is correct (cf. Borowiak 1999).

Under the assumption that nonlinear regularity conditions hold (errors normally distributed and independent, estimators unbiased, unique minimum of the objective function cf. Jennrich 1969)

$\Lambda = -2\ln(\lambda)$ is χ^2 distributed with $m_{complex} - m_{basic}$ degrees of freedom. (6-11)

m_{basic} = Number of parameters for model 1
$m_{complex}$ = Number of parameters for model 2

Example

n=9 data points

Model 1: SFO with two parameters, $m_{basic} = 2$
Model 2: bi- exponential model (DFOP) with four parameters, $m_{complex} = 4$

With RSS (SFO) = 22.1 and RSS (DFOP) = 10.04, the value of the test statistic is Λ=7.1. Since $\chi^2_{2,0.95}$ = 5.99 the hypothesis that the most basic model (here SFO) is the correct one is rejected at the 5% level of significance.

The F-statistic is given by F = 2.389 and the quantile $F_{2;4;0.95}$=5.79. Thus, the null hypothesis that the most basic model (here SFO) is the correct one cannot be rejected using the F-Test. Note that in this example, the F-Test and the generalised likelihood ratio test yield different results.

Limitations

Note that the conditions for applying the Likelihood test or the F-test are not always strictly met due to the small number of data points typical for degradation studies. Therefore, these two tests should not be used as a standard method. A comparison of the error levels at which the χ^2 test is passed should be made instead (see Section 6.3.1.2).

6.4 References

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7 RECOMMENDED PROCEDURES TO DERIVE ENDPOINTS FOR PARENT COMPOUNDS

This section gives recommendations on how to derive degradation endpoints for parent compounds. Guidance for metabolites and for parent compounds and metabolites in water-sediment studies is given elsewhere (Chapters 8 and 10).

Kinetic evaluation of a degradation study aims at identifying a model that is able to adequately describe the data. Details on visual and statistical methods used for this purpose are given in Chapter 6. Briefly, this involves:

- Visual assessment of fitted and observed data versus time
- Visual assessment of the residuals up to the DT90 to establish whether systematic deviations exist
- The estimation of the error percentage at which the χ^2-test is passed
- A t-test (or examination of the confidence intervals) to evaluate the confidence in all of the parameter estimates

The χ^2-test considers the deviations between measured and predicted values for each individual model relative to the uncertainty of the measurements. The measurement uncertainty is expressed as a % error that is used to scale the observed mean. The true error is unknown. The use of the test in this report is to determine the smallest error value at which the test is passed at the 5% significance level. This error value is calculated for each model and the model with the smallest error is considered the most appropriate. The χ^2 test is particularly sensitive to replicates, so the mean of the observed replicate values should be used for this statistical analysis. This prevents the test focusing on the degree of variability of replicates rather than the goodness of fit. Details are given in Chapter 6.

The best-fit model does not necessarily provide a good fit to the data, it is simply better than the other models tested. Thus how accurately the data are matched by the best-fit model must be evaluated. In addition to visual assessment (see above), the error at which the χ^2 test is passed at the 5% significance level can be considered (see the following sections). Also, the estimated parameters should significantly differ from zero.

7.1 Analysis of data sets without a lag phase

Chapters 7.1.1 and 7.1.2 give detailed guidance on how to derive endpoints for use as triggers for future work and for use as modelling inputs, respectively. For both assessments, one should:

- Eliminate any obvious artefacts arising from analytical or procedural errors prior to analysis
- Check the visual fit and calculate the error percentage at which χ^2 test passed for all models
- Check the confidence in parameter estimates
- Investigate if a bi-phasic pattern is due to a decline in microbial activity
- Aim at improving the fit by eliminating outliers, constraining M0 and / or weighting where necessary (as a second step)
- Interpret studies where DT50 and DT90 was not reached within the experimental period with care

The main differences between the two assessments are:

Triggers for additional work	**Modelling endpoints**
Run SFO and FOMC as a first step	Run SFO as a first step
Check visual fit and calculate error percentage at which χ^2 test passed If FOMC better than SFO, test other bi-phasic models	Check visual fit and calculate error percentage at which χ^2 test passed If error % **<** 15% and visual fit acceptable, use SFO DT50 If error % **>** 15% and visual fit not acceptable, run bi-phasic model
Use best-fit model	If 10% of initial reached in study period: Calculate DT50 as DT90 FOMC / 3.32 If 10% of initial **not** reached in study period: Use longer DT50 of HS or DFOP

Details are given in Sections 7.1.1 and 7.1.2.

7.1.1 Degradation parameters as triggers for additional work

The assessment always starts with the hypothesis that SFO is the most appropriate kinetics. DT50 and DT90 values that are intended for use as triggers should be derived by best-fit kinetics if this hypothesis is rejected. However, whether deviations from first-order kinetics are due to experimental artefacts or a decline in microbial activity during the laboratory study

must be established. DT90 values estimated with bi-phasic kinetic models are often unrealistically long for data sets in which the DT90 was not reached within the experimental period. The results of such assessments should be seen in the light of results from other studies.

The recommended procedure to derive endpoints for parent compounds is presented schematically in Figure 7-1.

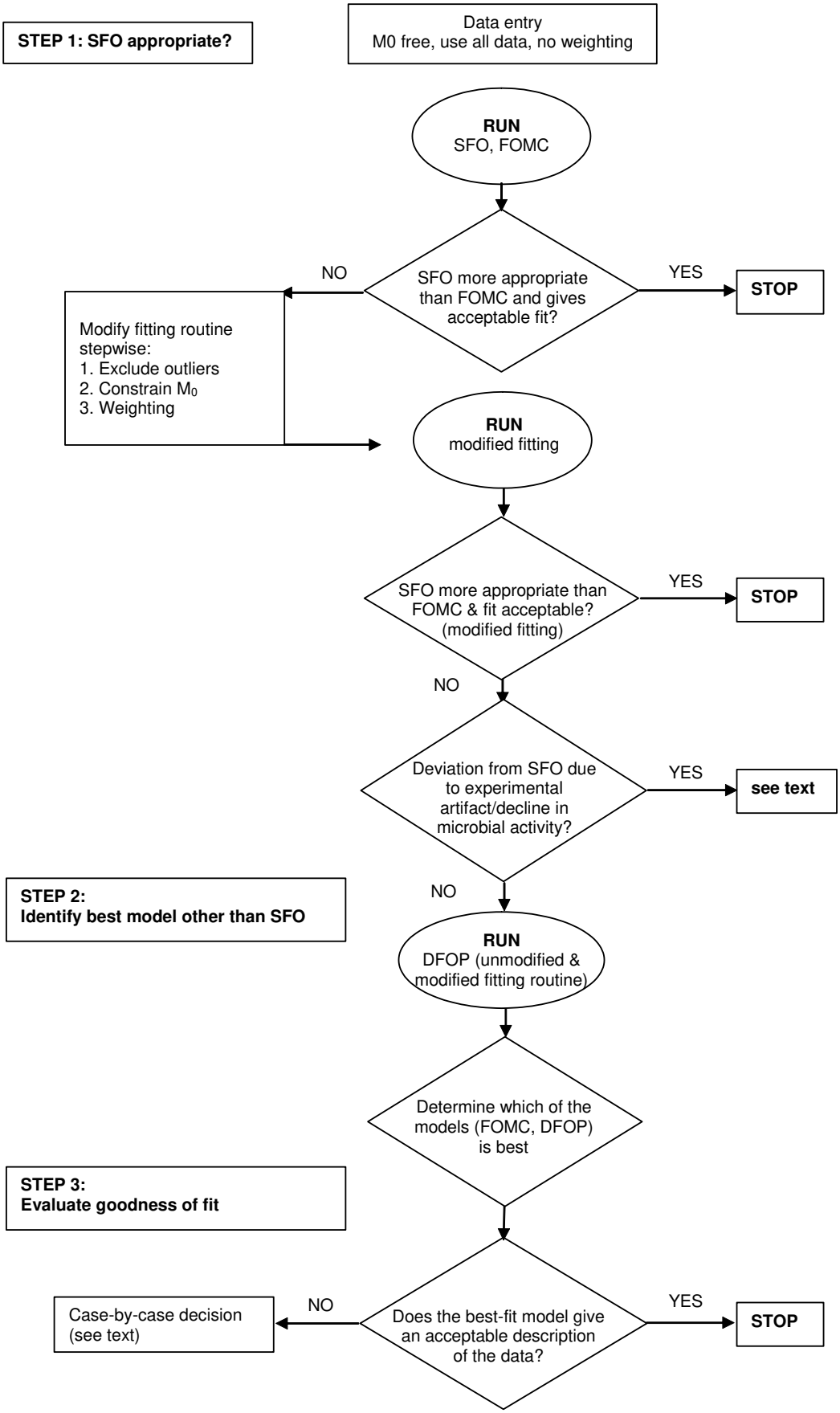


Figure 7-1. Recommended procedure to derive endpoints for use as triggers for additional work from degradation kinetics without a lag phase

The initial hypothesis is that the data are best described by SFO. In order to test this hypothesis, first-order kinetics should at first be fitted to all measured data. True replicate measurements should not be averaged prior to curve fitting (for a definition of true replicates see Section 6.1.2). Weighting or transformation of the raw data is not recommended at this stage. The initial concentration should be optimised during curve fitting, not fixed. Outliers should not be eliminated at this stage, since making an objective assessment of which samples are clear outliers is difficult because performing statistical tests is not usually possible due to the relatively small number of data points available. The decision on which samples to eliminate will thus be made by expert judgement. To restrict user subjectivity, all data points should be included initially and the fitting repeated after exclusion of outliers. Only measurements which are clearly influenced by known analytical or procedural errors can be eliminated prior to analysis. Any other outliers and artefacts due to a decline in the microbial activity during the study will be accounted for at a later stage (see below).

The SFO model is compared with a bi-phasic model. This aims to establish whether a degradation pattern is bi-phasic or not and any of the three models (Gustafson and Holden, hockey-stick model, bi-exponential) could have been chosen for this purpose. The Gustafson and Holden model (also known as FOMC model) was selected by the FOCUS work group because it has the least number of parameters of these three bi-phasic models.

A comparison between SFO and Gustafson and Holden kinetics will be made on the basis of visual assessment and a χ^2-test. If the SFO model fits the data better than the FOMC model and gives an acceptable fit, no further action is necessary and the results can be reported. If this is not the case, a modified fitting procedure can be adopted:

a) Eliminate any outliers from the data set.
b) Fix the initial concentration to the value measured on the day of treatment (after any corrections such as for the presence of metabolites) and fit the models to data remaining after the elimination of outliers.
c) Assign different weights or transform data remaining after the elimination of outliers.

If the FOMC model still fits the data better than the SFO model, additional bi-phasic models should be tested provided the deviation from first-order kinetics cannot be attributed to experimental artefacts such as a decline in microbial activity during the laboratory study. If a decline in microbial activity occurred, later data points should be discarded and the fitting procedure repeated.

Only the bi-exponential model (DFOP) is recommended to be tested in addition to the FOMC model. Alternative models should only be used in exceptional cases. The DFOP model will initially be fitted using unmodified data without constraints in the initial concentration. The bi-phasic model that gives the best fit to the data will be identified on the basis of visual assessment and a χ^2-test.

Ideally, the error value at which the χ^2-test is passed by the best-fit model (SFO, FOMC or DFOP) should be below 15% and the fit must be visually acceptable. However, this value should not be considered as an absolute cut-off criterion. There will be cases where the error value to pass the χ^2-test is higher, but the fit still represents a reasonable description of the degradation behaviour (see Appendix 3). Endpoints used as triggers that are extrapolated far beyond the duration of the experiment should be interpreted with care. For DT90 values this will be more often the case compared to DT50 values.

In field studies, the individual data points are often scattered around the curve, which results in a large error value. Visual assessment can be used in this case to establish whether the overall decline in pesticide concentrations is represented adequately by the model. When the derived endpoints are in line with the results from the remaining studies with the same compound, they may be considered acceptable.

The DT50 or DT90 values should not be used as trigger values if the measured data systematically deviate from the fitted curve (the shape of the curve cannot be described by the type of kinetics selected). This situation will rarely arise as degradation often follows first-order kinetics or a classical bi-phasic pattern that can be described well by the FOMC or DFOP model. In exceptional cases, alternative kinetics can be used. Model selection and the fitting procedure used must be justified and clearly documented.

In all cases, the reliability of the parameter estimate should be assessed using a t-test or by investigating if zero is included in the confidence interval. If a parameter does not differ significantly from zero, the endpoints derived from the parameter are uncertain and should be interpreted with caution.

7.1.2 Degradation parameters as input for pesticide fate models

Ideally, degradation should be described by the model that provides the best fit to the data. However, current versions of soil models used to assess movement of parent and metabolites into ground and surface water use first order kinetics. For the time being, a

pragmatic approach is recommended. Criteria for the acceptance of the resulting endpoints as input for simulation models are proposed. If these criteria are not met, half-lives for modelling can be calculated using correction procedures as described in Section 7.1.2.1. These half-lives should be used in PEC calculations for ground water, surface water, and sediment as a first step. Higher-tier approaches can be applied thereafter. A number of possible higher-tier approaches exist. Two examples are outlined in Section 7.1.2.2.

Note that the kinetic models generated using the procedures in this section are only for use in models. DT50/90 values used for comparison with trigger values must be generated with the procedures described in Section 7.1.1

7.1.2.1 Tier 1 calculations

The recommended first-tier procedure to derive endpoints for parent compounds is presented schematically in Figure 7-2. The approach is illustrated for several example data sets in Appendix 3.

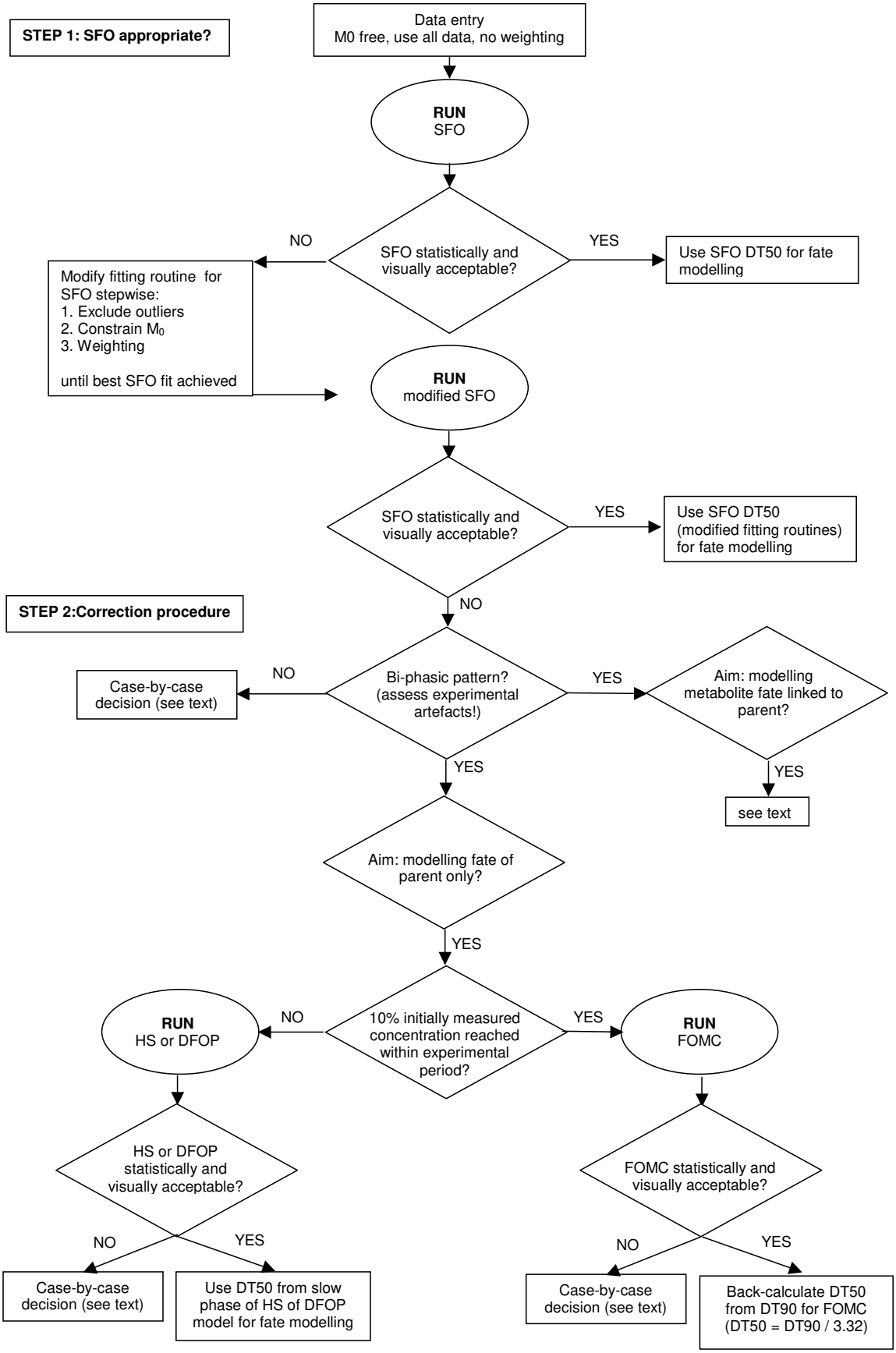


Figure 7-2. Recommended tier 1 procedure to derive degradation parameters for modelling the fate of a parent compound from degradation kinetics without a lag phase

First-order kinetics will be fitted to all unmodified data without constraints in the initial concentration. Only measurements which are clearly influenced by known analytical or procedural errors can be eliminated prior to analysis. Any other outliers and artefacts due to a decline in the microbial activity during the study will be accounted for at a later stage (see below). Field data should be normalised to a typical standard reference soil temperature and moisture content (e.g. 20°C and pF2) as described in Chapter 9. The goodness of fit is assessed using visual evaluation and a χ^2-test. Visual evaluation should be made for a plot of observed and calculated concentrations vs. time and a plot of the residuals up to the measured DT90. No further action is required and the half-life can be used for modelling if the fit is visually acceptable and passes the χ^2-test at an error level of 15% or less.

If these criteria are not met, attempts will be made to improve the first-order fit by eliminating outliers, fixing the initial concentration and/or data weighting. Depending on the outcome of this assessment, further action may be necessary.

a) Large inherent variability

The error term required to pass the χ^2-test may be larger if there is a large scatter in the data (e.g. field studies). In this case, a decision should be based on visual assessment. If the overall pattern of decline in pesticide concentrations is represented adequately by the model and the distribution of the residuals is random (no systematic deviations), the half-life from the SFO model may be used for modelling.

b) Bi-phasic degradation

If the pattern of degradation is clearly bi-phasic, systematic deviations of the residuals will occur. Additional information on the bi-phasic nature of the data is available from the comparison of the SFO and FOMC model carried out in order to derive DT50/DT90 values to be used as triggers for further work (see Section 7.1.1). If the deviations from SFO kinetics are due to a decline in microbial activity during the study, later sampling dates can be discarded and the procedure repeated with the modified data set. Otherwise, a stepwise procedure is recommended.

An error is introduced in pesticide fate modelling if a half-life is used as model input where degradation is truly bi-phasic. However, a pragmatic approach to derive worst case half-lives is necessary because current versions of soil models used to assess movement to ground and surface water use first-order kinetics. **Note that these**

corrected DT50 values can only be used to simulate the leaching of a parent compound. They must not be used to simulate the fate of the parent and a metabolite in a linked model run (i.e. the formation of the metabolite is directly calculated from the degradation of the parent). Information on how to proceed in this situation can be found in Chapter 8.

For experiments where concentrations decrease to 10% of the initial value within the study period, half-lives for pesticide fate modelling can be calculated from the DT90 value for the bi-phasic Gustafson and Holden model (FOMC). The DT90 value is divided by 3.32 for conversion to a half-life (for first-order kinetics, the half-life is 3.32 times shorter than the DT90 value). This recalculated half-life is longer than the original half-life and its use as an input for pesticide fate modelling will result in an over-estimation of pesticide residues in soil. The FOMC model was selected as the single standard option in this situation. Hockey-stick and bi-exponential kinetics are not tested at this stage, because these models have a larger number of parameters than the FOMC model. All three models are likely to result in similar DT90 values where measured concentrations decrease to 10% of the initial value within the study period. However, alternative models can be evaluated if the fit by FOMC kinetics is inadequate.

DT90 values from bi-phasic models are very uncertain and depend strongly on the model used if an extrapolation beyond the study period is necessary. The procedure described above is thus not considered appropriate for experiments where the last measured concentration is larger than 10% of the initial value. In this case the slower of the two degradation rates of the hockey-stick model (or the bi-exponential model) should be used in pesticide fate models. This results in an over-estimation of soil residues over the whole simulation period and introduces a safety factor into the modelling.

Half-life values derived from fitting the FOMC or the hockey-stick model should only be used for modelling if the fit to measured data is acceptable, based on visual assessment and statistical criteria. Ideally, the error level at which the χ^2-test is passed should not exceed 15%. Modified fitting routines can be used to improve the fits (eliminate outliers, constrain M0, data weighting). If the deviations are due to a large scatter of the individual data points around the curve, the DT50 values may nonetheless be acceptable, provided the overall pattern of decline is described well by the model. Thus, the 15% error value should not be considered as an absolute cut-off criterion. There will be cases where the error value to pass the χ^2-test is higher, but the fit still represents a reasonable description of the degradation behaviour (see Appendix 3).

c) Degradation neither first-order nor bi-phasic

If degradation deviates systematically from the pattern that can be described by either first-order or bi-phasic kinetics, the DT50 should be set to a conservative value in the model. This situation will rarely arise as degradation often follows first-order kinetics or a classical bi-phasic pattern that can be described well by FOMC and hockey-stick kinetics.

In all cases, the reliability of the parameter estimate should be assessed using a t-test or by investigating if zero is included in the confidence interval. If a parameter does not differ significantly from zero, endpoints derived from this parameter are uncertain. The reasons for the uncertainty in the parameter should be examined and the acceptability of the fit should be decided on a case-by-case basis.

7.1.2.2 Higher-tier approaches

As a first tier, half-lives for modelling should always be derived as outlined above. Thereafter, higher-tier approaches can be used. Two possible procedures are outlined in this report which aim at explicitly considering bi-phasic degradation in PEC calculations. The implementation of the Gustafson and Holden model, bi-exponential model and hockey-stick model into soil models simulating movement of parent compounds and their metabolites to ground and surface water (PEARL, PELMO, PRZM, MACRO, TOXSWA) is not universally valid. There are, however, approaches that provide a pragmatic solution.

The first approach discussed in this report (Section 7.1.2.2.1) is based on the assumption that the observed bi-phasic degradation pattern is caused by kinetic sorption (i.e. a decrease in the easily degradable fraction of a pesticide with time). Parameters for mathematical descriptions of long-term sorption and coinciding degradation are calculated from parameters derived by fitting empirical bi-phasic kinetics to degradation data. The calculated parameters are then used for higher-tier simulations with leaching models. The second approach (Sections 7.1.2.2.2 and 7.1.2.2.3) consists of two pragmatic techniques to implement bi-exponential degradation kinetics into pesticide leaching models.

Both methods outlined in this report should only be considered as examples. Alternative higher-tier modelling approaches may be used, provided the methodology is clearly documented.

7.1.2.2.1 Estimating parameters for two-site sorption / degradation models from bi-exponential degradation kinetics

Methods to estimate sorption parameters are outside the remit of this work group. There is, however, an interesting relationship between bi-phasic degradation patterns and long-term sorption kinetics that provides an opportunity to consider bi-phasic degradation in pesticide leaching models. This option was explored by the FOCUS group as one possibility to overcome the limitations of current versions of pesticide fate models, most of which only simulate degradation according to first-order degradation kinetics.

A relative increase in pesticide sorption with increasing residence time in soil is a well-known phenomenon (see for example the review by Wauchope *et al.*, 2002). This can be accounted for in pesticide fate modelling as a higher-tier option. For example, the leaching model PEARL considers an approach with two types of solid sites: a fast (equilibrium) site and a slow reacting, more strongly binding site. Degradation is limited to the compound in the liquid phase and in the equilibrium sorption site. Degradation is assumed not to occur in the more strongly sorbed site. This and similar two-site models result in a bi-phasic pattern of degradation of total residues in soil due to an increase in the non-degradable fraction with time. The observable bi-phasic pattern of degradation of bulk pesticide residues can be interpreted as the macroscopically visible system behaviour that results from the underlying "microscopic" processes. Fitting this macroscopic pattern with the bi-exponential model described in Section 5.2.3 yields four parameters. Interestingly, there is a direct mathematical relationship between 3 of these parameters (k1, k2 and g) and the "microscopic" parameters of the underlying mechanistic two-site sorption / degradation model. This relationship can be complex depending on the assumptions within the long-term sorption / degradation model (e.g. Freundlich or linear sorption), but relatively simple analytical equations exist for some special cases. There is, thus, a potential to use the parameters derived from fitting the bi-exponential model to degradation data in higher-tier modelling of pesticide leaching. It allows bi-phasic degradation and long-term sorption to be considered in higher-tier modelling without the need for complex experimental long-term sorption studies provided that the resulting parameters for long-term sorption kinetics are within the typical range of values (however, for regulatory applications, currently experimental sorption data are required). The approach will involve the following steps:

1. Fit bi-exponential kinetics to degradation data;
2. Calculate the parameters for the mechanistic long-term sorption / degradation model from the parameters of the bi-exponential model (some parameters must be derived from standard batch sorption data);
3. Check the validity of the parameters;

4. Run the leaching model with the calculated parameters to provide a higher-tier leaching assessment.

The approach is illustrated in Appendix 4 for higher-tier simulations with the leaching model PEARL. It can also be used for higher-tier simulations with other models. For example, the recently released MACRO 5.0 includes two-site sorption in the micropore region combined with equilibrium sorption in the macropores. The approach outlined in this chapter can be used for higher-tier modelling with MACRO 5.0, provided a revised relationship between the parameters of the bi-exponential model and the parameters of the long-term sorption / degradation model implemented in MACRO is established.

7.1.2.2.2 Implementation of bi-exponential kinetics (DFOP) into pesticide leaching models

As described in Box 5-2, the DFOP bi-exponential model consists of the sum of two exponential equations. The pesticide is assumed to be placed instantaneously into two independent pools or compartments, a fast and a slow degrading compartment. There is no exchange between the compartments and the total concentration of the pesticide is equal to the sum of the concentrations in each individual pool. The model has four parameters: the initial concentrations and degradation rates in each of the two compartments. The number of parameters can be reduced to three if the total initial mass is fixed. It is then only necessary to determine which percentage or fraction of the total amount is placed in the first compartment (the fraction in the second compartment is 1 – the fraction in the first compartment). The procedure for implementing the bi-exponential approach into a leaching model is to conduct two separate simulations. As an example, if the degradation rates corresponded to half-lives of 10 days and 100 days and 30 percent of the material went through the 10 day half-life, one simulation would consist of applications made at 30 percent of the total application rate with the compound degrading with a half-life of 10 days and the other simulation would consist of applications made at 70 percent of the total application rate with the compound degrading with a half-life of 100 days. The concentrations would then be summed to get the total concentration.

Breaking the pesticide into two fractions introduces a small error when the Freundlich exponent is not one. However, a conservative estimate can be made when the Freundlich exponent is not one by doubling the application rate and then dividing the final answer by two.

This approach is illustrated in Appendix 5 using the leaching model PRZM as an example. It can be implemented into any other leaching model in a similar way.

7.1.2.2.3 Implementation of bi-exponential kinetics (FOTC) into pesticide leaching models

A similar way to implementing bi-phasic kinetics also involves fitting the kinetic data to a two compartment model. However, in the FOTC (first-order, two compartment) approach, all of the compound is applied to the rapidly degrading compartment. There are two removal processes from the rapidly degrading compartment. One is the degradation of the compound to a metabolite or sink at a rate k_1. The other is the transformation to the slowly degrading compartment at a rate of k_2. In the slowly degrading compartment, the compound degrades to a metabolite or the sink at a degradation rate k_3.

In both the DFOP and FOTC approaches the first step is to fit the experimental data by the chosen model. This is relatively easy with commercially available software packages such as ModelMaker. The equation for the DFOP approach is given in Box 5-4. Details of the FOTC model are given in Chapter 5.4.

The fitted parameters are then used in the higher tier simulations with pesticide fate models. Unlike implementing bi-phasic degradation with the DFOP approach, the FOTC approach requires only a single model run, as illustrated in Appendix 5 with PRZM (this can be implemented in other leaching models in a similar manner). However, as with the DFOP approach, breaking the pesticide into two fractions introduces a small error when the Freundlich exponent is not one. However, a conservative estimate can be made when the Freundlich exponent is not one by doubling the application rate and then dividing the final answer by two.

As shown in Appendix 5, the DFOP and FOTC approaches give essentially equivalent answers in leaching models. However, implementing FOTC into the analytical solutions for the generation of soil PEC values described in Section 11.4 is difficult.

The DFOP and FOTC bi-phasic approaches should only be considered a pragmatic solution for representing bi-phasic kinetics, especially for evaluating the kinetics of metabolites formed from a parent or predecessor metabolite with bi-phasic kinetics for use in leaching models. The bi-exponential DFOP and FOTC equations are not mechanistically sound concepts and are entirely empirical in nature. In the DFOP equations there is no basis for the molecule to stay in its initial compartment as it undergoes sorption and desorption as it moves through the soil profile. Similarly in the FOTC approach there is no theory to distinguish why a molecule in the second compartment could not reverse to the first compartment. However, the bi-exponential models can empirically describe the slowing of degradation rates with time observed with some compounds. Having a good description of

the degradation or predecessor metabolite is extremely important for obtaining an accurate description of the kinetics of a metabolite so the use of a pragmatic approach can be justified.

Because of the empirical nature of these bi-exponential equations, care should be taken in extrapolating the results beyond the range of measurements. The procedures for normalisation and averaging of different kinetic results are also theoretically weak.

The two bi-exponential approaches are not higher tier approaches for assessment of leaching of parent, because increasing sorption with time is not considered. When potential leaching of a parent compound is indicated by the DT90/3.32 first tier approach, the assessment should consider increasing sorption and bi-phasic kinetics and Appendix 4 presents an example of such an approach. The pragmatic bi-exponential approaches proposed here, because of the conservative assumptions made regarding the sorption process, should be considered as worst-case and usually lead to even greater predicted leaching concentrations than the already conservative DT90/3.32 approach. However, these pragmatic approaches are necessary when metabolites are involved, so that the bi-phasic formation of the metabolite is properly described.

7.2 Analysis of data sets with a lag-phase

Two models to describe degradation patterns with a lag phase were selected (Section 5.3). The first model is a modified hockey-stick approach where the degradation rate before the breakpoint is set to zero. DT50 and DT90 values can be calculated by either including or excluding the length of the lag phase. The second option is recommended if the lag-phase can be attributed to inappropriate storage conditions or other experimental artefacts. The number of remaining data points must, however, be sufficient to allow robust parameter estimation. If a good fit is obtained for the period after the lag phase, the DT50 value for this period can be used as an input for pesticide fate models.

The second model (logistic model) is continuous with time. This model should only be used if a true lag-phase exists. DT50 and DT90 values are calculated from time zero onwards.

Both models should initially be fitted to all measured data points without weighting and without fixing the initial concentration. Modified fitting procedures and alternative kinetics can be used if no acceptable fit is achieved using standard procedures.

The DT90 value for data sets that show a true lag-phase could potentially be back-calculated to a DT50 value (DT90/3.32) which is then used for pesticide fate modelling. However,

standard modelling scenarios often assume repeated applications of the compound and it is often not known if the delayed onset of degradation will occur after each application or only after the first treatment. The use of the back-calculated DT50 value for all applications within the simulation period will, thus, give a worst-case situation.

7.3 References

Wauchope, R.D., Yeh, S., Linders, J.B.H.J., Kloskowski, R., Tanaka, K., Rubin, B., Katayama, A., Kördel, W., Gerstl, Z., Lane, M., Unsworth J.B., 2002. Pesticide sorption parameters: theory, measurement, uses, limitations and reliability. Pesticide Management Science 58, 419-445.

8 METABOLITES

8.1 Regulatory background

Overall, the same regulatory background already summarised in Chapter 4, for the parent compound, also applies to metabolites. Council Directive 91/414/EEC (Art. 2) establishes that by definition residues of plant protection products on plant or animal products and on the environment include metabolites, degradation and reaction products (the term metabolites will be used further on to refer to the three types of derivatives). In different parts of the directive and their amendments the concepts of major and relevant metabolites are used to indicate which metabolites would require further assessment or consideration. The process for determining if a metabolite is of toxicological, ecotoxicological, or environmental significance is outside the remit of this document, which is only concerned with technical guidance on how to measure kinetics endpoints. Therefore, all discussion on metabolites throughout this document makes no assumptions about whether they are relevant or not. Guidance on the relevance of metabolites is given in the Document on Relevant Metabolites as well as in the Guidance Documents on Terrestrial and Aquatic Ecotoxicology.

Kinetic endpoints are needed as triggers for subsequent studies for potentially relevant metabolites, and for the modelling of the metabolites in the different environmental compartments to help in determining their relevance.

This chapter provides guidance on how to derive kinetic endpoints for metabolites from a study performed with the parent substance or with a precursor of the metabolite (preceding metabolite) in the metabolism pathway. For metabolites applied as test substance, degradation kinetics should be derived following recommendations for parent (treated as parent substance) and the reader should refer to Chapter 7 for guidance.

8.2 Discussion of metabolite endpoints

As outlined in Chapter 4 of this report, a distinction needs to be made between kinetic endpoints for metabolites used as triggers for higher-tier experiments and kinetic endpoints used for modelling / PEC calculation.

8.2.1 Trigger endpoints

DT50 and DT90 values used as triggers as outlined by Commission Directive 95/36/EC amending 91/414/EEC for higher-tier experiments should always be derived by best-fit

kinetics unless deviations from first-order kinetics can be attributed to experimental artefacts. The trigger DT50 and DT90 values can be calculated from the estimated degradation rate of the metabolite using the equation corresponding to the best-fit kinetic model. When the degradation pathway is too complex or not sufficiently defined for a correct fitting of the metabolite degradation kinetics, or when the fitted degradation parameters are judged to be unreliable, a conservative estimate of the trigger DT50 and DT90 values can be obtained by estimating the disappearance of the metabolite from its observed maximum, by fitting the decline curve.

8.2.2 Modelling endpoints

Endpoints intended for use in environmental models and other PEC calculation methods should describe the complete behaviour of the metabolite. Hence, the kinetic description of the fate of the metabolite in laboratory or field studies needs to include both the formation and the degradation of the metabolite (i.e. the kinetic model should cover both formation and decline phases). This will allow in the exposure assessment to properly model the whole exposure range to a metabolite.

As a result, the required modelling endpoints for an individual metabolite are:

- Degradation kinetics and rate constant(s) of the parent and/or preceding metabolite(s) with formation fraction(s) of the metabolite
- Degradation kinetics and rate constant(s) of the metabolite

The formation fraction of the metabolite can either be estimated directly as a parameter, in combination with the overall degradation rate of the parent or preceding metabolite, or it can be calculated from the ratio of the individual degradation rate to the metabolite to the overall degradation rate of the parent or preceding metabolite (see equation in glossary). The metabolite formation fraction should not be confused with the maximum observed or modelled level of the metabolite. The maximum observed level will normally be lower than the actual formation fraction (except for persistent substances in which case it should be essentially equal) as a result of the simultaneous formation and degradation of the metabolite.

When first-order kinetics are considered, the modelling endpoints for a particular metabolite amount to a simple set of formation rate constants and formation fractions (one rate constant and one formation fraction per precursor of the metabolite) and one degradation rate

constant (for the overall degradation of the metabolite, this may be the result of degradation to several components, in which case the total (lumped) rate constant is used).

The kinetic endpoints for modelling should preferentially be derived from a study with the parent material or preceding metabolite, but could also be obtained from different studies (formation kinetics from study with the parent, degradation kinetics from study with the metabolite, although in that case same study conditions and same soil or same soil type are desirable unless enough soils are tested for average values to be considered). The technique used to derive modelling endpoints should not conflict with their intended use. Hence, the same kinetic model or models that is/are available in the environmental model (e.g. for gw or sw) or calculation tool (e.g. for soil) considered should be used to derive the modelling endpoints.

8.2.2.1 PEC_s

Predicted environmental concentrations in soil (PEC_s) for metabolites may be calculated by modelling the formation and degradation of the metabolites using the same or similar simple software tools (e.g. ModelMaker or other compartment models) or analytical solutions of integrated equations that are used to derive the kinetic endpoints. These calculations are not limited to first-order kinetics. The model that fits the experimental data best should be used to derive degradation parameters unless deviations from first-order kinetics can be attributed to experimental artefacts.

When the preceding approach is not feasible, an alternative approach may be used,. This consists of calculating the exposure starting from the peak (maximum) and using the kinetics of disappearance from the maximum (obtained from fitting of the decline phase of the metabolite).

8.2.2.2 PEC_{GW}

PEC in groundwater (PEC_{GW}) is calculated using environmental fate models such as the pesticide leaching models MACRO, PEARL, PELMO and PRZM. These models are currently limited in the first tier to first-order kinetics, and in some limited cases, hockey-stick kinetics. Higher-tier approaches may also be used to model bi-phasic kinetics, e.g. with the bi-exponential model or with time-dependent sorption in PEARL, as discussed in Section 7.1.2.2. In any case, the kinetics used to derive the degradation parameters from laboratory or field studies must be consistent with the kinetics used in the simulation model.

8.2.2.3 PEC_{sw}

Guidance to derive kinetic endpoints in aquatic systems for the calculation of PEC in surface water (PEC_{SW}) for metabolites are provided in the water-sediment section of this report. Kinetic endpoints in soil may also be needed as input parameter in runoff or drainage models, e.g. PRZM and MACRO. These models are in principle, similar/equivalent to the ground water models, and the same procedure recommended to derive kinetic endpoints in soil for ground water models should be valid for runoff/drainage surface water models. In any case, the kinetics used to derive the degradation parameters from laboratory or field studies must be consistent with the kinetics used in the simulation model.

8.3 General recommendations for metabolites

8.3.1 Data issues

8.3.1.1 Number and distribution of data points

The identification of a suitable model and the estimation of parameters for the description of the formation and degradation of metabolites are much more complex in comparison to the description of the degradation of a parent substance alone. The description of the concentration curve of one metabolite depends on a correct description of the degradation of the parent substance and/or other preceding metabolite(s), and of the degradation of the metabolite itself. The kinetic models for metabolites are therefore much more complex and require additional parameters to be fitted in addition to the parent degradation parameters (formation fractions and degradation parameters for the metabolites). In order for these metabolite parameters to be fitted, adequate data is required for the metabolites in addition to the parent substance.

The total number of data points necessary for parameter estimation depends on the number of parameters to be estimated, and therefore, will depend on the complexity of the metabolic pathway and complexity of the kinetic model(s) envisaged. While a minimum of 6 to 8 sampling dates should be available from the study, the number of data points available for a given metabolite may be much smaller due to non-detects before and after the metabolite is observed. As recommended in the general section, individual replicate values should be used for modelling in preference to average values. The higher number of data points with the replicate values will provide in most cases a better fit.

Ideally, a good distribution of the metabolite data points over the formation phase, area of maximum and decline phase of the metabolite should be available. However, the experimental design of a degradation study is usually optimised for the observation of the parent and not necessarily for metabolites. Metabolites may be formed in the later stages of the study, where the time between sampling dates is usually higher than in the beginning of the study, and therefore less appropriate for parameter estimation.

The natural uncertainty in the data and data scattering resulting from the sampling technique, study design, work-up procedure or analytical error, and other experimental artefacts may in some cases be higher for metabolites than for the parent substance. This may be because metabolites occur at lower concentrations and the study design and analytical methods are often optimised for the parent substance and main metabolites if known, and may be less appropriate for some of the metabolites. Other experimental artefacts in laboratory studies like decline in soil microbial activity or loss of aerobicity, generally increase with the duration of the study, and may affect the quality of the data for certain metabolites.

The influence of the number and distribution of data points on the quality of the estimation is illustrated with some generated data sets in Appendix 6. The impact of the number of data points was greater for metabolites that are formed at low amounts and for slowly forming and/or degrading metabolites, which are associated with the greatest uncertainty in the estimated parameters. In addition to the number of sampling times and distribution of data points, the quality of the data, that is the precision of the individual measurements and number of replicates, will certainly also play a major role for these metabolites. In contrast, metabolites formed at high amounts and fast-forming/degrading metabolites that exhibited a clear pattern of formation and decline were less affected by the number and distribution of data points, and should be less sensitive to data variability. Finally, if the maximum of the metabolite was not reached during the study, i.e. no observable decline or plateau, the uncertainty associates with the estimated degradation rates can be high, and the optimisation results should be interpreted with care, depending on their statistical significance and/or the goodness of fit.

8.3.1.2 Mass balance

The mass balance during the study should be discussed if available. Mass balance closure should normally be attained in laboratory studies conducted with radiolabeled substance, but is rarely attained in field studies when non-labeled substance is used, and volatiles and bound residues are not accounted for.

An appreciable loss of mass balance in any single sample replicate or time point should be examined and may justify discarding the point as outlier. A constant decrease in recovery with time needs to be discussed with regards to the validity of the data values for the later time points, for parent and any observed metabolite. Loss of mass balance due to not accounting for volatiles or bound residues would not affect the kinetic evaluation procedure as long as the sink data (sum of observed data for identified metabolites not specifically included in the fit as compartments, unidentified minor metabolites, organic volatiles, CO_2 and bound residues) is not included in the fit. However, losses specific to a particular substance, whether partly or completely unaccounted for, may not only impact the kinetic evaluation of the substance itself, but also any degradation products further down the metabolic pathway, as the route scheme would be affected (see Section 8.3.2).

8.3.1.3 Data treatment (outliers, time-0 values and points <LOQ/LOD)

Guidance on how to identify possible outliers and whether to include/keep them in the input data, on how to address time-0 values for degradates (including bound residues and identified or non-identified metabolites) when different than 0, and data points <LOQ/LOD are provided in the general/parent Section 5.1. The same recommended procedures are valid for metabolites, except that for points <LOQ/LOD, points before the formation phase of the metabolite should be considered in addition to the points at the end of the decline phase. In that case, the same principles as for the points at the end of the decline phase should be applied. Unless it corresponds to time-0, the last point before the first detectable amounts of substance should be included in the fit at ½ LOD if <LOD or ½ (LOQ+LOD) if <LOQ, and prior non-detects should be omitted. The initial amounts of metabolites at time 0 should be set to 0, unless another value can be justified (for example, a metabolite present in the application solution).

The approach is illustrated in Table 8-1.

Table 8-1. Example to illustrate the handling of concentrations below the limit of detection and quantification for metabolites (LOQ = 0.05, LOD = 0.02)

Metabolite Measured	Set to
< LOD [a]	0.00
< LOD	-
< LOD	0.01
0.03	0.03
0.06	0.06
0.10	0.10
0.11	0.11
0.10	0.10
0.09	0.09
0.05	0.05
0.03	0.03
< LOD	0.01
< LOD	-

* Time 0 sample

8.3.2 Description of the degradation pathway)

In order to obtain reliable formation and degradation endpoints for a metabolite from a study conducted with the parent substance or with a preceding metabolite, having a good knowledge of the degradation pathway up to this metabolite is essential. A kinetic analysis using compartment models may, in some cases, help in confirming or determining the extent of some specific pathways (e.g. flows to sink). All dissipation or degradation flows in the conceptual model must be realistic regarding the chemical or biological reactions and physico-chemical processes involved and should be justified accordingly.

When both rates are derived from the study with the parent, the formation rate and degradation rate are directly related as they happen simultaneously, which can result in correlation of the formation and degradation parameters. The correlation between parameters in a particular model may be checked in the correlation matrix of the fit. A value near 1 shows significant correlation between the two parameters in question, the effect of which needs to be addressed by testing different combination of starting values for these parameters. In any case, the degradation of the parent or preceding metabolite must be accurately described using the appropriate kinetic model (see Sections 7.1 and 8.4) in order for the degradation of the metabolite to be accurately described.

Another direct implication of the dependence of the degradation rate on the formation rate and fraction is that using an incorrect pathway may result in incorrect formation and therefore incorrect estimate of the degradation rate. This would result in incorrect kinetic endpoints for the metabolite.

One particularly important aspect of the degradation pathway that can be difficult to define clearly, especially in complex pathways involving numerous metabolites, is the formation of bound residues as well as the degradation to minor, undefined/unidentified metabolites (which in turn can form bound residues and/or mineralise to CO_2). Because of the undefined nature of these unidentified or bound residues, determining their source (parent and/or metabolite(s)), is usually difficult. Therefore determining a priori which flows to sink (from parent and from each metabolite) should be included in the pathway often is not possible. Not accounting for processes that actually occur results in an incorrect conceptual model that can severely impact the kinetic evaluation, as illustrated in the Example 8-1 below. On the other hand, including processes that do not actually occur, or that may occur at insignificant levels, results in unnecessarily complex and over-parameterized models, which can also lead to incorrect results. In the initial fitting, all possible flows to the sink compartment should be included, i.e. the flows from each substance, parent and metabolites, which is to be fitted in the compartment model. Based on the results of the initial estimation and on experimental evidence for or against such flow (e.g. depending on chemical reaction involved and by comparison with evidence from other studies), the flow may be kept, or should be removed for simplification of the conceptual model. In other words, the formation fraction of a metabolite should always be estimated at first, either directly as a free parameter, or by calculating it from the ratio of the rate to the metabolite to the overall rate of degradation of the precursor. Based on this first estimate and weight of evidence, a decision is made on whether it may be fixed to 1 (or 1 minus the formation fractions of the other metabolites in the case where multiple metabolites are formed from the same substance), in which case the flow to sink is removed. Further guidance on this procedure is given in Section 8.4.4.

In field studies, minor metabolites and metabolites considered non-relevant, but which could be involved in the metabolic pathway to major or potentially relevant metabolites, may not always be included in the analytical method. In laboratory studies, transient metabolites that occur at very low level are often difficult to identify, and therefore may not always be reported. In any case, if the presence of an intermediary metabolite is known from other studies or suspected from the chemistry involved in the degradation, a ghost compartment (without associated measured data) may be introduced in the model to fit the data of

observed metabolites further in the degradation route. Further guidance on this procedure is given in Section 8.5.2.

Example 8-1.

An example illustrating the importance of including flows to the sink compartment in the conceptual models to obtain the correct endpoints for a metabolite is shown below. In this example, the experimental data show a very rapid formation of bound residues, and a flow from the parent substance to the sink compartment is therefore justified. The degradation of the parent appears bi-phasic (see figures) and was therefore described with either SFO or FOMC kinetics, while the degradation of the metabolite was in both cases described with SFO kinetics.

The calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the SFO and FOMC kinetic model fits in ModelMaker 4.0, with or without including the flow from parent to sink, are listed in Table 8-2, and the description of the observed data for parent and metabolite with the different kinetic models and pathways is shown in Figure 8-1. With both SFO and FOMC models for the parent, the data for the metabolite can still be described reasonably well if the flow from parent to sink is not taken into account, although with the FOMC model, neither parameter alphaP nor betaP can be considered reliable as indicated by the high standard errors associated with the parameters. However, for both models the estimated parent initial amount is too low and considered unrealistic. Much better fitting is obtained when considering a flow from parent to sink. The initial decline of the parent is much better described, which results in improved statistical indices (χ^2 error of 21 versus 31 with the SFO model, and 10 versus 29 with the FOMC model). The relatively high standard error of the betaP parameter in the FOMC fit, together with the overestimation of the degradation at the later time points (systematic deviation in the plot of residuals, not shown here), indicates that FOMC may not be the best-fit model and that a different bi-phasic model such as DFOP should be tested. Comparing the calculated DT50 and DT90 values for the metabolite, these are much shorter if the flow of parent to sink is not considered (DT50 values of 13.5-16.0 days vs. 38.0-39.1 days). Hence, in this case, if the conceptual model is incorrect (no flow of parent to sink), the formation fraction of the metabolite and its degradation are overestimated and the estimated DT50 are overly short. The use of inadequate pathways in the compartment models can potentially result in appreciable error in the kinetic endpoints for metabolites. Note that since about 90% of the degradation of the parent, and therefore 90% of the formation of the metabolite occurs during the first phase of the parent's decline, the ability to describe the second phase of the parent degradation using FOMC versus SFO kinetics does not affect much the metabolite endpoints.

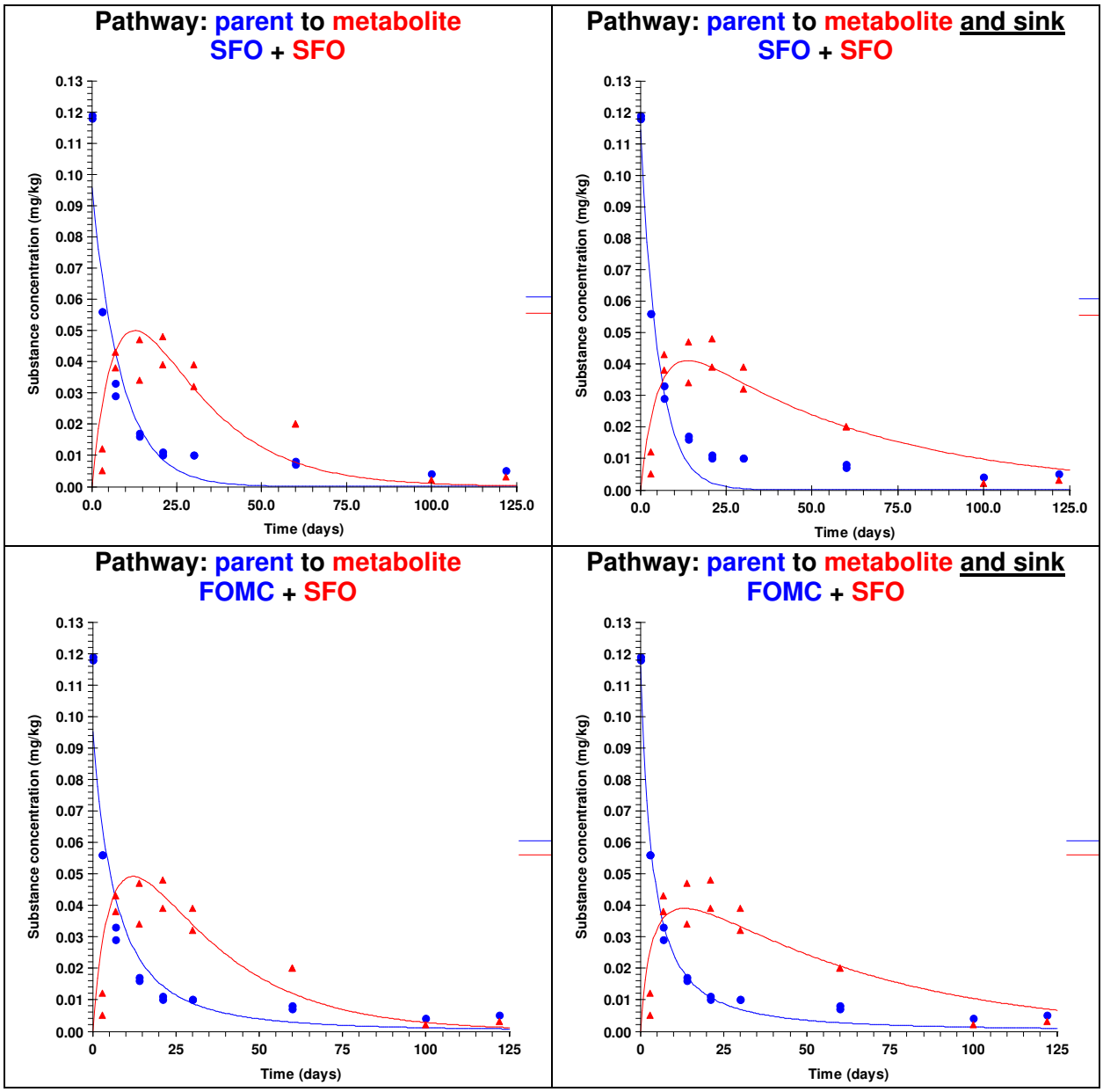


Figure 8-1. Description of the observed data for parent and metabolite from Example 8-1 with parent SFO (top) or FOMC (bottom) including (right) or not including (left) a flow from the parent to the sink (for any degradation processes other than to the main metabolite)

Table 8-2. Results of the fits with parent SFO or FOMC and metabolite SFO in Example 8-1, including or not including a flow from the parent to the sink (for any degradation processes other than to the main metabolite)

Pathway	**Parent to Metabolite (100%)**		**Parent to metabolite <u>and sink</u>**	
Kinetic model	Parent SFO Metabolite SFO	Parent FOMC Metabolite SFO	Parent SFO Metabolite SFO	Parent FOMC Metabolite SFO
Parameter (estimate±standard error)				
Pini (mg/kg)	0.0957 ± 0.0057	0.0952 ± 0.0054	0.1137 ± 0.0053	0.1158 ± 0.0047
kP (d^{-1}, SFO)	0.1140 ± 0.0169	-	0.1855 ± 0.0269	-
AlphaP (FOMC)	-	**2.242 ± 1.771***	-	1.541 ± 0.526
BetaP (FOMC)	-	**16.02 ± 15.92***	-	5.815 ± 2.946
ffM	1 (fixed)	1 (fixed)	0.4661 ± 0.0609	0.4778 ± 0.0513
kM (d^{-1})	0.0513 ± 0.0115	0.0434 ± 0.0082	0.0177 ± 0.0067	0.0183 ± 0.0041
Goodness of fit (χ^2 error)				
χ^2 error parent	31	29	21	10
χ^2 error metabolite	28	28	20	25
Kinetic endpoints (triggers)				
DT50 parent (d)	6.08	**5.80***	3.74	3.30
DT90 parent (d)	20.2	**28.7***	12.4	20.1
DT50 metabolite (d)	13.5	16.0	39.1	38.0
DT90 metabolite (d)	44.9	53.1	130	126

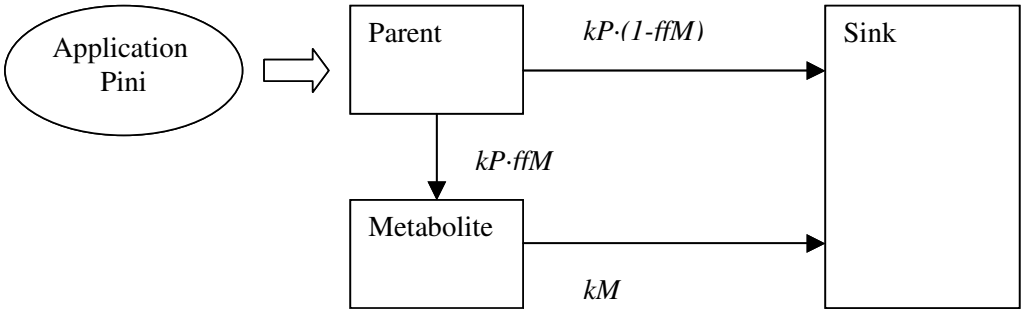
*The standard error associated to the FOMC shape and/or location parameter is very high compared to the parameter estimate, indicating these may not be reliable. Because of lack of confidence in the FOMC parameter estimates from this fit, the DT50 and DT90 values calculated from the parameters may not be reliable.

8.3.3 Types of kinetics (kinetic models) for metabolites

8.3.3.1 SFO model

Kinetic models with metabolites are much more complex than for parent only, involving additional parameters for formation and degradation of the metabolite. The parameters needed to describe simultaneous formation and degradation of metabolites can be correlated. The degree of complexity increases with the number of metabolites. Therefore, the SFO model, with its limited number of parameters (initial amount and rate constant for parent, formation fraction and rate constant for each metabolite), is by far the most robust model that can be used. The degradation of metabolites can, in most cases, be reasonably well described with single first-order kinetics, and this model should be used as a first choice. The SFO model for metabolite is described in Box 8-1 for the simple example of parent + 1 metabolite, with both substances following SFO kinetics.

Concept



Rate equations (differential form)

$$\frac{dParent}{dt} = -kP \cdot Parent$$

$$\frac{dMetabolite}{dt} = kP \cdot ffM \cdot Parent - kM \cdot Metabolite$$

$$\frac{dSink}{dt} = kP \cdot (1 - ffM) \cdot Parent + kM \cdot Metabolite$$

where

Pini	=	Total amount of parent present at time t = 0
Parent	=	Total amount of parent present at time t
Metabolite	=	Total amount of metabolite present at time t
kP	=	Rate constant of parent
ffM	=	Formation fraction of metabolite
kM	=	Rate constant of metabolite

Parameters to be determined

Pini, kP, ffM, kM

Endpoints

kP, ffM, kM

$$DT_{50\_Parent} = \frac{\ln 2}{kP}, \qquad DT_{50\_Metabolite} = \frac{\ln 2}{kM}$$

Box 8-1. Example of metabolite single first-order (SFO) kinetics with parent SFO

8.3.3.2 Bi-phasic models

Similarly to what is observed with parent substances, some metabolites may be subject to slower degradation with time. However, due to the gradual formation of the metabolites, especially if the formation is slow, a decrease in the degradation rate with time will be less evident than for the parent substance. An observable decrease in degradation rate that can be attributed to a decrease in soil microbial activity or other experimental artefacts should not be modelled. However, if the decrease is due to increased sorption, non-linear sorption or

other underlying mechanisms expected to influence degradation under field conditions in a similar manner, it should be described in the kinetic model. In that case, non-SFO models are needed to accurately describe the degradation of the metabolites and generate the trigger endpoints. Technically, the Gustafson-Holden (FOMC), bi-exponential model and hockey-stick models can all be applied to metabolites. Numerical solutions to the integrated forms of the models may be obtained using mathematical tools such as Mathematica or MatLab. Analytical solutions exist for some simple cases for parent/metabolite combinations, but are not provided in this document. The use of analytical or numerical solution to the model and method used to obtain/derive that solution should always be clearly documented in the kinetic evaluation report. In the cases of the FOMC and DFOP models, constituting autonomous differential equations, where the right hand side only contains state variables (variables such as concentration describing the state of the system at some instant of time; initial amount present and time are not state variables), do not exist. The differential forms proposed in Chapter 5 for these two models both contain time in the right hand side, and therefore are not appropriate for metabolites, which are formed gradually. An alternative formulation of the DFOP model with two sub-compartments and SFO kinetics for each sub-compartment is proposed below, which can be implemented in compartment models with differential equations.

8.3.3.2.1 Hockey-stick model

The hockey-stick model, with its single breakpoint time is not conceptually correct for a metabolite that is gradually formed over a period of time. Due to its continuous formation, deviations from SFO for a metabolite will appear to be gradual and smoothed. A clear break in the decline phase of a metabolite would imply that a change occurred in experimental conditions (e.g. loss of microbial activity) rather than in the metabolite bioavailability, and should therefore not be modelled. Hence, the hockey-stick model should not be used for metabolites.

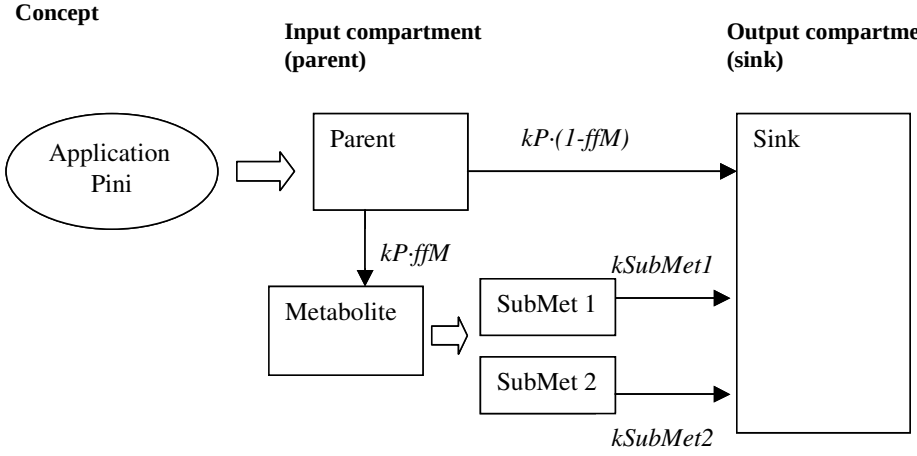
8.3.3.2.2 Bi-exponential model

The concept of the bi-exponential model for metabolites has the same limitations as previously discussed for parent compounds. The model is a pragmatic approach when the kinetics are bi-phasic. While the single differential equation proposed in chapter 5 cannot be used for metabolites because time is in the right-hand side, the DFOP model can be easily implemented for metabolites using two sub-compartments and a set of differential equations as described in Box 8-2. This model formulation implies that the precursor substance degrades to two sub-compartments, SubMet1 (fast degrading sub-compartment) and SubMet2 (slow degrading sub-compartment). The degradation for each sub-compartment is

then described with the constituting autonomous differential equation for SFO, so that none of the differential equations in the model have time in the right hand-side. The sum of the two sub-compartments is equal to the metabolite, which can be introduced in the compartment model as a separate variable.

One major disadvantage of this model is that DT50 or any DT values cannot be directly calculated from the model parameters although these trigger values can be derived using an iterative method, or by integrating the metabolite degradation (without formation) from a given initial amount (e.g. 100) and looking up the DT50 or DT90 from a table of calculated concentrations as the time at which the concentration has decreased to 1/2 or 1/10 the initial fitted value. Considering the modelling endpoints, the bi-exponential model cannot be directly implemented in environmental models, but the alternative methods presented in Appendix 5 for a parent substance may also be applied to metabolites.

Concept



Rate equations (differential form)

$$\frac{dParent}{dt} = -kP \cdot Parent$$

$$\frac{dSubMet1}{dt} = kP \cdot ffM \cdot g \cdot Parent - k1M \cdot SubMet1$$

$$\frac{dSubMet2}{dt} = kP \cdot ffM \cdot (1-g) \cdot Parent - k2M \cdot SubMet2$$

$$\frac{dSink}{dt} = kP \cdot (1-ffM) \cdot Parent + k1M \cdot SubMet1 + k2M \cdot SubMet2$$

where

Pini	=	Total amount of parent present at time t = 0
Parent	=	Total amount of parent present at time t
Metabolite	=	Total amount of metabolite present at time t, Metabolite = SubMet1 + SubMet2
kP	=	Rate constant of parent
ffM	=	Formation fraction of metabolite
g	=	fraction of Metabolite applied to sub-compartment 1
k1M	=	Rate constant of sub-metabolite 1
k2M	=	Rate constant of sub-metabolite 2

Parameters to be determined

Pini, kP, ffM, g, k1M, k2M

Endpoints

kP, ffM, g, k1M, k2M

$$DT_{50\_Parent} = \frac{\ln 2}{kP},$$

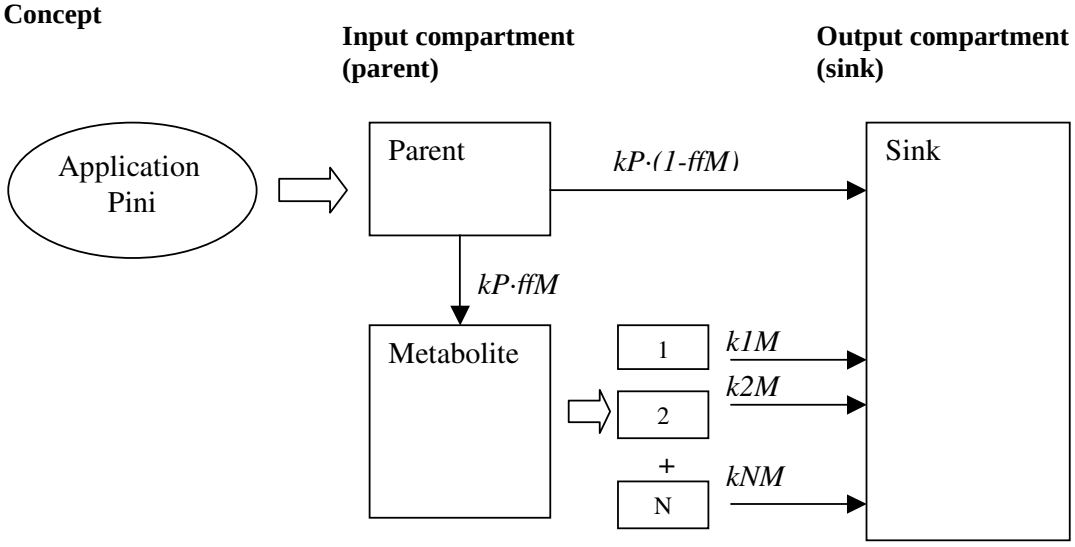
The DT_{50} value of the metabolite can be found by an iterative method, an analytical solution does not exist.

Box 8-2. Example of metabolite bi-exponential (DFOP) kinetics with parent SFO

8.3.3.2.3 FOMC model

The FOMC model has only one additional parameter compared to the SFO model and allows for straightforward calculation of DT values. However, the FOMC model can only be implemented for metabolites in an integrated form, which then needs to be solved analytically or numerically. The single differential equation proposed in chapter 5 cannot be used for metabolites because time is in the right-hand side. The FOMC model cannot be implemented in environmental models and is thus not valid for the determination of modelling endpoints. The only exception may be for terminal metabolites, for which a conservative estimate of the SFO DT50 may be obtained by dividing the FOMC DT90 by 3.32, in an approach similar to that described in 7.1.2 for the parent substance. This approach is only valid for terminal metabolites because otherwise it would affect the kinetics of formation of metabolites further down in the degradation pathway.

Concept



Because the FOMC model cannot be described with autonomous constituting differential equations it is not appropriate to use the differential form of the model for metabolites, which are formed gradually. Models including FOMC kinetics for metabolites need to be expressed in their integrated form and solved using e.g. mathematical tools such as Mathematica or MatLab. The solution for the above example is not provided here.

Parameters to be determined

Pini	=	Total amount of parent present at time t = 0
kP	=	Rate constant of parent
ffM	=	Formation fraction of metabolite
αM	=	Shape parameter (metabolite) determined by CV of *kiM* values
βM	=	Location parameter (metabolite)

Endpoints

kP, ffM, αM, βM

$$DT_{50\_Parent} = \frac{\ln 2}{kP}, \qquad DT_{50\_Metabolite} = \beta M \cdot \left(2^{\frac{1}{\alpha M}} - 1 \right)$$

Box 8-3. Example of metabolite Gustafson-Holden kinetics (FOMC) with parent SFO

Example 8-2

An example of metabolite exhibiting a bi-phasic degradation pattern is shown below. In this example, the experimental data show a very rapid degradation of the parent substance to one metabolite. The metabolite in turns degrades more slowly, with a marked decrease in the degradation rate over time. Although the data sampling continued far over the accepted limit

of 120 days for appropriate microbial activity, and hence experimental artefacts, i.e. a decline in microbial activity may be responsible for the metabolite bi-phasic pattern, it is assumed here for the sake of this exercise, that it is not the case, and therefore the bi-phasic pattern needs to be modelled. The data set was fitted with the parent-metabolite SFO-SFO and SFO-DFOP models in differential form described in boxes 8-1 and 8-2, except that the formation fraction of the metabolite, *ffM*, was fixed to 1, as in this particular case the metabolite is the only degradation product from the parent.

The calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the parent-metabolite SFO-SFO and SFO-DFOP kinetic model fits in ModelMaker 4.0 are listed in Table 8-3, while the description of the observed data for parent and metabolite with the two kinetic models is shown in Figure 8-2. The parent substance was described well with the SFO model, as reflected in the low χ^2 error values of 11-12. For the metabolite, although the χ^2 error values were also all low, with the lowest value of 11 obtained with the bi-phasic model versus 14 with the SFO model, reflecting the good description of the formation and peak of the metabolite, the plot of residuals for the SFO model shows a systematic error after 50 days, whereas the residuals from the DFOP fit appear to be randomly distributed. Assuming that the bi-phasic degradation pattern of the metabolite does not result from experimental artefacts, the correct trigger endpoints for the metabolite in this example are the DT50 of 15.6 days and DT90 of 113 days obtained from the SFO-DFOP model, as opposed to the DT50 of 18.3 days and DT90 of 60.9 days obtained from the SFO-SFO model.

Table 8-3. Results of SFO and DFOP fits of metabolite (parent SFO) in Example 8-2.

	Parent SFO Metabolite SFO	Parent SFO Metabolite DFOP
Parameter (estimate±standard error)		
Pini (% AR)	97.32 ± 3.19	100.2 ± 3.0
kP (d^{-1})	0.7385 ± 0.0570	0.7355 ± 0.0453
kM (d^{-1}, SFO))	0.0378 ± 0.0039	-
g fraction SubMet1 (DFOP)	-	0.6516 ± 0.1729
K1M (DFOS)	-	0.0738 ± 0.0260
K2M (DFOS)	-	0.0111 ± 0.0055
Goodness of fit (χ^2 error)		
χ^2 error parent	11	12
χ^2 error metabolite	14	11
Kinetic endpoints (triggers)		
DT50 parent (d)	0.94	0.94
DT90 parent (d)	3.1	3.1
DT50 metabolite (d)	18.3	15.6
DT90 metabolite (d)	60.9	113

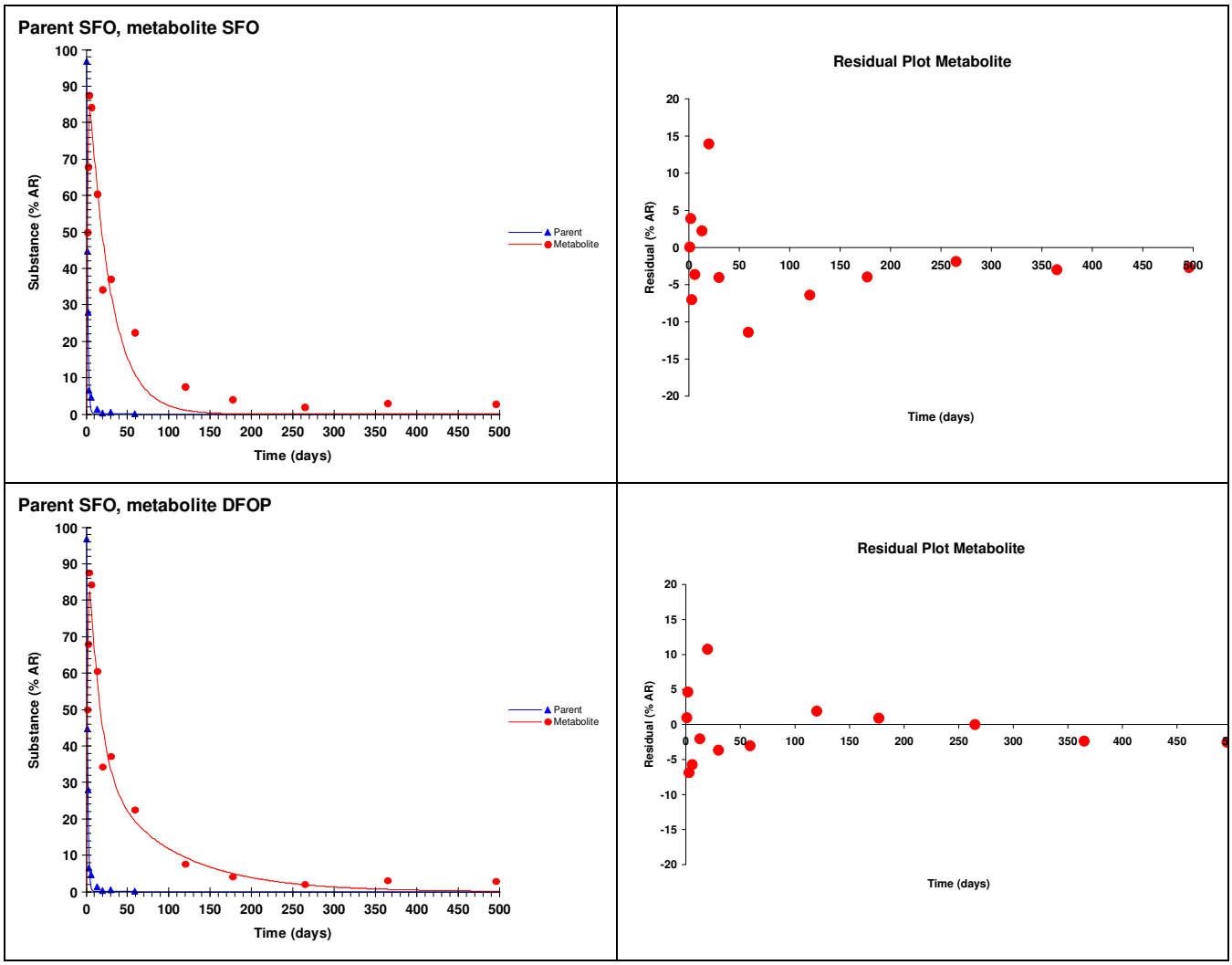


Figure 8-2. Description of the observed data for parent and metabolite from Example 8-2 with parent SFO and metabolite SFO (top) and DFOP (bottom) with corresponding residual plot of the metabolite (right)

8.3.4 Implementation of the conceptual model

The kinetic models listed in general metabolite section 8.3.3 are available in their integrated form or as rate equations in their differential form. Conceptual degradation models with parent and metabolites are generally implemented mathematically as a system of differential equations, but can also be expressed in their integrated form. Once the mathematical model has been defined, different fitting techniques can be used to estimate the kinetic endpoints for both parent and metabolites from the study data.

8.3.4.1 Analytically integrated models

For simple conceptual models, e.g. parent SFO plus one metabolite SFO, analytical solutions to the system of differential equations (mathematical model) are available that can be used in

most software tools to estimate the desired endpoints. Analytical solutions may also be derived for more complex, all-SFO models with multiple metabolites with or without parallel flows to a sink compartment, as well as for simple models with FOMC kinetics for the parent, for the metabolite, or for both parent and metabolite. The length and complexity of these analytical solutions mean that they are beyond the scope of this document, but where proper solutions exist they are equally valid for use.

8.3.4.2 Compartment models with differential equations

The most simple and flexible approach for implementing conceptual models for metabolites is to build compartment models with differential equation in software tools that can solve the systems of differential equations with analytical (e.g. Laplace transformation) or numerical (e.g. Runge-Kutta or Euler) methods. In such models, the substances are defined as compartments and dissipation processes (flows) are postulated between the compartments according to the proposed route of dissipation. Each flow is then described with a differential equation or a set of differential equations corresponding to the kinetic model to be applied. This approach can be applied to any system even with multiple metabolites, different kinetic models (as long as available as an autonomous differential equation, see kinetic model boxes in Chapter 5. and Section 8.3) and complex pathways. It is especially useful for sequentially building a model (see Section 8-4 for detailed guidance on the stepwise approach). The only limitation is the number of parameters that can be used with regards to the number of data points available. One major advantage of the compartment model approach is that it is transparent and relatively easy to report as long as the various compartments and flows are clearly defined. The quality of the estimation still depends on the quality of the numerical solver in the software tool (see software package section of this report).

In the case of pesticide dissipation or degradation in soil or other environmental systems, the above-mentioned models all represent simple and sensible approaches to mathematically describe the experimental data, and do not represent actual chemical reactions. The more complex the pathway and the type of kinetics used, the more parameters the model will require, and the more data points are needed for adequate parameter estimation. Therefore, the simplest model that can provide a sensible description of the proposed pathway and adequate description of the decline curves should always be preferred.

8.3.4.3 Metabolite formation fractions

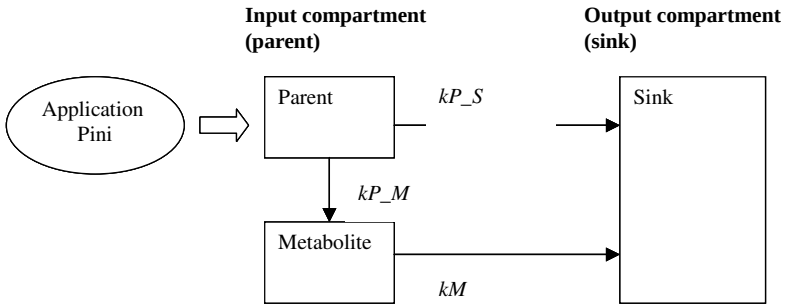
The degradation of a substance, parent or intermediate metabolite to metabolite(s) and sink can be formulated in two different ways, using individual degradation rates for each flow (one for each metabolite formed and one to the sink), or using formation fraction parameters to

split the overall degradation rate between the metabolites formed and the sink. The two approaches are illustrated in Box 8-4 for a simple example of parent substance degrading to one metabolite plus minor/unidentified residues (sink).

In the first case, the formation fraction of the metabolite is calculated from the ratio of the individual degradation rate to the metabolite to the overall degradation rate of the parent or preceding metabolite, while in the second case it is estimated directly as a parameter. Note that the first case is only applicable to SFO otherwise the formation fraction becomes time-dependent and cannot be used in modelling, while the second case implies that the degradation to each metabolite and to the sink follows the same kinetics, similar to what is assumed in most environmental models. Considering all-SFO kinetics as used in environmental modelling, the first approach allows the direct estimation of the actual endpoints for models formulated with individual rates, such as PELMO, while the latter approach with the introduction of formation fraction(s) allows the direct estimation of the actual endpoints for models such as PEARL and PRZM, i.e. degradation rate of parent or preceding metabolite, formation fraction and degradation rate of the metabolite. Still, the two approaches can be considered equivalent, as the formation fraction can be calculated from the individual rate constants and vice-versa.

The formation fraction parameter should be constrained between 0 and 1, or, if several metabolites are formed at once from the same substance, the sum of the formation fractions should be constrained to 1. Although in some cases the estimated value of the formation fraction may exceed 1 because of natural variability in the data and experimental error, these should be considered as artefacts, and it was therefore decided that the parameter should be constrained to its theoretical maximum of 1. The same natural variability of the data and experimental error would lead to an estimated value below 1 when the actual formation fraction should be 1. As a result, when the estimated value of the formation fraction is near one (e.g. ≥ 0.95), the likelihood of having a formation fraction of 1 should be assessed, based on the knowledge of the chemical reaction(s) involved and weight of evidence from other relevant studies. If fixing the formation fraction to 1 is justified, the conceptual model can then be simplified by removing the flow to the sink. The starting value of the formation fraction parameter should be initially set to its midpoint, i.e. 0.5. If the metabolite formation and degradation parameters are highly correlated, which should be reflected in high error associated to the parameter estimates, the optimisation should be repeated with a number of different initial combinations of parameter values to find the best starting values for the situation at hand (see section 6.2).

I: formulation with individual rate constants



Rate equations (differential form)

$$\frac{dParent}{dt} = -kP \cdot Parent$$

$$\frac{dMetabolite}{dt} = kP\_M \cdot Parent - kM \cdot Metabolite$$

$$\frac{dSink}{dt} = kP\_S \cdot Parent + kM \cdot Metabolite$$

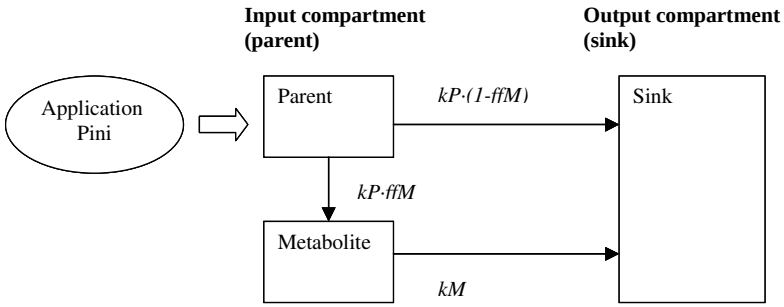
where

Pini	=	Total amount of parent present at time t = 0
Parent	=	Total amount of parent present at time t
Metabolite	=	Total amount of metabolite present at time t
kP_M	=	Rate constant of parent to metabolite
kP_S	=	Rate constant of parent to sink
kM	=	Rate constant of metabolite

and

$$\text{Metabolite formation fraction} = \frac{kP\_M}{kP\_M + kP\_S}$$

II: formulation with formation fraction



Rate equations (differential form)

$$\frac{dParent}{dt} = -kP \cdot Parent$$

$$\frac{dMetabolite}{dt} = kP \cdot ffM \cdot Parent - kM \cdot Metabolite$$

$$\frac{dSink}{dt} = kP \cdot (1 - ffM) \cdot Parent + kM \cdot Metabolite$$

where

Pini	=	Total amount of parent present at time t = 0
Parent	=	Total amount of parent present at time t
Metabolite	=	Total amount of metabolite present at time t
kP	=	Rate constant of parent
ffM	=	Formation fraction of metabolite
kM	=	Rate constant of metabolite

Box 8-4. Formulation of a simple conceptual model of parent + 1 metabolite and sink with individual rate constants (top) and formation fraction (bottom)

8.3.5 Weighting method

The method of weighting the data can affect the description of the degradation of the parent compound, and in the process will affect the description of the metabolites. Correctly describing the formation of the metabolite to be able to also describe its degradation is essential. In that regard, obtaining a good fit of the earlier stages of the parent's degradation curve or preceding metabolite's decline is more desirable than its later stages so as to describe most of the formation of the metabolites. Therefore, as recommended in Section 5.1.3 for the parent, unweighted fits (same absolute error assigned to each point) are recommended as a first step.

Example 8-3

The example below illustrates the impact that the weighting method may have on the kinetic evaluation results for metabolite(s). In this example, the parent substance degrades to two main metabolites and also to some minor metabolites and/or bound residues (sink). The three substances were fitted simultaneously with a four-compartment model with all-SFO degradation flows from the parent to the two metabolites and the sink, and from each metabolite to the sink. Note that in this example the model was formulated with individual rate constants (one for each flow from the parent to metabolite 1, *kP_M1*, to metabolite 2, *kP_M2*, and to the sink, *kP_S*), rather than using formation fractions. Further guidance on the 2 approaches is given in Section 8.4.1. In one case, the fitting was performed with ordinary least-squares (unweighted), while the second fit was performed with weighted least-squares with a fractional error.

The parameter estimation results and calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the unweighted and weighted fits in ModelMaker 4.0 are listed in Table 8-4, and the description of the observed data for parent and metabolite with the different kinetic models is shown in Figure 8-3.

With the ordinary least-squares method (unweighted), the description of the parent experimental data with SFO is good, except for the last sampling times, which show a slight tailing that cannot be described with SFO, and very good for both metabolites. The use of a bi-phasic model may help improve the fit for the parent substance, but considering that >90% of its degradation is appropriately described by the SFO fit, and considering the very low χ^2 error value of 5, the SFO model can be considered appropriate. The two metabolites are

also adequately described with the SFO model in the unweighted fit, as reflected in the low χ^2 error values of 10-11 and random distribution of the residuals.

The weighted least-squares with fractional error approach does not properly describe the experimental data, which is reflected in the higher χ^2 error values obtained for the parent and metabolites, and systematic error in the residual plots, in the early time points for the parent and around the observed maximum for the metabolites. Because of the slight tailing of the parent substance, the weighting was strongly on the last sample points, which resulted in a gross underestimation of the initial percentage and first points of parent and therefore of the formation of the metabolites (observed maxima are not reached for both metabolites). In addition, the t-test indicates that the degradation rate constant parameter for metabolite 2, kM2, is not significantly different from zero, so the DT50 for this metabolite would not be considered reliable.

The unweighted fit, which provided a good description of the degradation of the parent substance, is the appropriate fit to derive the kinetic endpoints for the metabolites.

Table 8-4. Results of unweighted and weighted all-SFO fits of the parent and two metabolites in Example 8-3

	Ordinary least-squares	Weighted least-squares with fractional error
Parameter (estimate±standard error)		
Pini	100.5±0.7	69.3±7.6
kP_M1	0.008±0.001	0.007±0.001
kP_M2	0.0071±0.0004	0.006±0.001
kP_S	0.040±0.001	0.027±0.004
kM1_S	0.017±0.002	0.015±0.002
kM2_S	0.005±0.001	**0.002±0.002**[Φ]
Goodness of fit (χ^2 error)		
χ^2 error parent	5	30
χ^2 error metabolite 1	10	21
χ^2 error metabolite 2	11	22
Kinetic endpoints (triggers)		
DT50_P	12.7	17.6
DT50_M1	41.5	47.3
DT50_M2	132.5	**369***

[Φ]The probability corresponding to the calculated t-value for the highlighted parameter is far above the significance level of 5% (0.161), indicating that the parameter is not significantly different from zero.
*Because of lack of confidence in the rate constant parameter estimate for M2 from this fit, the DT50 value for M2 calculated from the parameter may not be reliable.

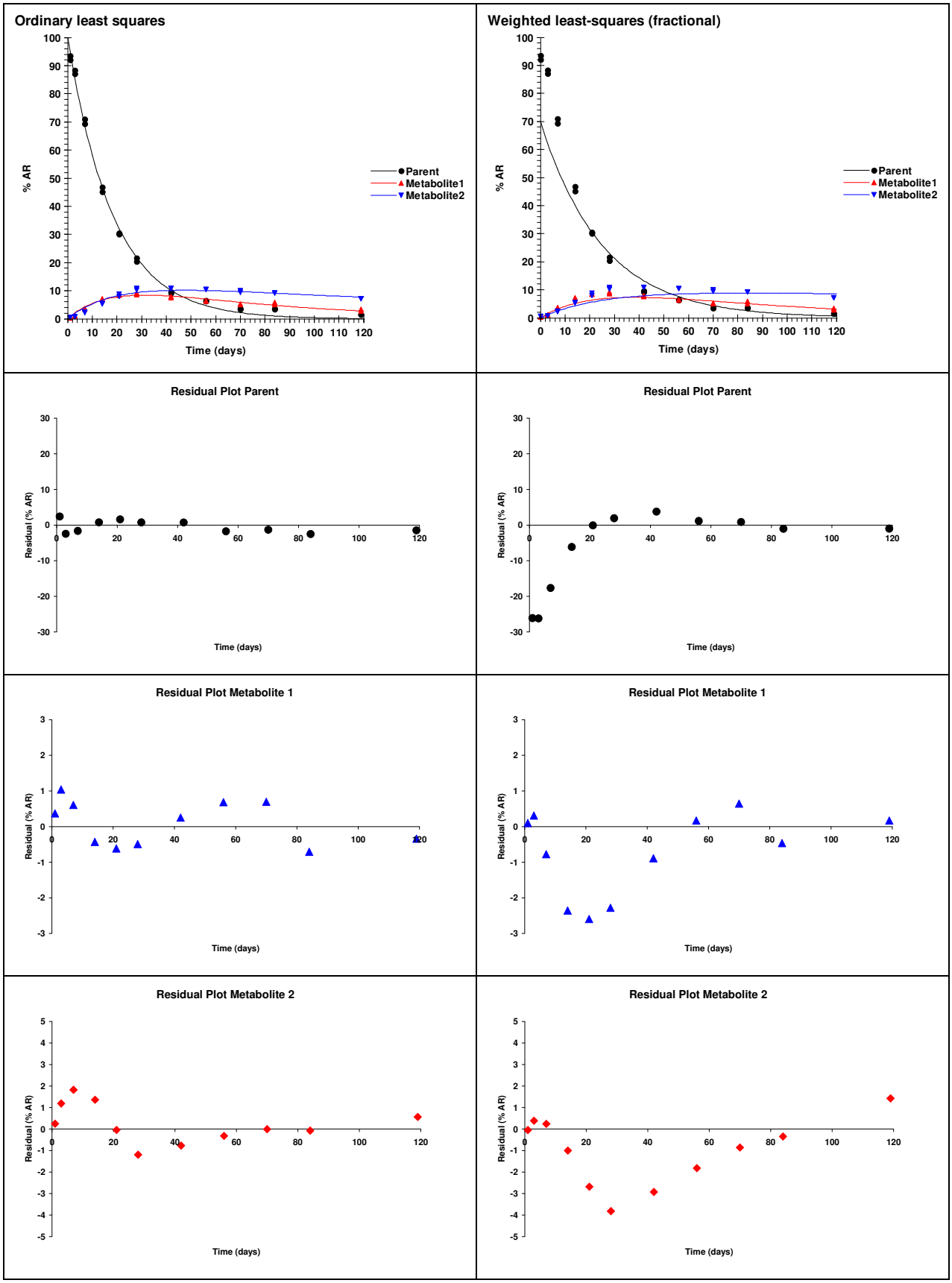


Figure 8-3. Description of the observed data for parent and metabolites from Example 8-3 with parent and metabolites all-SFO, fitted with ordinary least-squares (unweighted fit, left) or weighted least-squares with fractional error (right), with corresponding residual plots.

8.3.6 Use of sink data

Considering that the number of data points strongly impacts the quality of a parameter estimation, including the sink data in the fitting procedure may be desirable to increase the number of degrees of freedom in the model and improve the overall fit and reliability of the estimated parameters. However, fitting the sink data in addition to the parent and metabolite(s) will also introduce in the overall fit the experimental error associated to this data. This error can be very appreciable considering that the sink actually consists of a number of different fractions that are sometimes difficult to measure accurately (especially with regard to minor unidentified metabolites, bound residues and CO_2) and that the error would be additive. Non-closure of the mass balance in the input data may force the model to unrealistic results. Even small variations in the overall mass balance recoveries can have a considerable impact on the fitting of metabolites, especially considering the unweighted fitting method. Indeed, if the sink data values are higher than the metabolite values, the sink data points in an unweighted fit would in effect carry more weight than the metabolite of interest (see example with minor metabolite in section 8.4.5.1). Therefore, fitting the sink data is not recommended initially (the model is formulated with a sink compartment and flows from all substances to the sink; there are no experimental data associated with the sink compartment). The sink data may only be included in refined fits, and only if a complete mass balance is provided in the study and the total recovery remains constant (and relatively close to 100%) throughout the study.

8.4 Recommended procedure to derive metabolites endpoints

8.4.1 Stepwise approach

In many cases simultaneous fitting of all substances will be feasible even for complex reactions schemes. However, if simultaneous fitting results in equivocal estimates, in cases where the pathway is not fully defined with regards to the formation of minor metabolites and bound residues, in cases where non-SFO kinetic models are considered, or for data sets with scattered or limited data points, a stepwise approach might be preferred. In a sequential fit, compartments/substances are gradually added to the model and the parameters for the newly added substance are fitted while the parameters for the other substances (previously fitted) are fixed to their estimated value. In a last step, all parameters may be fitted simultaneously, using their previously estimated value as initial value (this allows to evaluate the potential correlation between parameters by generating a complete correlation matrix). In

any case, the procedure followed needs to be clearly recorded and the results easily reproducible.

Detailed guidance on the implementation of the stepwise approach in compartment models is provided below and illustrated in Figure 8-4. An illustration of the approach with an example data set of a parent substance with three successive metabolites is presented in Appendix 7.

Step 1/ The degradation rate of parent substance is estimated with a two-compartment model (parent and sink compartments). The model is fitted to the observed data of the parent substance. This step should be performed following the general recommendations provided in the general/parent section, and according to the desired endpoint (best-fit for trigger DT50 or PEC_s calculation versus SFO and other kinetic models that can be implemented in the environmental models). The estimates of the degradation parameters and initial amount of substance if estimated need to be statistically reliable in order to proceed to step 2 (the correct description of the parent is a prerequisite for a correct description of the metabolites).

Step 2/ The first metabolite is included in the model by adding a compartment. If the parent substance degrades to several metabolites, these should be all added in as many compartments. The initial amount of parent substance is fixed to the value estimated in step 1, and the degradation flow is split between flow(s) to the metabolite(s) formed and flow to the sink compartment to account for the formation of minor metabolites and incorporation in the soil matrix. When dealing with SFO kinetics, the rate of degradation of the parent substance estimated in step 1 is split between the metabolite and sink compartment(s). A condition could be included in the model setting the sum of the different rates equal to the overall rate estimated in step 1. Alternatively, or if kinetics other than SFO are used for the parent substance, the model formulation with formation fractions may be used (see Section 8.3.4.3). This approach allows the result of the previous step estimation of the overall degradation rate of the parent to be used as the initial value. The degradation of the metabolite(s) to the sink is described with the appropriate kinetics (initially SFO, but FOMC, DFOP or other bi-phasic kinetics may be required depending on the SFO results and type of endpoints needed). The model is fitted to the observed data of the parent substance and metabolite(s). The parameters to be estimated in step 2 are the metabolites formation (individual formation rates or formation fraction) and degradation parameters. The flow to the sink may be removed at this point if the estimated value of the degradation rate of the parent substance to the sink is negative or not significant, or if the estimated formation fraction(s) indicates that there is no significant flow to the sink (upper constraint of 1 violated, or

estimate close to 1), and a new simulation performed. The elimination of flow to the sink should always be in accordance with the degradation pathway and therefore should be justified based on knowledge of the chemical reaction(s) involved and weight of evidence from other studies. If the goodness of the fit is not satisfactory, or statistically non-significant (no confidence or very low confidence level) or unrealistic (e.g. negative) estimates are obtained for the parameters, the compartment model / pathway may need to be modified.

Step 3/ All parameters in the compartment model used in step 2 are optimised with starting (initial) values set to the estimates obtained in step 1 (for initial amount and degradation rate of parent substance) and step 2 (formation fractions and degradation rates of metabolite(s)). This step is useful in identifying possible correlation between parameters.

Step 4/ Metabolite(s) formed from the first metabolite(s) are added to the model. The procedure is identical to the procedure described in step 2. Flows of the first metabolite(s) to the sink should be initially included and may be later removed depending on the optimisation results. The new parameters for the formation and degradation of the added metabolite(s) are optimised while the other parameters are fixed to the values estimated in step 3.

Step 5/ All parameters in the compartment model in step 4 are optimised with starting (initial) values set to the estimates obtained in step 3 and 4.

This stepwise approach is continued until all metabolites are included. A final optimisation is then conducted with all parameters with initial values set to the estimates obtained in the previous two steps (same procedure as described in steps 3 and 5).

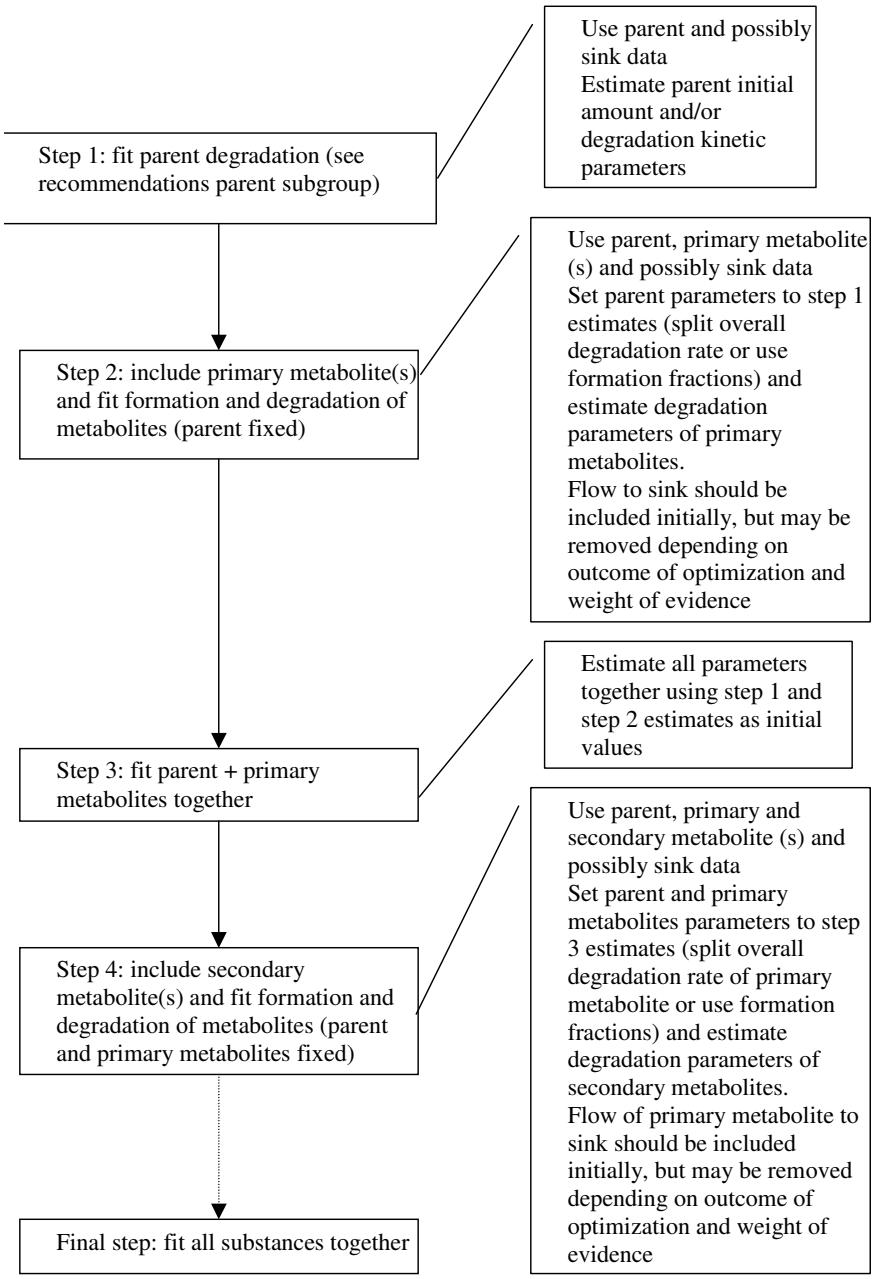


Figure 8-4. Recommended stepwise approach for complex models including metabolites

For complex models (considering the pathway and type of kinetics involved), the stepwise approach can be particularly useful in helping to identify the most accurate kinetic model at each step, starting with the parent substance, so that the metabolites can be described in the best way. This step-by-step approach may also be helpful in confirming the conceptual model or obtaining a more appropriate model for the data, as it can be used to identify if and where compartment and/or flows (e.g. to sink) need to be added or deleted. Finally, for software tools that are very sensitive to the starting values of the parameters to be fitted (e.g. ModelMaker), a step-by-step approach should help provide better starting values, and may be the only possible way to reach a realistic solution.

8.4.2 Metabolites decision flow charts

8.4.2.1 Derivation of metabolite endpoints for pesticide fate modelling

The recommended procedure to derive modelling endpoints for metabolites is presented schematically in Figure 8-5. This approach is only valid if the parent kinetics are SFO or bi-phasic. If the parent degradation exhibits a lag-phase, the kinetic evaluation should be performed for the metabolites disregarding the data points in the lag-phase, and moving the time 0 to the start of the parent decline. A separate evaluation and modelling is then needed for the parent alone, following the recommendation in Section 7.2.

① As a first step, the parent substance should be fitted with SFO as outlined in Section 7.1.2 to determine if SFO kinetics are appropriate for description of the degradation of the parent / formation of the first metabolites. The SFO model is deemed appropriate if the χ^2-test for goodness of fit yields an acceptable error value and the plot of residuals indicate no systematic error (see section 6.3.1). The SFO model is considered appropriate for modelling the metabolites if > 90% of the degradation of the parent (i.e. > 90% of the formation of the first metabolite(s) formed) is adequately described, as can be assessed visually and looking at the distribution of the residuals. While the initial fitting of the parent should be performed with Pini free, using all data and without weighting, the SFO fit can be refined stepwise by first excluding outliers, then constraining Pini and finally data weighting, provided these steps are justified by the experimental data, until best-fit is achieved. Guidance on these refinement steps is provided in Sections 6.1 and 7.1.

② If the SFO model is appropriate for the parent, the metabolites are then added to the model and the data are fitted with all-SFO, using the most appropriate conceptual model to describe the degradation pathway, and including use of a sequential/stepwise approach as recommended. The goodness of the fit for each metabolite is assessed with the χ^2 test and the distribution of the residuals (see Sections 6.3.1 and 8.4.3). The validity of the estimated rate constant parameters is assessed using the recommended t-test (see section 6.3), and if deemed acceptable, the SFO modelling endpoints (i.e. degradation rate of parent and formation fraction and degradation rate of metabolites) can be used for fate modelling. While the initial fitting of the metabolites should be performed using all data and without weighting, the fit can be refined stepwise by excluding outliers, and data weighting (of specific points or of complete metabolite series, based on available data on uncertainty associated with a specific data point or component), provided these steps are justified by the experimental data, until the best-fit is achieved. If in the final fit some parameters are not fully reliable or

cannot be estimated (e.g. when the decline phase of a metabolite is not reached or is not clearly defined during the experiment, or if the model is too complex compared to the data), a case-by-case decision (③) is necessary:

③ If both the formation fraction and degradation rate of the preceding substance(s) are reliable, but the degradation rate of a metabolite is not reliable although a decline can be observed, the degradation rate could be estimated separately from the decline curve. This provides a conservative estimate of the degradation rate. If a reliable decline rate can still not be obtained, the degradation rate could be set to a conservative default value (e.g. corresponding to a DT50 of 1000 days).

If the degradation rate of the preceding substance(s) is reliable, but the formation fraction of the metabolite and its degradation rate are not, the formation fraction may be set conservatively to 1 (unless other metabolites are formed from the same predecessor, in which case it would be 1-formation fraction(s) of the other metabolite(s)), and used in combination with a conservative estimate of the degradation rate, from the decline curve or using a conservative default value.

If there is a clear overestimation of observed metabolite residues using the default assumptions of formation fraction of 1 and DT50 of 1000 days, alternative -but conservative- estimates should be allowed that better describe the observed patterns. The worst-case nature of the selected estimates for the study of interest should always be discussed in details, and compared to available information from other studies for weight of evidence.

If none of the endpoints are reliable for a particular metabolite, the conceptual model may not be appropriate and would then need to be revised, or the experimental data simply does not support the fitting of this metabolite (see section on data quality), and the metabolite should be removed from the fit.

In case of a bi-phasic degradation pattern of a metabolite, higher-tier approaches for this metabolite may be used similar to what is proposed for the parent substance in section 7.1.2. For example, the metabolite may be described/fitted with DFOP and implemented with the same approach in the environmental model, or, in the case of a terminal metabolite that can be described/fitted with a bi-phasic model, a half-life may be calculated from the bi-phasic DT90 divided by 3.32.

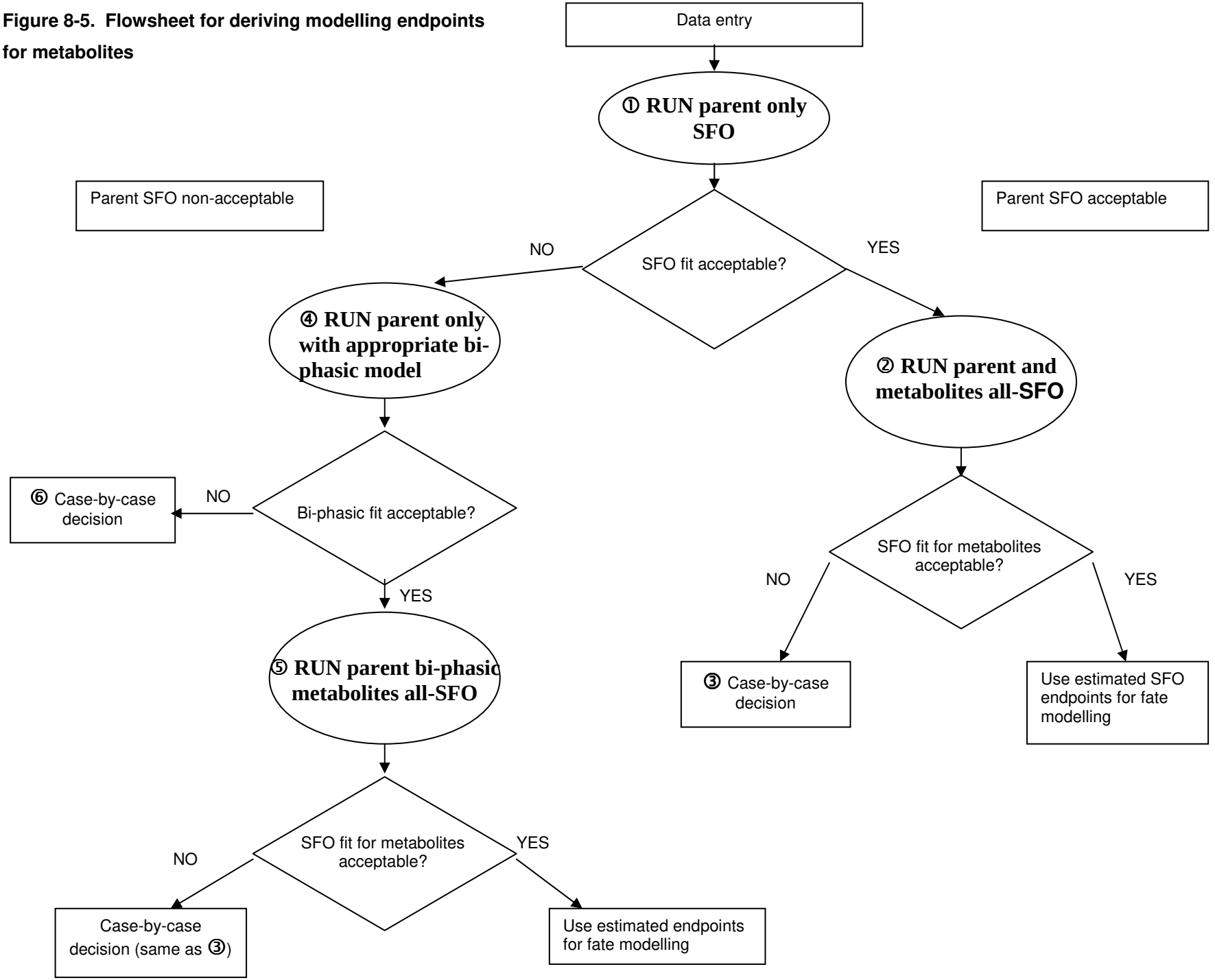
④ If the SFO model is not appropriate for the parent, and the FOMC model is shown to be more appropriate as outlined in section 6.3.1 (indicating a bi-phasic degradation pattern), the parent should then be fitted with an appropriate non-SFO model that may be implemented in environmental models, as recommended in Section 7.1.2. The option of back-calculating a half-life from a bi-phasic DT90 is limited to modelling of the parent alone, and is not appropriate for deriving the kinetic endpoints of metabolites. The bi-phasic model is deemed appropriate if the goodness of fit criteria(s), i.e. χ^2 error and random distribution of residuals, are met and the validity of the estimated bi-phasic rate constant parameters has been checked using the t-test. While the initial fitting should be performed with Pini free, using all data and without weighting, the bi-phasic fit can be refined stepwise by excluding outliers, constraining Pini, and data weighting, provided these steps are justified by the experimental data, until best-fit is achieved. If the experimental data do not show significant degradation in the second phase, the rate constant for the second phase of the HS model or slow phase of the DFOP model could be set to a conservative default (e.g. 0.0007 d^{-1} corresponding to a DT50 of 1000 d).

⑤ If an acceptable bi-phasic fit can be obtained for the parent, the metabolites are then added to the model and the data are fitted with metabolites (all with SFO kinetics), using the most appropriate degradation pathway, and including use of a stepwise approach as recommended. The goodness of the fit for each metabolite is assessed with the χ^2 test and the distribution of the residuals (see sections 6.3.1 and 8.4.3). The validity of the estimated parameters is assessed using the recommended t-test (see section 6.3), and if deemed acceptable, the parameters can be used with the appropriate environmental model (depending on selected bi-phasic approach for the parent). While the initial fitting should be performed using all data and without weighting, the fit can be refined stepwise by excluding outliers, and data weighting (of specific points or of complete metabolite series), provided these steps are justified by the experimental data, until the best-fit is achieved. If in the final fit some parameters are not fully reliable or cannot be estimated (e.g. when decline phase is not reached during the experiment or is not clearly defined, or if the model is too complex), a case-by-case decision as detailed in ③ is necessary.

⑥ If the bi-phasic approaches that can be implemented in the environmental models are not appropriate for the parent, as assessed with the recommended statistical indices and visual assessment, the experimental data simply may not support the fitting, for example because of excessive scattering of the data. Case-by-case decisions need to be made on the metabolites depending on the available data for each metabolite, and on their potential

relevancy. Conservative degradation endpoints may be derived by fitting the decline curve of the metabolite from its observed maximum, and modelling of the metabolites may be performed likewise. In case no bi-phasic approach can be implemented in the environmental model, the modelling of metabolites may be performed based on the decline curve from the maximum. Another pragmatic approach may be to model the parent with HS or DFOP (whichever provides the best fit) and the metabolites all with SFO kinetics to derive the endpoints for modelling (the bi-phasic formation of the first metabolite(s) needs to be accounted so as to adequately determine the formation fractions and degradation rates). The modelling can then be performed using two sets of all SFO endpoints: 1/ first-order degradation rate of parent in the first phase of HS or fast compartment of DFOP, formation fraction and SFO degradation rate of metabolites, and 2/ first-order degradation rate of parent in the second phase of HS or slow compartment of DFOP, formation fraction and SFO degradation rate of metabolites. The highest concentrations of the two sets may then be used in the risk assessment.

Figure 8-5. Flowsheet for deriving modelling endpoints for metabolites



8.4.2.2 Derivation of metabolite endpoints for triggers and PEC_s calculations

The recommended procedure to derive trigger or PEC soil endpoints for metabolites is presented schematically in Figure 8-6. In both cases, parent and metabolites should be described with the best-fit model, i.e. SFO, FOMC or DFOP for parent, as recommended in parent Section 7.1.1, and SFO, FOMC, or DFOP for metabolites. One must note that when using differential equations to formulate the model (e.g. in compartment models), the FOMC kinetic model cannot be used and the bi-phasic kinetic model of choice is then DFOP. If the parent degradation exhibits a lag-phase, the kinetic evaluation for the metabolites should be performed disregarding the data points in the lag-phase, and moving the time 0 to the start of the parent decline. A separate kinetic evaluation is then needed for the parent alone, following the recommendation on lag-phase for parent (Section 7.2). The special case of lag-phase for metabolites is discussed in Section 8.5.5.

①As a first step, the parent substance should be fitted with SFO and FOMC models as outlined in Section 7.1.1 to determine if SFO are appropriate for description of the degradation of the parent (i.e. of the formation of the first metabolites), or if bi-phasic kinetics should be used. The fits are compared based on the χ^2 test and distribution of the residuals (see Section 6.3.1). While the initial fitting should be performed with Pini free, using all data and without weighting, the SFO fit can be refined stepwise by excluding outliers, constraining Pini and data weighting, provided these steps are justified by the experimental data, until best-fit is achieved.

② If the FOMC model is more appropriate than SFO, the parent is then fitted with the bi-exponential (DFOP) model to determine if a bi-phasic model is acceptable, and if so, which model, either DFOP or FOMC, can be considered best-fit for the parent.

③ If neither of the bi-phasic models is appropriate for the parent, the experimental data simply may not support the fitting, e.g. because of excessive scattering of the data for the parent. Case-by-case decisions need to be made for the metabolites at this point. If a clear decline phase of the metabolite can be observed, conservative degradation endpoints may be obtained by fitting the decline curve of the metabolite from its observed maximum. In such case, the PEC_s need to be calculated likewise.

④ Once the best-fit model for the parent has been determined, the metabolites are then added to the model and the data are initially fitted with all-SFO kinetics, using the most

appropriate conceptual model to describe the degradation pathway, following the guidance provided in Section 8.3.2, and if necessary including use of a sequential/stepwise approach as recommended in Section 8.4.1. Performing the kinetic evaluation for the metabolites in a stepwise approach allows for checking the adequacy of the SFO model at each new step, for each additional metabolite(s) added. The SFO model is deemed appropriate if the χ^2-test for goodness of fit yields an acceptable error value, the plot of residuals indicate no systematic error, and all parameter estimates are deemed reliable (see Section 6.3.1). A shift in the peak between observed and fitted values and tailing, which would result in the residual plot in a systematic error around the maximum and at the later time points, are indications that SFO kinetics may not be appropriate for the metabolite.

⑤ If the SFO fit of a metabolite is not satisfactory, the metabolite should then be fitted with DFOP or FOMC kinetics as described in the metabolite general Section 8.3.3.2. The bi-phasic fit is deemed appropriate if the χ^2-test for goodness of fit yields an acceptable error value, the plot of residuals indicates no systematic error, and all parameter estimates are deemed reliable (see Section 6.3.1).

While at each step the initial fitting should be performed using all data and without weighting, the fit can be refined stepwise by excluding outliers, and data weighting (of specific points or of complete metabolite series), provided these steps are justified by the experimental data, until best-fit is achieved. Once the best-fit model has been determined for each metabolite, the metabolite trigger endpoints (DT50 and DT90 values) or PEC soil endpoints (formation rate parameters, formation fraction and degradation rate parameters) are obtained from the final fit of the stepwise approach, with all parameters estimated together. If in the final fit some parameters are not fully reliable or cannot be estimated, a case-by-case decision (⑥) is necessary:

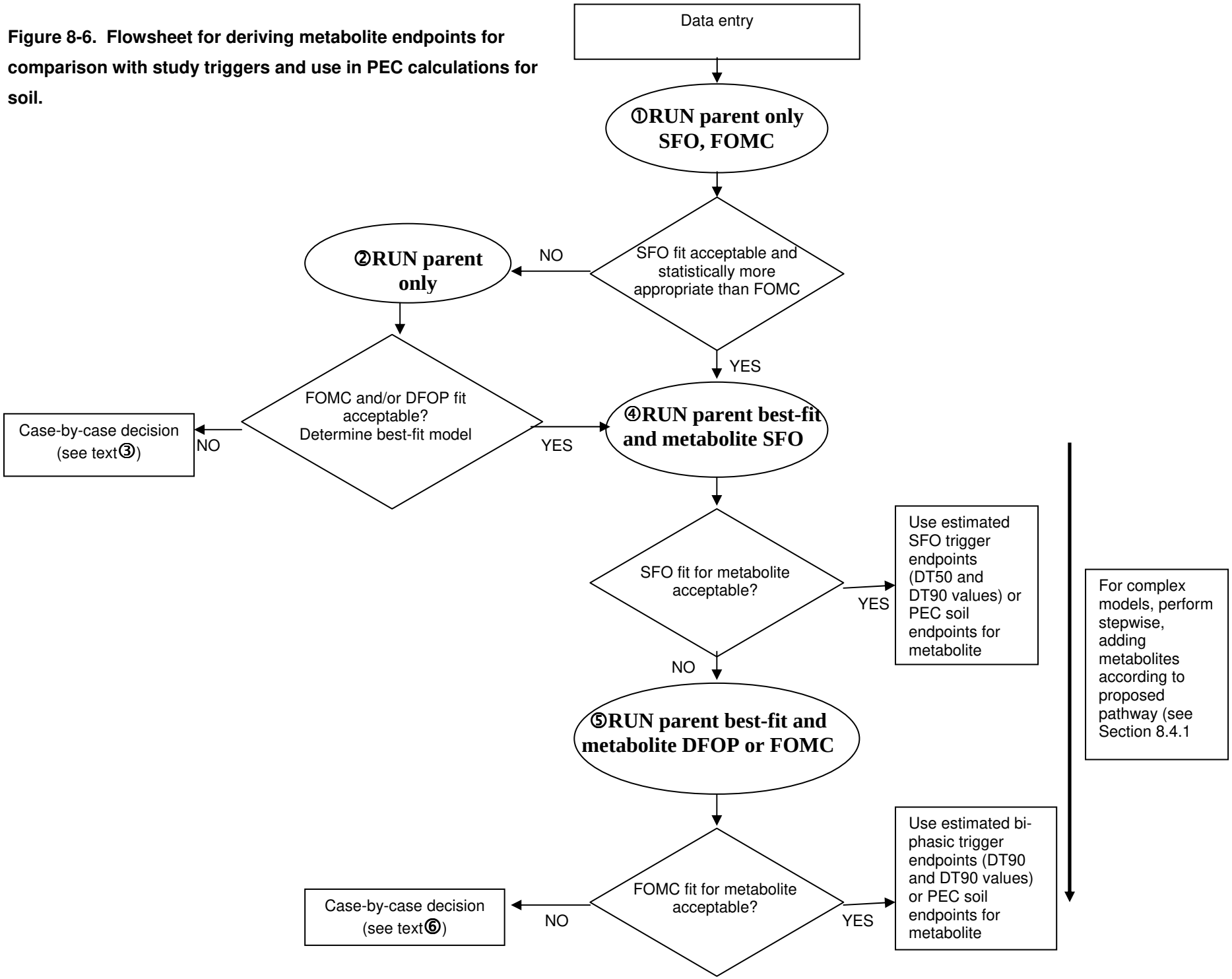
⑥: <u>Trigger endpoints:</u>

- If the degradation parameters of a metabolite are not reliable while a decline can be observed, the DT values could be estimated separately from the decline curve of this metabolite, from the maximum observed and onward (this will provide conservative estimates of the DT values)
- If the degradation rate of a metabolite is not reliable and no decline can be observed, trigger values may not be obtained for this metabolite from the study with the parent substance.

⑥: PEC soil endpoints:

- If both the formation fraction and degradation parameters (e.g. rate constant if SFO) of the preceding substance(s) are reliable, but the degradation parameters of a metabolite are not reliable while a decline can be observed, the degradation parameters of the metabolite could be estimated separately from its decline curve. This provides a conservative estimate of the degradation rate. If the degradation rate could still not be estimated, the degradation rate could be set to a conservative default value (e.g. corresponding to a generic conservative DT50 of 1000 days), or corresponding to a conservative yet more realistic DT50 for the particular substance, based on available information from other studies. Unless the generic value of 1000 days is used, the worst-case nature of the selected estimate should always be discussed in detail.
- If only the degradation parameters of the preceding substance(s) are reliable, the formation fraction of the metabolite may be set to an absolute worst case of 1 (unless another metabolite is formed from the same predecessor, in which case it would be 1-formation fraction of the other metabolite(s)) and used in combination with a conservative estimate of the degradation rate, from decline curve if there is an observable decline, or using a conservative default value. If there is a clear overestimation of observed metabolite residues using the default assumptions of formation fraction of 1 and DT50 of 1000 days, alternative -but conservative- estimates should be allowed that better describe the observed patterns. The worst-case nature of the selected estimates for the study of interest should always be discussed in details, and compared to available information from other studies for weight of evidence.
- If none of the endpoints are reliable for a particular metabolite, the conceptual model may not be appropriate and would then need to be revised, or the experimental data simply does not support the fitting of this metabolite, and the metabolite should be removed from the fit (see section on data quality/requirements).

Figure 8-6. Flowsheet for deriving metabolite endpoints for comparison with study triggers and use in PEC calculations for soil.



8.4.2.3 Experimental artefacts

As discussed in general Section 6.1.7 on experimental artefacts, when facing a bi-phasic degradation pattern for a substance, the reason why degradation kinetics diverge from SFO kinetics should be determined, at least tentatively. The bi-phasic degradation pattern may result from a number of experimental artefacts (see Table 6-3), which should not be accounted in the determination of kinetic endpoints.

8.4.2.3.1 Experimental artefacts affecting the parent substance

In general, experimental artefacts can be assumed to impact all substances present at the time of the artefact. A loss of microbial activity during the study would affect the degradation of the parent substance but also that of all metabolites formed at the time of the soil "dying-off" as long as all degradation steps are microbially mediated. Hence, time points affected by experimental artefacts should be eliminated for all substances before carrying-on the parameter estimation. However, in some cases, to be addressed on a case-by-case basis, kinetic endpoints for metabolites may still be derived from data sets affected by experimental artefacts, if it can be shown that these endpoints can be considered conservative, or that the metabolite would not be affected by the experimental artefact (for example, a change in pH or extraction method may not affect all substances). In such cases, the decrease in the degradation rate of the parent substance would still need to be described with the most appropriate bi-phasic kinetic model in order to determine the correct formation and degradation kinetics for subsequent substances, and while the derived parent endpoints would not be valid and may need to be estimated separately according to the guidance in 6.1.7, the endpoints for metabolites may be considered valid or even conservative.

8.4.2.3.2 Experimental artefacts affecting metabolites only

A loss of or decline in microbial activity is more likely to occur at the later sampling times of a study, and may therefore occur after the parent substance has already fully degraded and affect the degradation of metabolites only, especially of terminal metabolites measured at the later stages of a study. The reason why degradation kinetics of metabolites diverge from SFO kinetics should be determined, at least tentatively. However, the impact of experimental artefacts on metabolites may be difficult to address, as for metabolites there should not be two major phases with a distinct breakpoint time since there is continuous formation. The DFOP model may be employed in this case, to try and extract the normal degradation of the metabolite (fast compartment) from experimental artefacts (slow compartment). This approach should be restricted to obvious cases of bi-phasic degradation attributed to experimental artefacts (e.g. in cases, where the microbial activity has decreased during the laboratory experiment).

8.4.3 ***Goodness of fit***

The same methods recommended for evaluating the goodness of fit for the parent substance (section 6.3.1) are also applicable to metabolites. The work group felt that the goodness of fit should be performed for each compartment separately. While it is true that the data on the formation of metabolite is linked to the degradation of the parent and may therefore contain supportive information for the parent, examining the overall fit to all species is inconclusive with regards to the individual species. In the overall fit to all species, the species with the highest measured levels would carry more weight than species at lower level, and as a result an overall fit may still appear acceptable while one or more of the individual species may not be well fitted.

The visual assessment is the main tool for assessing goodness of fit. The plots of residuals should be used to determine if the residuals are randomly distributed or whether any systematic error is apparent during the formation, maximum or decline of the metabolite, which would indicate that the pathway or kinetic model used for parent or metabolite is maybe not appropriate.

The χ^2 test is recommended as a tool for model comparison and as a supplementary tool for assessing the goodness of fit of an individual model. The χ^2 error value should be calculated for each metabolite using all data used in the fit (after averaging), including the sampling points below LOD or LOQ before the formation phase and after the decline phase that are included as ½ LOD or ½ (LOQ+LOD). The time-0 sample however, if set to 0 should not be used in the χ^2 error determination. Since the χ^2 statistics are calculated separately for each substance, parent and each individual metabolite fitted, only the parameters specific to the metabolite are considered in the metabolite χ^2 calculation. These are the formation fraction and degradation parameters of the metabolite, while the degradation rate of the precursor(s) are only considered for the precursor χ^2 calculation. The number of model parameters for selected model fits is given in Table 8-5. Ideally, the error value at which the χ^2-test is passed for the metabolite should be below 15%, like for parent substance, and the fit must be visually acceptable. However, this value should only be considered as guidance and not absolute cut-off criterion. There will be cases where the error value to pass the χ^2-test for a metabolite is higher, but the fit still represents a reasonable description of its formation and degradation behaviour.

Table 8-5. Number of model parameters for selected kinetic model fits.

Kinetic model	Number of model parameters	Fitted parameters
SFO	1	kM, (ffM fixed to 1)
SFO	2	kM, ffM
FOMC	2	αM, βM (ffM fixed to 1)
FOMC	3	αM, βM, ffM
DFOP	3	g, k1M, k2M, (ffM fixed to 1)
DFOP	4	g,k1M, k2M, ffM

In addition to these goodness of fit indices, the reliability of the individual rate parameter estimates needs to be evaluated as outlined in Section 6.3, based on the results of the t-test or confidence intervals of the parameters. This is particularly important for metabolites that do not show a clear decline, to discern between metabolites that are persistent and metabolites that are degrading and forming at the same time at a similar rate. Note that to calculate the t-test for the individual parameter, the total degrees of freedom are used, which depends on the total number of parameters estimated in the fit, as opposed to the metabolite parameters only as used for the χ^2 calculation. Whenever fits are performed with the stepwise approach, the reliability of the individual parameters needs to be assessed at the final step, when all parameters are estimated at once, which is when the degrees of freedom will be the lowest and the uncertainty of the estimated parameters should be the greatest.

The χ^2 statistics, plots of residuals, and t-test of all individual rate constant parameters were performed and discussed for parent and all metabolites in the examples in Chapter 8 and Appendices 7 and 8. Parameters for which the calculated t-value (single-sided) was greater than the significance level of 5 percent are highlighted, indicating that the parameter is not significantly different from zero (in cases where the probability is between 0.05 to 0.10, the. parameter may still be considered acceptable, however further discussion and justification is then necessary). All other parameters showed a probability lower than the significance level. These examples clearly show that unreliable parameters can still be obtained while χ^2 statistics and plots of residuals indicate a good fit, and vice-versa, the t-test may be passed for all parameters while the χ^2 statistics and/or plots of residuals indicate an unacceptable fit. As a general rule, all statistical indices, χ^2 statistics, plots of residuals, and t-test of individual rate constant parameters would need to be addressed in order to accept metabolites endpoints as fully reliable. However, on a case-by-case basis, the metabolite endpoints may still be considered acceptable even though one or more of the indices are not met, as long as

the endpoint value can be considered conservative, or can be justified based on weight of evidence from other studies.

8.5 Special cases

8.5.1 Minor metabolites

This section concerns metabolites that are observed at levels lower than 10% of the applied parent throughout the study. Depending on the quality of the data, deriving reliable kinetic endpoints may still be possible for minor metabolites, especially if there is a clear formation and decline pattern with enough data points. Alternatively, an estimate of the degradation of minor metabolites can be obtained by fitting the decline curve of the metabolite from its observed maximum.

Because of the low levels observed, the relative experimental error for minor metabolites may be higher than for the parent or major metabolites, which would affect the kinetic evaluation. The uncertainty in the measurements depends on the LOQ, LOD and overall precision of the method with regards to the metabolite of interest (e.g. quality of peaks in HPLC). Losses of mass balance with time or high variations in mass balance may also have more impact on the kinetic evaluation of minor metabolites compared to other substances, especially if the minor metabolites are observed at later times, unless the losses can be attributed to specific recovery deficiencies that would not affect the metabolite (e.g. inefficient CO_2 or other volatile trapping, losses during combustion of bound residues). The study LOQ/LOD, mass balance, and any scattering of the data should be discussed in details with regards to the minor metabolites prior to conducting their kinetic evaluation.

Unweighted fits naturally give more weight to the points with the highest concentration/levels, and minor metabolites fitted together with major metabolites and/or the parent substance will be less precise. Therefore, whenever possible, fitting minor metabolites together with high-level metabolites carrying more weight should be avoided. Obviously, the preceding metabolite or parent still needs to be included to be able to estimate formation fraction, but sub-models may be created, which only include the portion of the degradation pathway that is pertinent to the formation of the minor metabolite of interest. The use of a stepwise procedure (see Section 8.4.4) is also recommended, so as to permit the estimation of parameters specific to the minor metabolite while all other parameters for the metabolite precursor(s) set to the values estimated in the previous step(s). The sink data should not be

used in the fit of minor metabolites, as it would in most cases carry more weight than the minor metabolite (see Example 8-4).

The degradation pathway leading to the minor metabolite of interest should be clearly defined/understood to be able to derive reliable kinetic endpoints. The pathway to minor metabolites can be unclear, in which case a kinetic evaluation of their formation and degradation may not be possible.

Example 8-4

The example below illustrates the potential error introduced when including the sink data with a minor metabolite. In this example, the parent substance degraded to one minor metabolite (maximum level of 7% of applied), and other metabolites and/or bound residues. The data were described with the same conceptual model as in Box 8-1, with all-SFO kinetics. Note that in this example, the initial amount of parent substance at time 0 was fixed to 100 because no data were available for the time-0. This is considered a modified fitting routine as the initial fit should be performed with Pini included in the parameter optimisation procedure.

The parameter estimation results and calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the fits in ModelMaker 4.0, including or not the sink data, are listed in Table 8-6, and the description of the observed data for parent and metabolite with the model are shown in Figure 8-7. The degradation of the parent substance and formation and degradation of the minor metabolite, can be modelled with great accuracy with SFO kinetics if the sink data are not included in the fit (top figures). The estimated parameters are reliable and the kinetic endpoints for modelling and triggers may be derived with confidence for this metabolite. However, when including the sink data in the fit, the degradation of the metabolite is not properly described, as reflected in the high χ^2 error value of 33 obtained, and systematic error in the residuals (bottom figures), and the estimated degradation rate constant may not be reliable. In this case, the fitting of the sink data, which amounts for levels above 10 times that of the minor metabolites, and introduced additional error due to small decline in overall mass balance recovery, carries much higher weight than the metabolite. As a result, the degradation of the metabolite is grossly underestimated, and an unrealistic long DT50 would be calculated.

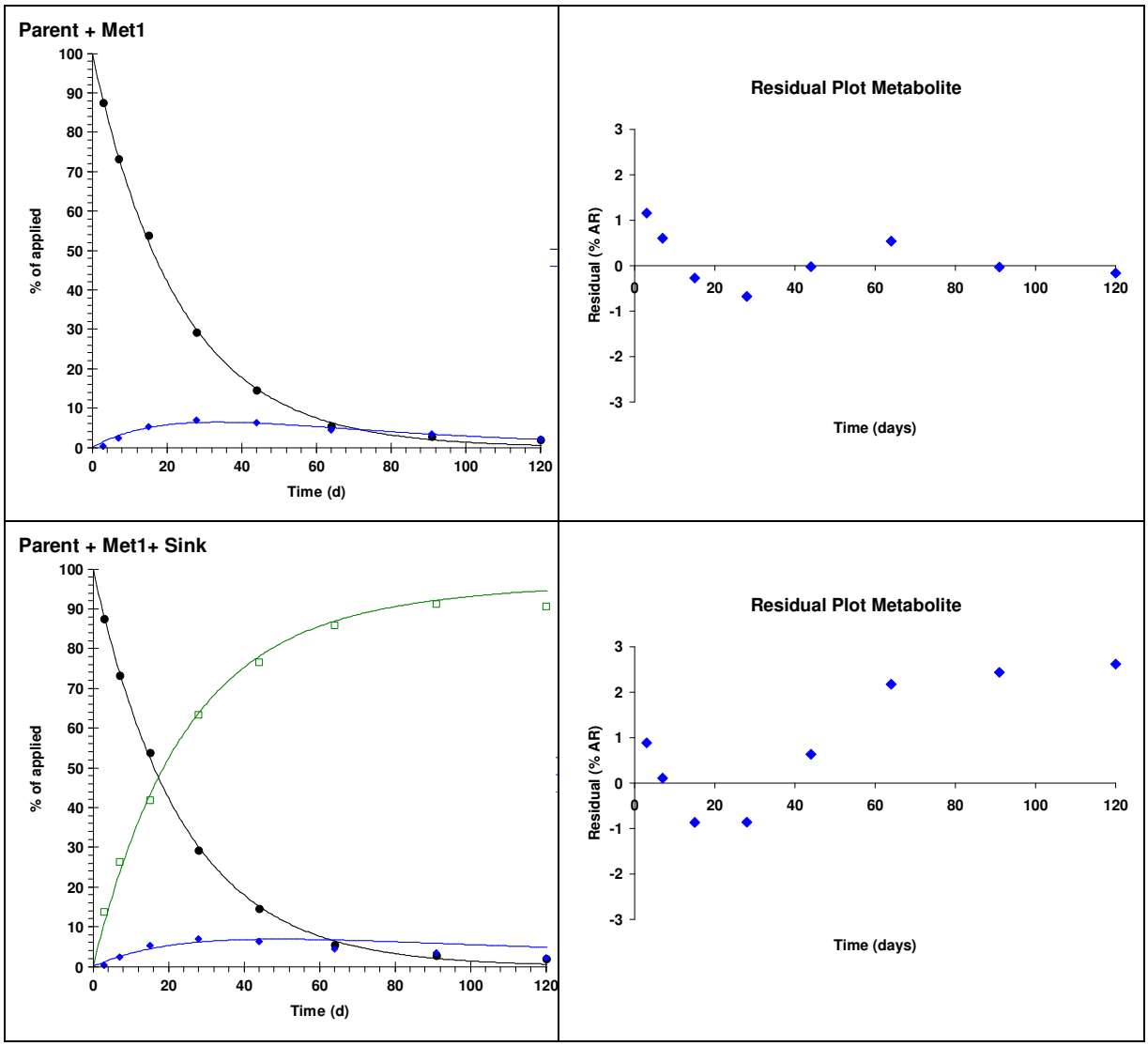


Figure 8-7. Description of the observed data for parent and metabolite from Example 8.-4 with corresponding residual plots for the metabolite, all-SFO fits performed without using the sink data (top), or including the sink data (bottom).

Table 8-6. Results of the fits of the parent and metabolite in example 8-4, without and with the sink data included

	Sink data not fitted	Sink data fitted
Parameter (estimate ± standard error)		
kP (1/d)	0.0432±0.0006	0.0428±0.0009
ffM1 (-)	0.1234±0.0144	0.0994±0.0164
kM1 (1/d)	0.0195±0.0038	0.0075±0.0033
Goodness of fit (χ^2 error)		
Parent	3	3
Metabolite	12	33
Sink	-	3
Kinetic endpoints		
DT50 parent (d)	16.1	16.2
DT90 parent (d)	53.4	53.8
DT50 metabolite (d)	35.5	92.2
DT90 metabolite (d)	118	306

Example 8-5

The example below illustrates the potential error in the parameter estimation for a minor metabolite resulting from uncertainty of measurements. The measured levels of the example minor metabolite (measured maximum of 7.5% of applied) are fairly scattered, suggesting high relative uncertainty/experimental error for the metabolite (see Figure 8-8). The data were described with the same conceptual model as in Box 8-1, with all-SFO kinetics.

The parameter estimation results and calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the fits in ModelMaker 4.0, for the parent and metabolite, and for the decline of the metabolite from its maximum observed level, are listed in Table 8-7, and the description of the observed data for parent and metabolite with the model is shown in Figure 8-8. The degradation of the parent substance and formation and decline of the metabolite can be reasonably well described with a SFO model. However, the uncertainty associated with the estimate of the degradation rate constant of the metabolite is very high. The t-test indicates that kM1 is not significantly different from zero, and the degradation DT50 for the metabolite may not be considered reliable. A fit of the decline curve of the metabolite from the maximum gives a reliable estimate of the disappearance rate constant that suggests faster degradation. The DT50 calculated from the fit with the parent, although unreliable, may be considered conservative and kept as such. Alternatively, the DT50 estimated from the decline curve, which can also be considered conservative as a disappearance rate including both formation and degradation, and which is calculated from a reliable parameter estimate, may be used as the endpoint.

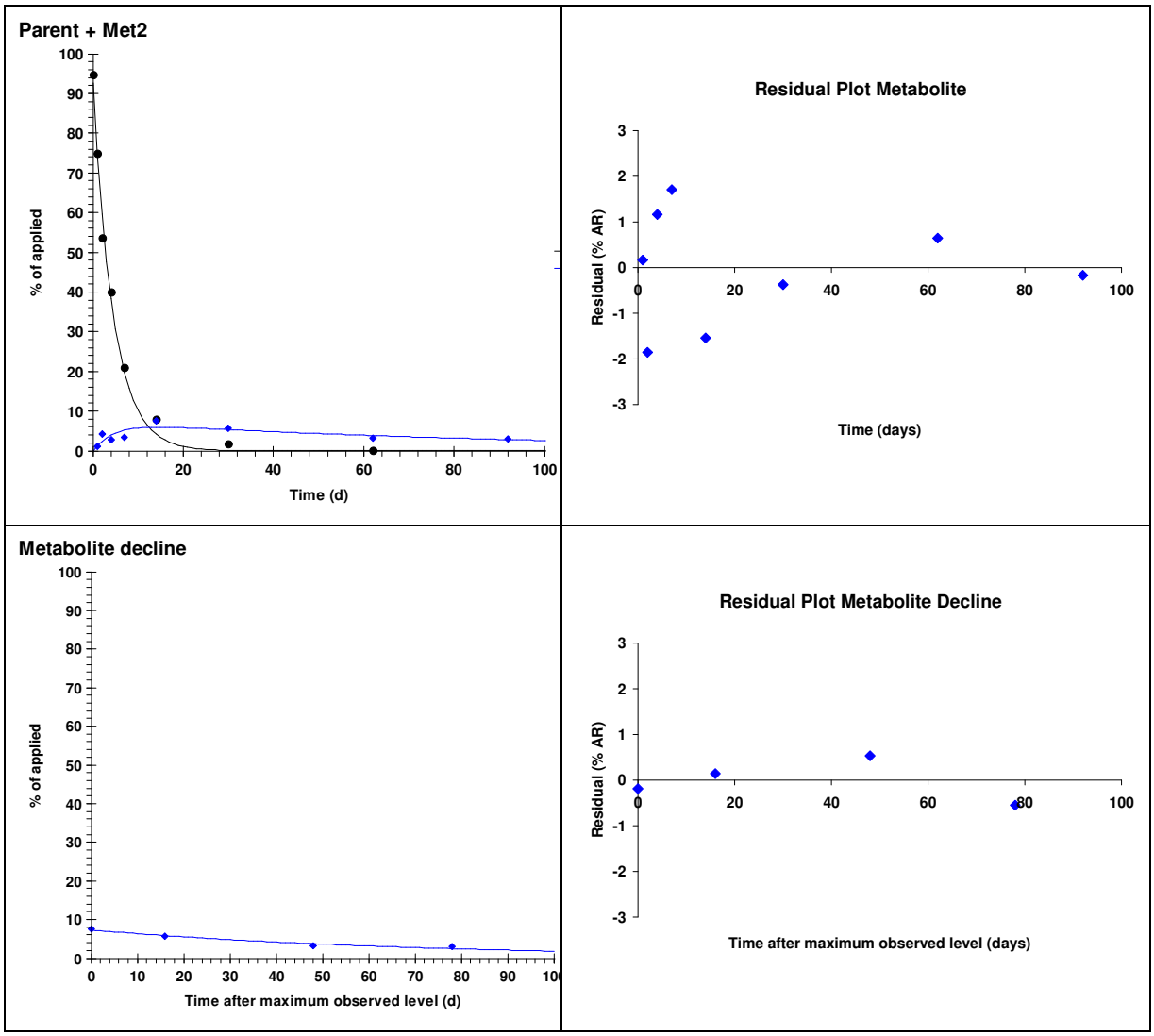


Figure 8-8. Description of the observed data for parent and metabolite from Example 8-5 with corresponding residual plots for the metabolite, all-SFO fits performed for parent and metabolite (top), and for the decline of the metabolite from the maximum observed (bottom).

Table 8-7. Results of the fits of the parent and metabolite, and of the metabolite decline from the maximum, in Example 8-5.

	Parent and metabolite	Metabolite decline
Parameter (estimate ± standard error)		
Pini (% AR)	92.6 ± 2.0	-
kP (1/d)	0.2205 ± 0.0118	-
ffM2	0.0747 ± 0.0206	-
kM2 (degradation) (1/d)	**0.0102 ± 0.0088**[①]	-
kM2 (decline) (1/d)	-	0.0140 ± 0.0025
Goodness of fit (χ^2 error)		
Parent	6	-
Metabolite	24	7
Kinetic endpoints		
DT50 parent (d)	3.14	-
DT90 parent (d)	10.4	-
DT50 metabolite (d)	**67.8***	49.5
DT90 metabolite (d)	**225***	165

[①]The probability corresponding to the calculated t-value for the highlighted parameter is above the significance level of 5% (0.134), indicating that the parameter is not significantly different from zero.
*Because of lack of confidence in the degradation rate constant parameter estimate of M2, the DT50 and DT90 values calculated from this parameter may not be reliable.

8.5.2 Transient metabolites

Transient metabolites relate to very rapidly degrading, unstable metabolites that are intermediary in the metabolic pathway. Depending on their formation rate, these metabolites may be observed at very low levels throughout the study or may be only observed at a few successive time points, potentially at high levels. Reliable kinetic endpoints may be difficult to obtain for these transient metabolites. In the first case (slow formation), the formation and decline phases of the transient metabolite may not be well defined from the measured data, if available, because of the low observed levels. Because of the low observed levels, transient metabolites are not always identified or reported in the study, in which case a ghost compartment may need to be implemented in the model (see Section 8.5.4). In the second case (rapid formation), the transient metabolite may be observed as a pulse, in which case there may not be enough data points available to provide a correct kinetic evaluation. Furthermore, the actual maximum of the metabolite may occur between sampling times and thus not be measured. In both cases, even if the transient metabolite may not be environmentally relevant due to its instability in the system, a correct description of its kinetics of formation and degradation is still necessary for a correct kinetic evaluation of the metabolites further in the degradation route.

The kinetic endpoints for a transient metabolite may not always be estimated accurately based on the data alone, because of potential high correlation between its formation and

degradation parameters. Granted that the degradation pathway is clear up to the transient metabolite and its transformation product(s), and that the conceptual model is correct, assumptions may still need to be made on at least one of the parameters (e.g. by fixing the formation fraction or degradation rate). Any assumption about the formation fraction must be realistic, considering the chemical or biological reactions and physico-chemical processes involved and should be justified accordingly, ideally based on supporting data or weight of evidence.

8.5.3 Field data

This section provides some general recommendations with regards to the kinetic evaluation of metabolites in field studies.

The trigger endpoints obtained from field studies are the dissipation DT50 and DT90 values that can be derived directly from the dissipation curves. Conservative estimates of the dissipation DT50 and DT90 values for metabolites can be obtained by estimating the disappearance of the metabolite from its observed maximum, by fitting the decline curve. Refined values can be obtained by simultaneously fitting the parent and metabolites, to separate formation and dissipation processes, assuming that the complete pathway up to the metabolite(s) of interest can be described in the model.

Considering kinetic endpoints to be used in modelling, the first step is determining that degradation is the main route of dissipation for the metabolite, i.e. other routes of dissipation than transformation should be negligible. Field data may help in refining degradation parameters for a more realistic situation compared to laboratory data. However, the dissipation of pesticide substances and their metabolites in field experiments may result from a number of simultaneous processes. The same approach used to determine if parent kinetic endpoints are suitable (see Section 7.1) is also valid for metabolites that are monitored during the field experiments. However, determining whether degradation is the main dissipation process may be more difficult in the case of metabolites. The design of a field study is usually focused to solve some concerns arising from lower tier approaches. Therefore, interpretation of the study must take into account the purpose for which the field study was designed and conducted. Field studies are often designed for the parent substance, and it must be assessed if sampling intervals and sampling depths are also appropriate to evaluate the formation and degradation of the metabolites and distinguish other dissipation routes. Volatility and mobility parameters (Henry's Law constant, Water solubility, Kow, Koc…) should be used to interpret field data. When this information is not available from laboratory studies for metabolites, estimates may be generated with the

appropriate QSPR (Quantitative Structure Properties Relationships) model. However, the uncertainty of these estimates is usually high and laboratory data are preferred, especially if the metabolite of interest is envisaged to be relevant.

The number of metabolites analysed in field samples is usually limited to the ones envisaged as being present in concentrations greater than 5 to 10 percent of the amount of active ingredient applied or those that are important with respect to toxicology or ecotoxicology. Therefore, field dissipation studies may not provide a complete picture of the degradation route in the field. The kinetic evaluation of a metabolite for modelling purposes can only be performed correctly if the actual degradation route up to that metabolite is included in the model. If one known intermediate metabolite from the laboratory studies is not monitored in the field study, a ghost metabolite with no associated measured data may need to be included in the model for the kinetic evaluation (see Section 8.5.4).

If the route of degradation has been well established by laboratory studies and degradation processes are envisaged to be the main routes of dissipation for parent and metabolites (with low mobility and low volatility), quantitatively modelling field behaviour may be possible based on the same general approaches given for the modelling of laboratory results.

The number of data points might be less in field studies as compared to laboratory studies, especially when considering metabolites. This implies a higher uncertainty for the parameters calculated on the basis of field data. This uncertainty should be taken into account before adopting a parameter calculated from field data in preference to the same parameter calculated on the basis of laboratory data. However, if the visual assessment and the statistical endpoints from the kinetic evaluation in the field study are deemed satisfactory, the metabolite kinetic endpoints should be valid, and may be used for comparison with triggers or for modelling purposes.

Considering the effect of soil temperature and moisture content on the degradation of the metabolites, a similar standardisation method as those recommended and described for the parent in Chapter 9 may be used for metabolites.

In conclusion, to be able to use the field data to derive kinetic endpoints for metabolites to be used for modelling, degradation needs to be clearly identified as the main route of dissipation for parent and metabolites, or clearly and quantitatively separated from other dissipation processes. Kinetic interpretation of the field study for metabolites can only be carried out in accordance with the information already available from laboratory studies or QSPR

estimates. Parameters calculated from field data should always be checked for consistency with the overall degradation/dissipation route of the substances and for their degree of uncertainty. Their validity as trigger values or modelling endpoints should be carefully evaluated and justified so as not to derive misleading conclusions for metabolites from field data.

8.5.4 Ghost compartments

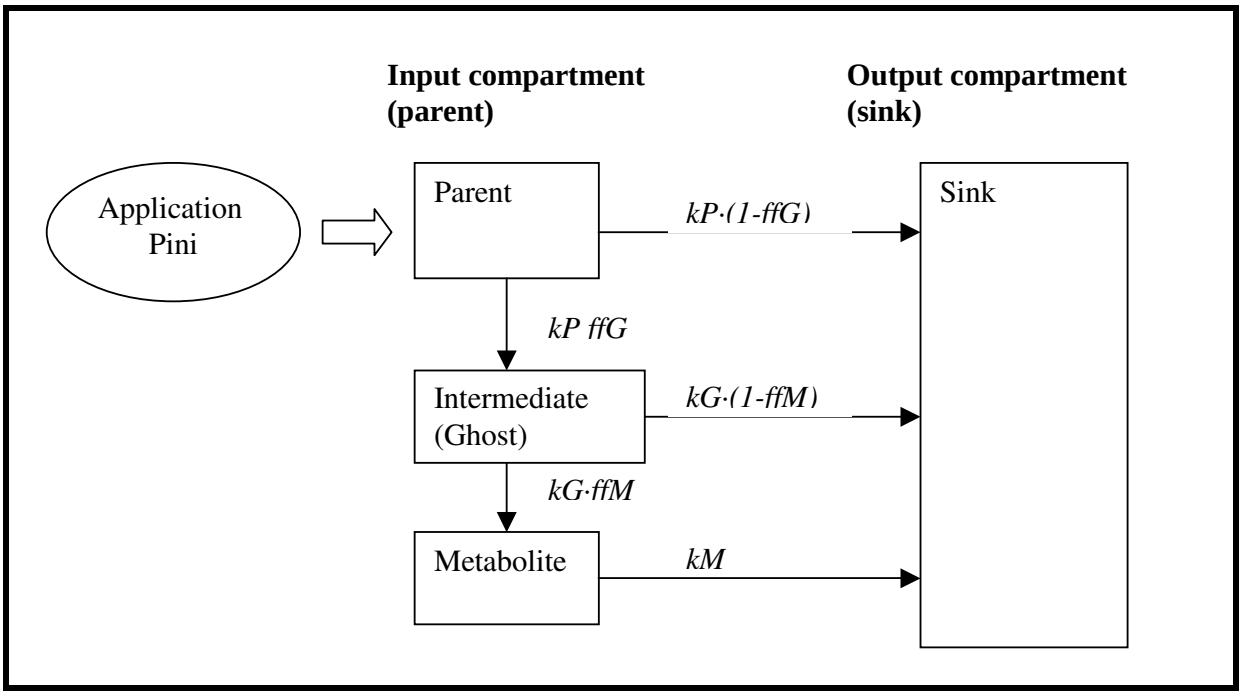
In some cases, the experimental data for an intermediate metabolite may be missing or may not be usable, for example in the case of transient metabolites that are observed at very low levels or cannot be detected (< LOD), in field studies when an intermediate may not be included in the analytical method, or again in cases when the analytical method does not permit to separate the metabolite from other components. The formation and degradation of metabolites further in the transformation pathway may not be properly modelled if the intermediate metabolite is neglected, leading to an incorrect conceptual model with a gap in the transformation pathway. In such cases, a ghost compartment, without associated data, may be needed in the model to represent the intermediate metabolite.

The formation and degradation parameters for the ghost metabolite are estimated together with the precursor(s) and metabolite of interest. Due to the lack of data for the ghost metabolite, its parameters may be highly uncertain. Therefore, the fit must be performed in a step-by-step approach, starting with the parent and other precursor(s) to the ghost metabolite, fixing the estimated parameters for these compartments, then estimating the parameters of the ghost metabolite and metabolite of interest together, and finally fitting all parameters together. Because the formation fraction of the ghost metabolite and formation fraction of the metabolite of interest are strongly correlated, the formation fraction of the ghost metabolite may be fixed to 1, but the formation fraction of the metabolite of interest always should be estimated. This should be adapted for more complex cases when another metabolite is formed from the precursor of the ghost metabolite. Then the formation fraction of the ghost should be set to 1-ffMi (Mi being the other metabolite).

The trigger endpoints (DT50 and DT90 values) for the metabolite can be considered valid if the goodness of fit criteria are met. The situation is more complex when considering kinetic parameters to be used in environmental models. While the degradation rate of the metabolite can be directly estimated, the estimate of the formation fraction needs to be discussed further. Furthermore, the modelling may not be performed without considering the intermediate metabolite.

Example 8-6

The following example illustrates the case of an intermediate not included in the analytical method in a field study. The next metabolite in the degradation pathway cannot be described properly when the intermediate is not included in the conceptual model, which may lead to incorrect endpoints because the conceptual model is incorrect. The data was described with all-SFO kinetics, with the same conceptual model as in Box 8-1, assuming that the metabolite forms directly from the parent, or with the conceptual model shown in Box 8-4 below when considering an intermediate metabolite (ghost) in the pathway. For this example, the conceptual model with the ghost compartment was simplified with regards to the formation of the intermediate, as it was assumed that the parent degraded exclusively to the intermediate, with no flow to the sink (in other words, the formation fraction of the intermediate, *ffG*, is set to 1, which in effect removes the flow from parent to sink). This was done because of the otherwise high correlation between parameters for the formation and degradation of a ghost substance without data. The formation fraction of the metabolite of interest, *ffM*, is still estimated in order to provide a correct description of its formation.



Box 8-4. Conceptual model of parent and metabolite with intermediate (ghost) compartment with all-SFO kinetics.

The parameter estimation results and calculated degradation DT50 and DT90 values (trigger endpoints) obtained from the fits in ModelMaker 4.0, for the parent and metabolite without intermediate, and including an intermediate as ghost compartment, are listed in Table 8-8,

while the description of the observed data for parent and metabolite with the models and plots of residuals for the metabolite are shown in Figure 8-9. Without the intermediate, the metabolite cannot be described properly. The high χ^2 error of 34 and plot of residuals of the metabolite showing a systematic error in the formation phase and around the maximum indicate a poor fit of the observed data. When a ghost compartment is added to the model (bottom), the metabolite can be described with accuracy, as reflected by the low χ^2 value of 9 and random distribution of the residuals. All parameter estimates are deemed reliable and the DT50 and DT90 values of the metabolite can be determined from the fit with the ghost compartment.

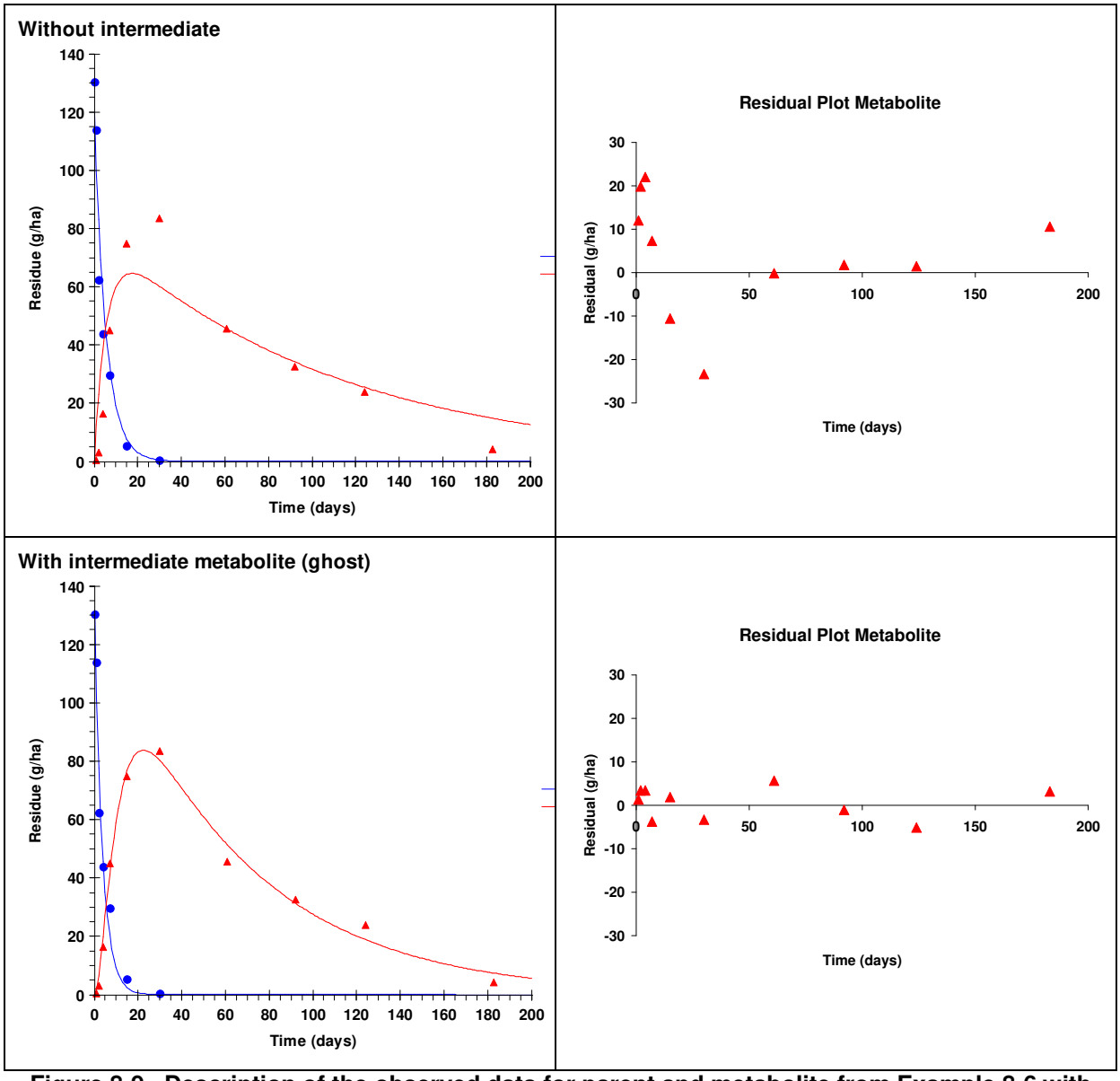


Figure 8-9. Description of the observed data for parent and metabolite from Example 8-6 with corresponding residual plots for the metabolite, all-SFO fits performed for parent and metabolite only (top), and for parent and metabolite including an intermediate metabolite as ghost compartment (bottom).

Table 8-8. Results of the fits of the parent and metabolite, in Example 8-6, including or not an intermediate metabolite as a ghost compartment

	Parent and metabolite only	With ghost compartment for intermediate metabolite
Parameter (estimate ± standard error)		
Pini (g/ha)	119.6 ± 11.3	131.8 ± 6.1
kP (1/d)	0.1816 ± 0.0416	0.2646 ± 0.0295
Formation fraction metabolite (from parent)	0.6327 ± 0.1345	-
kM (1/d)	0.0092 ± 0.0035	0.0159 ± 0.0030
kG (1/d)	-	0.1420 ± 0.0411
Formation fraction metabolite (from ghost)	-	0.8566 ± 0.1100
Goodness of fit (χ^2 error)		
Parent	17	13
Metabolite	34	9
Kinetic endpoints		
DT50 parent (d)	3.82	2.62
DT90 parent (d)	12.7	8.70
DT50 metabolite (d)	75.4	43.6
DT90 metabolite (d)	250	145

8.5.5 Lag-phase

In principle, the degradation of a metabolite may follow a lag-phase kinetic in the same manner and for the same reasons as for a parent substance (see Section 5.3). However, due to the fact that for metabolites formation and degradation occur simultaneously, a lag-phase pattern may often be difficult to identify. Unless the lag-phase period is fairly long, and there is a drastic change in degradation rate between the lag-phase (slow degradation or no degradation) and the second phase, the metabolite data can probably be described reasonably well with SFO kinetics. The kinetic endpoints obtained this way could be used for modelling and as well as for triggers (the DT values will be somewhere in between the values for the two phases).

However, in some cases a lag-phase pattern is evident from the metabolite curve, with a smooth or flat stationary maximum with no or little degradation, followed by a more or less abrupt decline. This may usually happen when the metabolite is rapidly formed and the difference in the two degradation rates is important. When a lag-phase is identified, determining whether it may be attributed to experimental artefacts is essential. If so the lag-phase should be omitted from kinetic analyses, and conservative estimates of the trigger and modelling endpoints may be derived by fitting the decline phase (second phase) of the metabolite. Information from other laboratory degradation studies with the parent substance or with the metabolite, and field studies if available, can be used to determine whether a lag-phase occurs in other soils and under field conditions. If a true lag-phase is identified for the

metabolite, because of slow adaptation of the degrading microflora or inhibition of the degrading microflora at high concentrations, a separate degradation study with the direct application of the metabolite to soil may be necessary in order to derive kinetic endpoints for the metabolite following the recommendations in Section 7.2.

8.6 References

A link to Council Directive 91/414/EEC can be found at http://europa.eu.int/comm/food/plant/protection/index_en.htm.

Guidance Document on the assessment of the relevance of metabolites in groundwater of substances regulated under Council Directive 91/414/EEC, Guidance Document on Terrestrial and Aquatic Ecotoxicology (as well as other Guidance Documents generated under the EU work on Plant Protection Products) can be found at http://europa.eu.int/comm/food/plant/protection/evaluation/guidance_en.htm.

9 NORMALISATION OF FIELD DISSIPATION HALF-LIVES TO REFERENCE TEMPERATURE AND MOISTURE CONDITIONS

The time-course of the dissipation of pesticide residues in the field can often be approximated by single first-order kinetics. The value derived from a single first-order dissipation curve is most often described as the dissipation half-life, the time required for dissipation of half of the amount of the pesticide. Dissipation kinetics obtained from laboratory studies conducted under dark conditions are believed to represent only chemical and biological degradation thus being equivalent to degradation kinetics, whereas the observed dissipation in the field may also include photodegradation and transfer processes like volatilisation, leaching, plant uptake (if plants were present), run-off or erosion.

A clear advantage of field over laboratory results is that they are determined under conditions specific for the intended use of a pesticide in an agricultural field (i.e. unsieved soil, fluctuating soil temperature and moisture conditions, and often the presence of crops) and thus closely match the situation which is to be modelled. Field DT50 and DT90 values also can reflect the variation in degradation due to seasonal changes in climatic conditions. The best-fit endpoints derived from measured residue data may trigger additional work provided the field study is relevant to the proposed usage conditions.

Eventually, observed dissipation in the field soil can be attributed exclusively to degradation if the study design fulfils requirements outlined in 9.1. As a consequence degradation half-lives for parent and metabolites derived under realistic field conditions may be used in pesticide fate modelling.

The power of models is that from a limited set of input parameters, they allow the predictions of degradation for a wide variety of conditions and situations. In order to permit the broadest possible use of field dissipation data, it is useful to normalise this data using a reference temperature and moisture condition (e.g. 20^{o}C and pF2). Normalised input parameters permit field dissipation data collected under one set of environmental conditions to be used to simulate likely behaviour under different conditions if dissipation was mainly due to degradation. There are practical limits to this recommended extrapolation procedure and caution should be used in applying normalised data from extremely wet settings to extremely dry settings, for example.

9.1 Assessment of field study design and results

A properly conducted field soil dissipation study should fulfil the criteria as outlined e.g. by the CTB (Risico voor milieu: Uitspoeling naar grondwater, Bijlage 3). The most important points to consider are:

- A critical assessment of the significance of photodegradation and specific transfer processes to the overall dissipation is recommended as a first step in evaluating the appropriateness of field study results for modelling purposes, i.e. deriving a degradation kinetics for parent and/or metabolites. If these processes play an important role in the overall dissipation, techniques such as inverse modelling and information from mechanistic laboratory scale studies (e.g. soil photolysis studies) may be used to estimate parameters needed to model the individual processes, rather than lumping all the transfer process into one rate constant.
- If such losses can be considered unimportant or can be properly addressed as separate processes, a further evaluation of the field study or deriving degradation half-lives for pesticide fate modelling is possible.
- Proper measurement of the applied dose.
- The soil should be well characterised at different depths.
- The soil sampling depth and analytical method should allow to capture the bulk of the applied material.
- Meteorological measurements should be available at least for the duration of the field experiment.
- The history of pesticide use in preceding years is available. The active substance or a chemical analog should not have been applied on the plot prior to the experiment.

The following are several approaches to consider when normalising field soil dissipation study results. The choice of one versus another rests with the modeller, although the time-step or rate constant normalisation methods are generally preferred due to their transparency. If properly performed, both the time-step normalisation and the rate constant normalisation should result in similar normalised values and can therefore be considered to be equally valid approaches.

9.2 Normalisation of field degradation half-life values to reference conditions

Field half-lives are normalised to reference conditions reflecting the major influence factors on field dissipation, i.e. in most cases soil temperature and soil moisture. The reference

conditions for soil temperature and moisture would be 20°C and 100% FC (pF2) unless scientific reasoning requires other values. The normalisation is conducted using measured or simulated values for soil temperature (air temperatures are not considered a suitable surrogate for soil temperatures) and moisture, e.g. daily values. During the parameter estimation at least the correction for temperature, preferably the correction for both temperature and moisture are activated. Deviations should be properly justified. The functional relationships and default parameters describing the dependence of soil degradation on soil temperature and soil moisture defined by FOCUS are applicable, unless better scientific knowledge, e.g. substance specific parameters is available. In any case the fits should fulfil the following criteria:

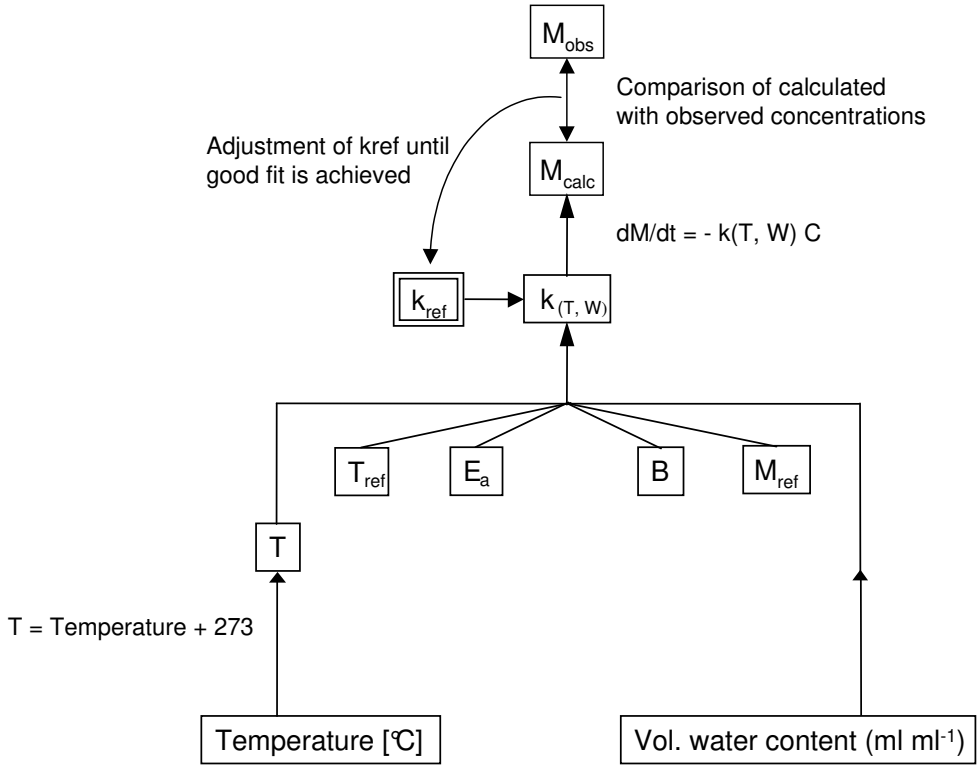
- The concentration curve calculated by the model is a good description of the data points. The goodness of fit should be demonstrated by a statistical evaluation.
- The normalised degradation half-lives estimated from different field studies are plausible.

9.2.1 Time-step normalisation approach

In this approach a normalised 'day length' is calculated based on daily variations in soil temperature and moisture content using the standard FOCUS equations and assumptions. For example a daily soil temperature of 25°C and moisture content of 20% (vs. 25% for pF2) gives a normalised day length of 1.27 days at 20°C and pF2. The daily values are calculated and the cumulative time between sampling points determined and used as input into a standard kinetic evaluation. For an example see Appendix 8 and Hardy *et al*. (2003).

9.2.2 Rate constant normalisation approach

The effect of variations in soil temperature and moisture content on the degradation rate constants is evaluated over standard time points, for instance based on daily values. The approach was proposed by Gottesbüren (1991), and Dressel and Beigel (2001) successfully used this technique to fit data from seven field studies to derive normalised parameters for parent and four metabolites. The degradation rate at reference conditions (kref) is back-calculated using daily soil temperatures and water contents in the field. The concept is illustrated below for an example where the Arrhenius equation and the Walker equation (1973) are used to describe temperature and moisture dependence, respectively.



with $k(T,W) = k_{ref}\ e^{\frac{Ea\,(T-T_{ref})}{R\,T\,T_{ref}}} \left(\frac{M}{M_{ref}}\right)^{B}$

where

k(T, W)	degradation rate at temperature T and water content W (d^{-1})
k_{ref}	degradation rate at standard temperature and water content (d^{-1})
E_a	activation energy (J mol^{-1})
T	absolute temperature (K)
T_{ref}	reference temperature (^oC)
R	gas constant (J mol^{-1} K^{-1})
M	actual soil moisture (m^3 m^{-3})
M_{ref}	standard soil moisture (m^3 m^{-3})
B	moisture exponent (-)

The optimisation of k_{ref} was achieved by minimising the sum of the squared differences between modelled (M_{calc}) and observed pesticide residues in soil (M_{obs}). Soil temperatures and water contents were imported from an external database. The parameters T_{ref}, E_a, M_{ref} and B, are held constant. The procedure can be implemented into available software packages such as ModelMaker.

Alternatively, pesticide leaching models which simulate the concentration of the pesticide in soil at different depths (e.g. PEARL, PELMO) can be used to simulate soil residues under the conditions of the actual field study. An automated optimisation technique referred to as inverse modelling can be adopted to optimise the standard degradation rate. This consists of repeatedly running the model and automatically adjusting the reference degradation rate until

the fit between simulated and measured soil residues is considered acceptable on the basis of pre-defined statistical criteria. Software packages that can be linked to most pesticide leaching models include PEST (Doherty *et al.*, 1994) and UCODE (Poeter & Hill, 1998). When using an inverse modelling approach, caution must be taken to avoid unrealistic results obtained by numerical artefacts.

9.3 Normalisation of field degradation half-life values to average soil temperature and moisture conditions during the experiment

The approaches described in Sections 9.1 and 9.2 should be used wherever possible. If measured soil temperatures and water contents are not available, they can often be calculated from standard weather data using a pesticide leaching model. Only in cases where the available data do not permit the procedures described above to be followed, a third approach may be used. This consists of normalising field degradation half-lives to average conditions that were present during the study in the field. These conditions will then in turn be used as base values for the calculations with both temperature and moisture corrections turned on. However, the overall degradation at average conditions may differ from that at fluctuating conditions due to the non-linearity of the relationship between degradation and soil temperature and moisture.

In this approach a representative **average soil temperature** for the field trial is used as the **reference temperature** in the model. An appropriate period for averaging would include the period that comprises all sampling dates that will be used in the kinetic analysis. When considering metabolites, this period should be selected to ensure good account of both the formation and decline. Average values or conservative estimates (e.g. 100% FC) for soil moisture conditions during the field study can be used as a reference moisture content. During winter periods, the soil water content is generally more uniform compared to the summer season due to lower evapotranspiration losses. When implementing this approach in PEC calculations, the soil and moisture references conditions are used and the temperature and moisture correction routines should be activated. **This approach is only recommended where the averaging period is short and the climatic conditions within this period are stable**.

9.4 General recommendations

- The equations and parameters describing the general dissipation kinetics and those used to normalise degradation for temperature and moisture effects must be consistent with those used for the subsequent calculation of PEC values with pesticide fate models. The current FOCUS groundwater leaching models use the Arrhenius and Walker equations for soil temperature and moisture corrections, respectively (Note that the Arrhenius equation uses temperatures measured in Kelvin).
- Soil moisture content must be specified as absolute or relative water content, not in tension units.
- The normalisation of field degradation half-lives requires measured or calculated soil temperature and moisture data. Both are highly variable with time and are depth dependent. Optimisation with ModelMaker or similar software is only recommended if a reliable estimate can be made of the actual daily soil temperature and moisture conditions within the layer of soil containing the bulk of the pesticide residues. Pesticide leaching models can simulate soil temperature and moisture at different depths in soil from standard weather data and their use is recommended if detailed measurements are not available. Routines like those included into PERSIST (Walker and Barnes, 1981) that predict soil temperatures and moisture from measured air temperatures have been validated, however, calculated data should be checked against measurements where possible.
- Normalisation of field degradation half-lives and calculation of PEC values should be made using the same concepts and assumptions.
- The adopted normalisation approach and steps must be clearly outlined in the report.
- Using a DegT50 field value without normalisation is not recommended for leaching assessments when the period for calculating the kinetics covers only the spring and summer period. Leaching potentially takes place on a time scale of years and degradation rates in autumn and winter will be lower compared to those in spring and summer, leading to an underestimation of leaching in such cases.

9.5 References

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Dressel, J., Beigel, C., 2001. Estimation of standardized transformation rates of a pesticide and its four soil metabolites from field dissipation studies for use in environmental fate

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10 WATER SEDIMENT STUDIES

10.1 Introduction

The main purpose of this chapter was to provide guidance about how to estimate and use the disappearance times (kinetic endpoints) that describe the various aspects of parent and metabolite fate in water-sediment systems. There are two general types of kinetic endpoints needed for parent and metabolite substances:

- *Persistence Endpoints* to determine whether various aquatic ecotoxicology studies are triggered; and
- *Modelling Endpoints* to use in calculating Predicted Environmental Concentrations as part of an aquatic risk assessment, e.g. with FOCUS surface water scenarios.

Certain aspects of parent and metabolite behaviour in water-sediment systems are more complex than in laboratory soil systems, and they have an influence on the meaning and/or estimation of some endpoints that must be taken into account. For example, water-sediment systems comprise two *interacting* compartments (the water column and the sediment) subject to different physical, chemical and biological conditions. This can result in quite different degradation behaviour in each compartment, e.g. in the sediment compartment, degradation rates may vary much more than in the water column, due to the variations in the sediment such as the redox gradient from aerobic conditions at the interface with the water column to strong reducing conditions towards the bottom of the sediment. Furthermore, degradation is sometimes thought to occur mainly in an interfacial region between the water column and the sediment. Distinguishing where most of the degradation occurs may be possible; however, to determine exactly what occurs in each compartment and how quickly necessarily becomes somewhat arbitrary. The net result of this complexity means that while some endpoints remain relatively straightforward, e.g. degradation in the whole water-sediment system or dissipation of parent from the water column, some of the persistence and modelling endpoints become more difficult to define and estimate.

First, some of the kinetic endpoints have not been explicitly defined in study and modelling guidelines, so this chapter also provides definitions as well as some guidance on their use. This is particularly important for persistence endpoints where current definitions do not distinguish whether the required endpoint is disappearance by dissipation (as DT50/90) or by degradation (as DegT50/90). For example, the work group was not clear which endpoint was required for disappearance times of parent substance from the sediment (persistence endpoints). Was it by dissipation, i.e. the times for parent substance to decline by 50% and

90% from the peak concentration in sediment? Or was it by degradation, i.e. the times to degrade 50% and 90% after entry into the sediment? Neither endpoint will be consistently shorter or longer than the other, as can be seen in the examples shown in Section 10.2.4. Hence, in this and other cases, the work group decided that both dissipation and degradation endpoints should be calculated. The endpoint that is used should be decided on a case-by-case basis between the registrant and the regulator.

Secondly, in addition to the somewhat arbitrary separation into water column and sediment, estimation of degradation rates in these compartments is made difficult because all kinetic models are very sensitive to the fundamental correlation between the estimated transfer rates (from the water column to sediment and *vice versa*) and estimated degradation rates. Hence, if the transfer rate to sediment is over-estimated for a parent substance, this will tend to result in an over-estimate of the degradation rate in sediment and an under-estimate of the degradation rate in the water column. The situation is reversed if the transfer rate to sediment is under-estimated. Unless some of the parameters can be constrained, there is a greater likelihood that they take on extreme (physically implausible) values to obtain a best fit, e.g. zero degradation in the water column or the sediment, making degradation effectively occur in only one compartment. Therefore the work group thoroughly examined several options to try constraining parameter values to more realistic values. The main methods included constraining either the degradation rates in the water column and sediment, or the transfer rates between the water column and sediment. The former was rejected because no clear scientific basis could be made. A scientific basis for the latter was examined and some preliminary testing conducted (see Appendix 9). However, the general conclusion of the work group was that it was not possible within the time frame to develop simple, robust and reliable constraint procedures, though it is possible to check that the transfer rates are plausible. For constraints, the exception may be when transfer rates can be estimated independently, e.g. when a water-sediment study is also run under conditions in which degradation does not occur.

Therefore, the approach taken here for these extreme cases was to use default worst-case parameter values for degradation in the water column and sediment if the initial parameter estimates indicate a lack of degradation, implausible transfer rates, or inconsistency with other environmental fate studies. The defaults, degradation half-lives of 1000 days in the water column or sediment, are considered to be conservative. These conservative defaults apply equally to parent and metabolite substances. The work group regards these conservative default values only as modelling endpoints to enable aquatic risk assessments

to be conducted. The conservative default value for sediment should therefore not be automatically regarded as triggering further aquatic ecotoxicology studies.

When using default values as a modelling endpoint in one compartment, the work group decided to use the system half-life for the other compartment. This approach ensures that a conservative set of modelling endpoints is used, since the overall degradation rate is underestimated. Furthermore, a degradation rate below the overall degradation rate in one compartment requires that the degradation rate be above the overall degradation rate in the other compartment. Appendix 10 provides more details about why this approach is conservative, but avoids unrealistic combinations of water column and sediment degradation rates as far as possible.

The overall approach to estimate and use the disappearance times in water sediment systems is outlined in Tables 10-1A&B and 10-2A&B for parent and metabolites, respectively, and in more detail in Sections 10.2 and 10.3. Tables 10-1B and 10-2B are based on FOCUS SW modelling at EU level. Although this chapter primarily deals with endpoints required for EU registration, much of the information is applicable to calculating similar endpoints required by individual Member States for national registrations. In general Tables 10-1A and 10-2A will be applicable to persistence endpoints and Tables 10-1B and 10-2B will be applicable to modelling endpoints. One exception might be for PEC calculations using models in which first-order kinetics are not required (similar to the situation described for PEC calculated for soil described in Section 11.4). In this case the kinetic models derived for estimating persistence endpoints may be more appropriate.

Two levels[7] of kinetics are used:

- Level I is for one-compartmental approaches to estimate the kinetics endpoints such as degradation in the whole system as a single compartment, dissipation from the water column compartment, and dissipation from the sediment compartment; and
- Level II is for two-compartmental approaches to estimate degradation in the water column and sediment compartments.

For parent substances these levels are denoted P-I and P-II, and for metabolites are denoted M-I and M-II. However, no Level M-II has been developed (due to the complexities experienced in resolving Level P-II, so only an outline of how it may be developed in future is given in Section 10.3). Therefore, Table 10-2A&B only shows how to use the results from Level M-1 and should be updated when Level M-II is developed. For substances that require

[7] The term "Levels" is used to avoid confusion with the term "Steps" used in FOCUS surface water assessment.

further consideration beyond that given in these levels, refinements or alternatives can be considered on a case-by-case basis.

For the persistence endpoints (Tables 10-1A and 10-2A), all of them can be derived from Level I, with the exception of the sediment DegT50 for parent. Also, these endpoints can all be estimated from different types of kinetics, ranging from Single First Order (SFO) kinetics to First Order Multi Compartment (FOMC) kinetics, Double First Order in Parallel (DFOP) kinetics and Hockey-Stick (HS) kinetics, again with the exception of the sediment DegT50 for parent (the reasons for this are discussed in Section 10.2.4), and HS kinetics for metabolites for reasons discussed in Chapter 8.

For several of the endpoints, options are given about whether to use one endpoint *or* another against study triggers and in fate modelling (the associated type of kinetics are also indicated in the same manner). In general, the reason is that the most appropriate endpoint to use is a matter for discussion between registrants and regulators. For the persistence of parent in sediment, the DT50/90 endpoint is not consistently shorter or longer than the DegT50/90 endpoint. The endpoint that should be used is the one that best represents the persistence in the sediment compartment. For the persistence of metabolites in the water column and sediment, the system DegT50/90 is given as an alternative endpoint to the compartment DT50/90 because estimating whether a decline actually occurs is often very difficult to determine, except for major metabolites that peak early in a study at significantly >10% of applied radioactivity and then undergo a clear decline.

For the modelling endpoints, options are provided for two reasons. First, in FOCUS Step 2 the use of such options is recommended, e.g. for parent at Step 2. Secondly, conservative default positions need to be used, when robust degradation rates cannot be estimated for both compartments, to ensure that an appropriate aquatic risk assessment can be conducted.

In conclusion, Tables 10-1 and 10-2 summarise the recommendations for which kinetic endpoints should be estimated and used for the majority of situations, although some deviations can be made if they can be justified. In addition to this, Table 10-3 summarises the data to which models are fitted in order to derive the various endpoints, i.e. DT values that represent dissipation due to various combinations of processes, and DegT values that only represent the degradation aspect of dissipation.

Finally, the kinetic concepts presented here only apply to “water-sediment systems” used in laboratory or semi-field studies. If plants are also present in other laboratory or semi-field studies, these concepts do not apply and should not be used for estimating disappearance times, unless the plants are unlikely to have an appreciable impact on the kinetics, e.g. because they adsorb/absorb relatively little parent or metabolite.

Table 10-1A. Estimation and use of *persistence endpoints* for parent compounds.

Approach	Compartment		
	System	**Water Column**	**Sediment**
Kinetic Level	Level P-I System DegT50/90	Level P-I Water column DT50/90	Level P-I Sediment DT50/90 **or** Level P-II Sediment DegT50/90
Type of Kinetics	Best-fit model SFO/FOMC/DFOP/HS	Best-fit model SFO/FOMC/DFOP/HS	Best-fit model SFO/FOMC/DFOP/HS **or** SFO

Table 10-1B. Estimation and use of *modelling endpoints* for parent compounds.

Approach	FOCUS Step		
	1	**2**	**3**
Kinetic Level	Level P-I System DegT50/90	Level P-II Water Deg50/90 + Sediment Deg50/90 **or** Level P-I System DegT50/90 for both compartments	Level P-II Water Deg50/90 + Sediment Deg50/90 **or** Level P-I System DegT50/90 + Default DegT50/90 for Water and/or Sediment **or** in case of no backtransfer and degradation faster in sediment, Level P-I System DegT50/90 for sediment + Level P-II Water Deg50/90
Type of Kinetics	SFO	SFO	SFO

Table 10-2A. Estimation and use of *persistence endpoints* for metabolites.

Approach	Compartment		
	System	**Water Column**	**Sediment**
Kinetic Level	Level M-I System decline DT50/90 **or** Level M-I System DegT50/90	Level M-I Water decline DT50/90 **or** Level M-I System decline DT50/90 **or** Level M-I System DegT50/90 As Justified	Level M-I Sediment decline DT50/90 **or** Level M-I System decline DT50/90 **or** Level M-I System DegT50/90 As Justified
Type of Kinetics	Best-fit model SFO/FOMC/DFOP	Best-fit model SFO/FOMC/DFOP	Best-fit model SFO/FOMC/DFOP

Table 10-2B. Estimation and use of *modelling endpoints* for metabolites.

Approach	FOCUS Step		
	1	**2**	**3**
Kinetic Level	Level M-I System decline DT50 **or** If no decline observed then use Default DT50 of 1000 days	Level M-I System decline DT50 for both compartments **or** If no decline observed then use Default DT50 of 1000 days	Level M-I System DegT50 for main degrading compartment where justified + Default DT50 of 1000 days for other compartment
Type of Kinetics	SFO	SFO	SFO

Table 10-3. Disappearance times from different compartments, the processes influencing them, and the data to which kinetic models need to be fitted for parent and metabolites.

Substance	Compartment	Endpoint	Processes	Fit model to
Parent	System	DT50/90[1]	Degradation Volatilisation	All data for parent in total system (P-I)
	Water	DT50/90	Degradation Volatilisation Partitioning	All data for parent in water (P-I)
	Sediment	DT50/90	Degradation Partitioning	Data for decline of parent in sediment from max. onwards (P-I)
	Water	DegT50/90	Degradation	All data for parent in water and sediment (P-II)
	Sediment	DegT50/90	Degradation	All data for parent in water and sediment (P-II)
Metabolite	System	DT50/90[1]	Degradation Volatilisation	All data for parent and metabolite in total system (M-I)
	System	DT50/90	Formation Degradation Volatilisation	Data for decline of metabolite in total system from max. onwards (M-I)
	Water	DT50/90	Formation Degradation Partitioning Volatilisation	Data for decline of metabolite in water from max. onwards (M-I)
	Sediment	DT50/90	Formation Degradation Partitioning	Data for decline of metabolite in sediment from max. onwards (M-I)

[1]DT50 = DegT50 where corrected for volatilisation as described in Appendix 11.

10.2 Goodness of fit

The methods recommended for evaluating the goodness of fit for the parent substance (Section 6.3.1) are also applicable to water-sediment evaluations. The work group felt that the goodness of fit should be performed for each compartment separately. While it is true that the data on the water and sediment compartments are linked, examining the overall fit to both compartments is inconclusive with regards to the individual compartments. In the overall fit to both compartments, the one with the highest measured levels would carry more weight than the other, and as a result an overall fit may still appear acceptable while either the water column or sediment may not be well fitted.

Visual assessment is the main tool for assessing goodness of fit. The plots of residuals should be used to determine if the residuals are randomly distributed or whether any systematic error is apparent during the formation, maximum or decline in the sediment, which would indicate that the pathway or kinetic model used may not be appropriate.

The χ^2 test is recommended as a tool for model comparison and as a supplementary tool for assessing the goodness of fit of an individual model. The χ^2 error value should be calculated using all data within a compartment used in the fit (after averaging), including the sampling points below LOD or LOQ that are included as ½ LOD or ½ (LOQ+LOD). The time-0 sample however, if set to 0 should not be used in the χ^2 error determination. Since the χ^2 statistics are calculated separately for each compartment, only the parameters specific to that compartment are considered in the χ^2 calculation. For the water column these are the initial amount (M_0), the degradation rate (k_w) and the transfer parameter ($r_{w\text{-}s}$). For the sediment these are the degradation rate (k_s) and the transfer parameter ($r_{s\text{-}w}$). The number of model parameters for selected model fits is given in Table 10-4.

For metabolites, as for parent, only the parameters specific to that compartment are considered in the χ^2 calculation. When fitting the metabolite decline in water column, sediment or total system, the relevant parameters are the maximum level of metabolite (M_{max}) when fitted, and the parameters specific to the kinetic model, e.g. the degradation rate (kM) for SFO, and shape and location parameters (αM and βM) for FOMC. When fitting the metabolite formation and degradation in the total system, the relevant parameters for the metabolite are the formation fraction, ffM, and the parameters specific to the kinetic model, e.g. the degradation rate (kM) for SFO, and shape and location parameters (αM and βM) for FOMC. The number of model parameters for metabolite for selected model fits is given in Table 10-5.

Ideally, the error value at which the χ^2-test is passed should be below 15% and the fit must be visually acceptable. However, this value should only be considered as guidance and not absolute cut-off criterion. There will be cases where the error value to pass the χ^2-test for a metabolite is higher, but the fit still represents a reasonable description of its formation and degradation behaviour.

Table 10-4. Number of model parameters for parent for selected kinetic model fits

Level	Kinetic model	Number of model parameters	Fitted parameters
Level I	Water column or total system SFO	2	M_0, kP
	Water column or total system FOMC	3	M_0, αP, βP
	Water column or total system HS	4	M_0, k1P, k2P, t_b
	Water column or total system DFOP	4	M_0, gP, k1P, k2P
Level II	Water column SFO	3	M_0, k_wP, r_{w-s}
	Sediment SFO	2	k_sP, r_{s-w}

Table 10-5. Number of model parameters for metabolite for selected kinetic model fits

Level	Kinetic model	Number of model parameters	Fitted parameters
Level I	Metabolite decline water column, sediment or total system SFO	2	M_{max}, kM
	Metabolite decline water column, sediment or total system FOMC	3	M_{max}, αM, βM
	Metabolite decline water column, sediment or total system DFOP	4	M_{max}, g, k1M, k2M
	Metabolite degradation in total system SFO	2	ffM, kM
	Metabolite degradation in total system FOMC	3	ffM, αM, βM
	Metabolite degradation in total system DFOP	4	ffM, g, k1M, k2M

In addition to these goodness of fit indices, the reliability of the individual degradation rate parameter estimates needs to be evaluated as outlined in Section 6.3, based on the results

of the t-test or confidence intervals of the parameters. Note that to calculate the t-test for the individual parameter, the total degrees of freedom are used, which depend on the total number of parameters estimated in the fit, as opposed to the compartment parameters only as used for the χ^2 calculation. Whenever fits are performed with the stepwise approach, the reliability of the individual parameters needs to be assessed at the final step, when all parameters are estimated at once, which is when the degrees of freedom will be the lowest and the uncertainty of the estimated parameters should be the greatest.

Due to the inherent uncertainties of fitting water sediment data, a significance level of 10 percent ($p< 0.1$) for the t-test (single-sided) is considered appropriate to decide if the degradation rate parameter is significantly different from zero[8]. As a general rule, all statistical indices, χ^2 statistics, plots of residuals, and t-test of individual degradation rate constant parameters would need to be addressed in order to accept water sediment endpoints as fully reliable. However, on a case-by-case basis, the water sediment endpoints may still be considered acceptable even though one or more of the indices are not met, as long as the endpoint value can be considered conservative, or can be justified based on weight of evidence from other studies.

10.3 Parent kinetics

10.3.1 Introduction

Many kinetic approaches can be used to describe the disappearance of parent[9] compounds from water-sediment systems. The work group decided to use compartmental approaches, rather than more detailed mechanistic approaches, which allowed the use of similar approaches for estimating persistence and modelling endpoints. Two levels were used: Level P-I for one-compartmental approaches and Level P-II for two-compartmental approaches. For substances that require further consideration beyond that outlined here, refinements or alternatives can be considered on a case-by-case basis. The details of Levels P-I and P-II should adequately cover the majority of cases, so refinements and alternative approaches should only be used as a last resort.

[8] Note that the transfer rate coefficients are not subject to a t-test, because intrinsically they should be greater than zero, and because the Fsed test is used to assess them.
[9] The term “parent” here is used in its broadest sense: it simply denotes the test substance applied, so it may also refer to the metabolite of a pesticide.

Within this overall approach, there are several aspects that are similar to those used for kinetics in other test systems (see Chapters 7 and 8). Hence, the methods of fitting kinetics to data are similar with respect to data entry, selection of fitting routine, selection of constraints, data exclusion, statistical evaluation, and types of kinetics considered.

At Level P-II, however, there are a number of special points that need to be made. First, in the handling of data for day zero, parent residues are often found in the sediment, particularly for highly sorbing compounds, due to sampling a short time after zero time. These residues should be treated as if they were in the water column, i.e. add them to the residues in the water column.

Secondly, since Level P-II uses a two-compartmental approach, parent data should be handled in terms of mass or equivalent, e.g. % applied radioactivity, remaining in each compartment. Using concentration data is not recommended, because mass balance is not preserved, unless the compartments are the same size and concentration in the water column and the sediment are defined with respect to the total volume of each compartment, rather than the volume of liquid or mass of sediment.

Thirdly, there are special considerations about how the default approach should operate when the initial parameter estimates indicate a lack of degradation in the water column or sediment; these are given later in Section 10.3.3 and in further detail in Appendix 9.

Likewise, the methods used to make kinetic decisions (see Chapters 7 and 8) are similar to those for kinetics in other test systems. These decisions include details of what needs to be reported and where in the study report / raw data (see Chapter 12), particularly over the logic of the kinetic approach taken and the recording of the approach taken.

10.3.2 Level P-I

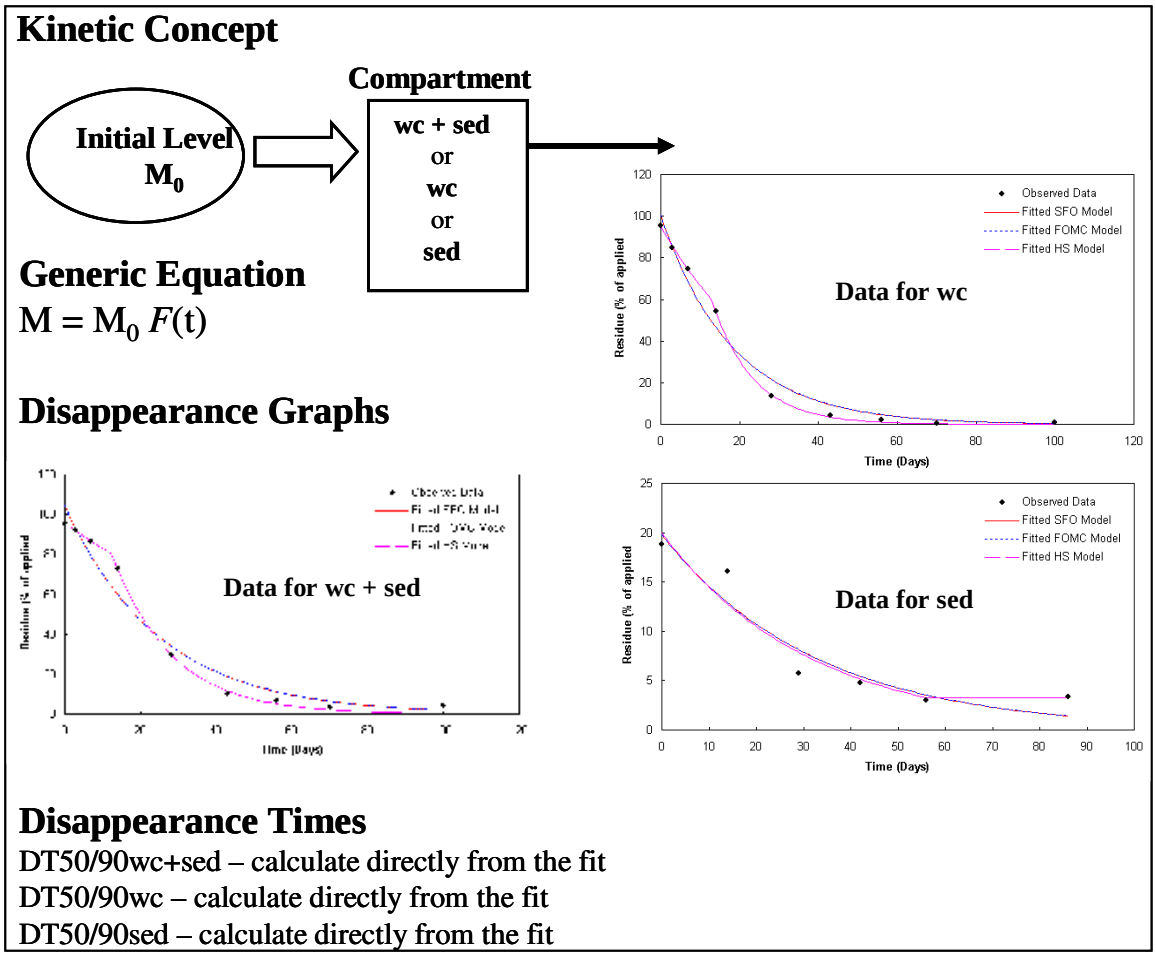
At Level P-I, both persistence and modelling endpoints are estimated using one-compartmental approaches that represent the whole water-sediment system, or just the water column or sediment, and from which degradation or dissipation is estimated (see Box 10-1) from an arbitrary time zero, i.e. after an application to the compartment or after the level in sediment reaches its peak value.

For degradation in the whole water-sediment system as DegT50/90, estimation requires kinetics to be fitted to the whole system data. However, the Level P-I approach described here is only valid for non-volatile compounds that only undergo losses by degradation. It can

also be used for slightly volatile compounds if volatile losses can be accounted for by trapping, identification and quantification of volatiles (see Appendix 11 for details of the correction procedures).

For dissipation from the water column as DT50/90, estimation requires kinetics to be fitted to the water column data. Similarly, estimating dissipation from sediment as DT50/90 requires kinetics to be fitted to the sediment data, but with time zero taken as the time that the peak concentration in the sediment is reached.

As shown in Box 10-1, these endpoints are estimated using a generic equation describing the degradation or dissipation from the compartment after an initial values of M0 in the compartment at time zero, where the function *F*(t) describes the rate of dissipation or degradation and is determined by the kinetics. For example, the function *F*(t) = exp (-kt) for SFO kinetics.



Box 10-1. Parent Kinetics at Level P-I

Four types of kinetics (SFO, FOMC, DFOP, and HS kinetics) are recommended. SFO kinetics are recommended as the default first choice and because FOCUS Surface Water modelling requires the use of SFO kinetics. FOMC kinetics are used to help evaluate whether the data depart appreciably from SFO kinetics, and DFOP kinetics are used because they have more flexibility in shape than FOMC kinetics due to having one more parameter. Hockey-stick kinetics are used because the data, particularly for the whole system, sometimes appear to have some form of "breakpoint" from one rate to another and provide the best fit to the data.

Box 10-1 shows a generic kinetic equation describing disappearance from the compartment, where the function *F*(t) describes the rate of dissipation or degradation and is determined by the type of kinetics. For substitution of all the various types of kinetics into this generic equation, please see Chapter 5 (note the limitations presented in Chapter 5 for bi-phasic kinetic models). The kinetic endpoints (DT50/90 and DegT50/90 values) can be calculated directly from the fits of these equations as described in Chapters 7 and 8.

Box 10-1 also shows an example of disappearance patterns from the whole water-sediment system, plus from the water column and from the sediment for a moderately adsorbing compound, plus the fits of SFO, FOMC and HS kinetics to the data (Note that the fit of FOMC kinetics was very similar to that of SFO kinetics, so the two fits are more-or-less superimposed and cannot be distinguished easily). The graphs indicate that dissipation from the water column (DT50≈15 days) is somewhat faster than degradation in the whole system (DegT50≈20 days), and that dissipation from the sediment (DT50≈22 days) is somewhat slower than degradation in the whole system. For further details of this example, please see Section 10.2.5 under Compound 1.

The recommended procedures to estimate the *persistence endpoints* are outlined in Figure 10-1 for level P-I. The procedures essentially operate in the same way as those described in Chapter 7, so they are not repeated here. These procedures need to be used three times, to cover degradation in the whole system, plus dissipation from the water column and from the sediment. Next, the recommended procedures to estimate the *modelling endpoints* are outlined in Figure 10-2 for level P-I. Again, the procedures essentially operate in the same way to those described in Chapter 7, so they are not repeated here. Two sets of procedures are used because best fit kinetics are needed to estimate persistence endpoints, while SFO kinetics fits are required to estimate modelling endpoints (including how to derive a half-life when SFO kinetics do not provide the best fit to the data). Examples of how to use these procedures are given in Section 10.3.5.

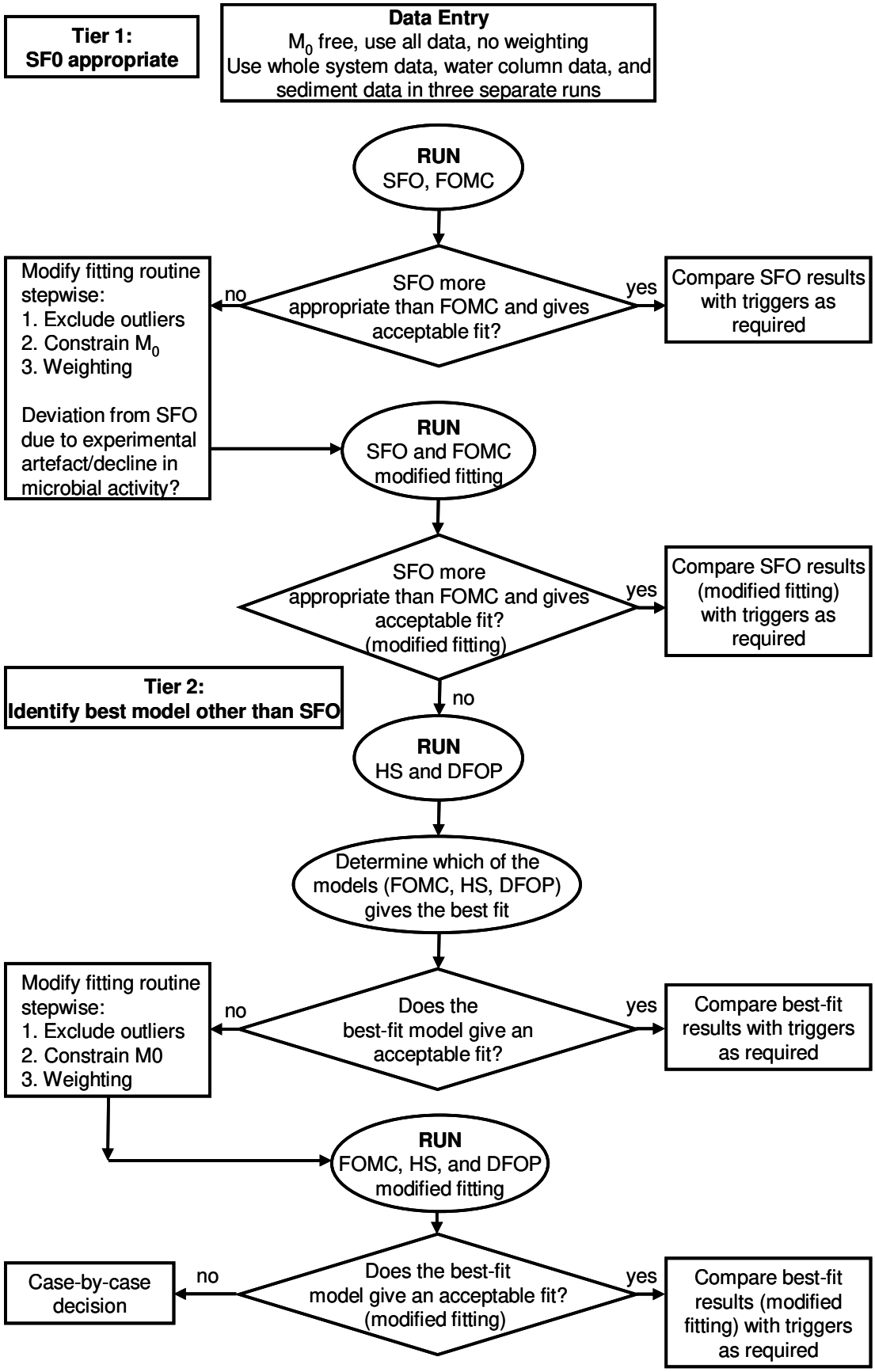


Figure 10-1. Recommended procedure at Level P-I to estimate persistence endpoints.

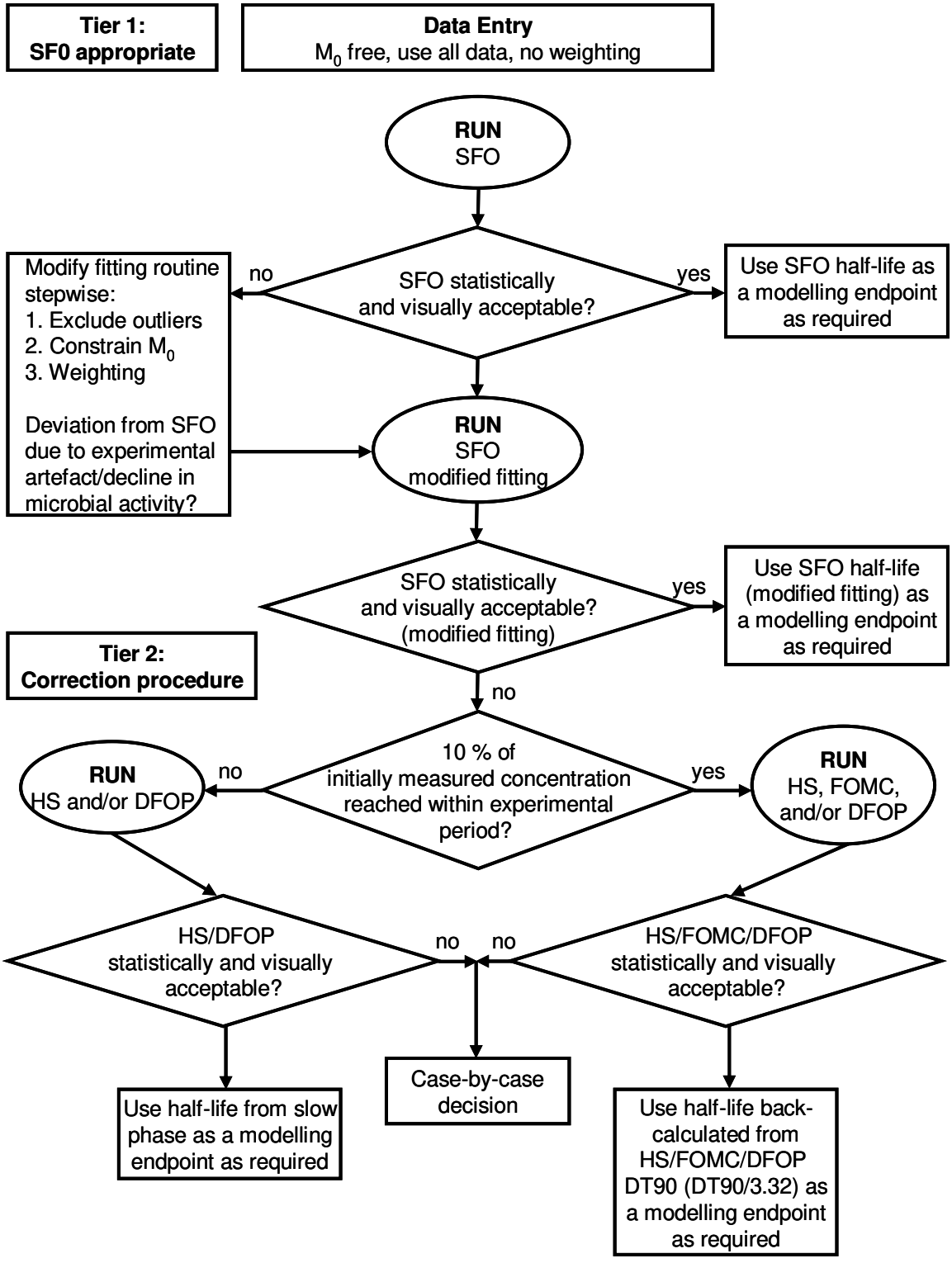
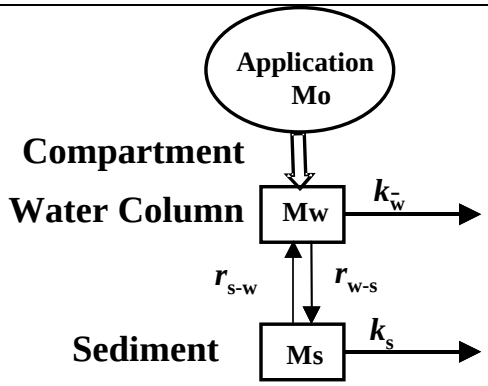


Figure 10-2. Recommended procedure at level P-I to estimate modelling endpoints.

10.3.3 Level P-II

At Level P-II, both persistence and modelling endpoints are estimated using a two-compartmental approach, comprising water column and sediment compartments. As shown in Box 10-2, after an application is made to the water column, first-order kinetics are used to describe degradation in these compartments (rate constants k_w and k_s) as well as reversible transfer between these compartments (rate constants $r_{w\text{-}s}$ and $r_{s\text{-}w}$). Only first-order kinetics were used for degradation due to the complexities of implementing biphasic kinetics, which is a limitation when degradation rate slows down over time.

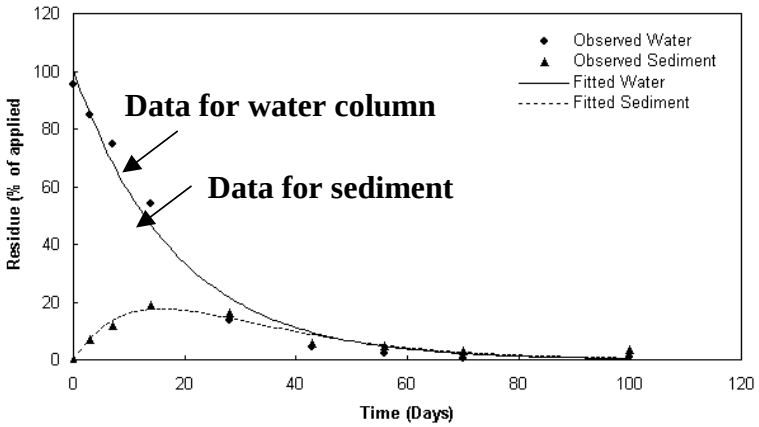
Kinetic Concept



Generic Equations

$$\frac{dMw}{dt} = -r_{w\text{-}s}\,Mw + r_{s\text{-}w}\,Ms - k_w\,Mw \qquad \frac{dMs}{dt} = -r_{s\text{-}w}\,Ms + r_{w\text{-}s}\,Mw - k_s\,Ms$$

Disappearance Graph



Disappearance Times

DegT50/90w – calculate directly from the fit
DegT50/90s – calculate directly from the fit

Box 10-2. Kinetics at Level P-II

First-order kinetics was used to represent transfer for three reasons. First, it can approximate the empirical pattern of transfer between the water column and sediment as shown in Appendix 9. Secondly, treating the sediment as a compartment whose detailed internal mechanisms do not need to be known eliminates the need for knowledge of the spatial concentration gradients down through the sediment[10]. In contrast, diffusion-based approaches which would be highly complex if sediment comprises both aerobic and anaerobic regions in which degradation rates differ. In other words, representing transfer processes with first order kinetics appears to have the appropriate level of detail before considering alternatives or refinements. Thirdly, first-order transfer processes are relatively simple to implement.

Box 10-2 shows the kinetic equations describing disappearance from the water column and sediment compartments based on first-order kinetics. Box 10-2 also shows a graphical example of the dissipation patterns from the water column and from the sediment compartment, plus fits of the first-order degradation and reversible transfer kinetics to the data. For the water column, the graph shows that the parent only goes through a decline phase. However, for the sediment, the graph shows that the parent undergoes a transfer phase before the onset of the decline phase. Sometimes an apparent plateau phase occurs before the decline phase, when the entry and exit rates to the sediment are in balance. Finally, Box 10-2 indicates that DegT50 (or half-life for modelling endpoints) for the water column and sediment can be calculated directly from the fit as $ln2/k_w$ and $ln2/k_s$ ($ln10/k_w$ and $ln10/k_s$ for DegT90).

Similar to Level P-I, this approach is valid for non-volatile compounds that only undergo losses by degradation. It can also be used for slightly volatile compounds if volatile losses can be accounted for by trapping, identification and quantification of volatiles (see Appendix 11 for details of the correction procedures). If such a correction needs to be made, it is recommended here that only the degradation rate for the water column is amended, for the pragmatic reason that the loss will occur via the water column.

[10] Most water-sediment studies involve gentle stirring or agitation to keep the water column well-mixed under aerobic conditions.

The recommended procedures to estimate the *persistence endpoints* and *modelling endpoints* are outlined in Figure 10-3 for level P-II (note that sediment DegT50 is the only persistence endpoint). The initial fitting of the data is much the same as described in Chapter 7. First, an unweighted fit with all of the data and with the starting value unconstrained is performed. If the fit is not satisfactory, then a variety of actions can be taken to see if an acceptable fit can be obtained.

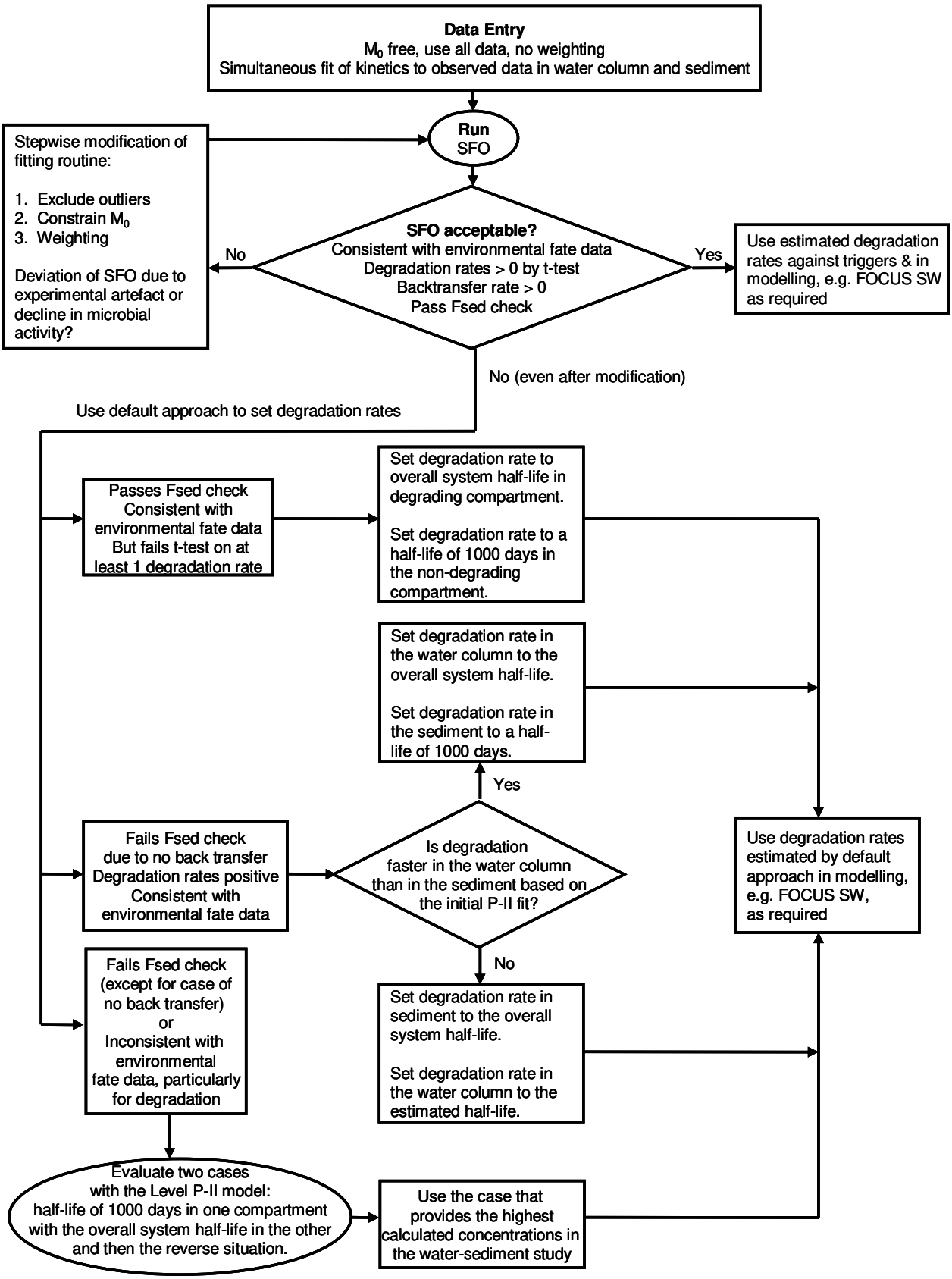


Figure 10-3. Recommended procedure at level P-II to estimate both persistence and modelling endpoints.

To be considered an acceptable fit, the fit must be visually and statistically acceptable as described in Chapter 6. This assessment may include checking that the DegT50/90 for the whole system, DT50/90 from the water column and DT50/90 from the sediment are similar to the results obtained at Level P-I. DegT50/90 in the whole system is simply calculated by summing the amount in the water column and the sediment and finding when 50% and 90% has degraded. DT50/90 in the water column calculated in a similar way, except that only the amount in the water column is used. And DT50/90 in the sediment is calculated in a similar way, but with time zero taken as the time at which the residues reach a peak in the sediment. In addition, three other criteria must be met:

- The results must be consistent with environmental fate data. For example, lack of degradation in the water column would be inconsistent with a rapid degradation rate observed in a distilled water hydrolysis study (assuming the absence of other factors such as pH). Also, the compartment in which degradation is faster according to the results from the Level P-II fitting must also be the faster compartment predicted from the results of the environmental fate studies. If the compartment in which degradation is faster cannot be determined from the environmental fate data, then the Level P-II fitting is not considered to be consistent with the environmental fate data (except when little degradation occurs in either compartment or perhaps when degradation is predicted to be similar in each compartment and the Level P-II fit gives a similar answer).
- The degradation rates in both compartments must be greater than zero as shown by the t-test. While a zero degradation rate is certainly a possibility for some compounds, this introduces considerable uncertainty into the parameter optimisation.
- The Fsed check must be passed. In particular, the back transfer rate (rate of transfer from sediment to water, $r_{s\text{-}w}$) must be greater than zero to pass the check. This test, described in Appendix 9, checks the ratio of the transfer rates to see if they are consistent with the properties of the compound.

When the fit is not considered acceptable due to failure of at least one of the criteria, one of three default approaches is used to set the degradation rates to be used in FOCUS surface water modelling. The basis for these default approaches is described in Appendix 10, in particular why they result in worst-case PEC values for both the water column and the sediment, including some testing of the implications in FOCUS Step 3 TOXSWA runs.

The first default approach is used when at least one degradation rate is zero or is not significantly different from zero (as shown by the t-test) but the predicted degradation rates

(or lack of degradation) in both compartments are consistent with the available environmental fate data and the predicted transfer rates pass the Fsed test. The default approach is to set the degradation rate in the compartment with the higher degradation rate to the total system degradation rate calculated in Level P-1. The degradation rate in the other compartment is set to a half-life of 1000 days. This is a conservative approach because the overall degradation rate will be less than the total system degradation rate calculated in Level P-1. If both compartments show degradation rates not significantly greater than zero or if the environmental fate data do not rule out that the degradation rate could have been higher in the compartment showing no degradation, the third default approach should be used.

The second default is used when the Fsed test fails due to no back transfer, but the degradation rates are positive and consistent with the available environmental fate data. In this case the lack of back transfer results in a higher than actual degradation rate in the sediment and a lower than actual degradation rate in the water column. If the degradation rate in the sediment is faster than in the water column (initial Level P-II fit after modification), then the degradation rate in the sediment is set to the total system degradation rate calculated in Level P-1 and the degradation rate in the water column is set the value obtained in the initial Level P-II fit after modification. If the degradation in the water column is faster than in the sediment, the degradation rate in the water column is set to the total system degradation rate calculated in Level P-1 and the degradation rate in the sediment is set to a half-life of 1000 days.

The third default approach is used when the Fsed test fails (except for the case of no back transfer or when the results from the initial fit (after modification) are not consistent with available environmental fate data. As a result, there is no confidence in the kinetic analysis of the water-sediment experiment. In this case, the following two cases are evaluated using the transfer rates and initial concentrations from the initial P-II fit (after modification):

- The degradation rate in the sediment is set to the total system degradation rate calculated in Level P-1 and the degradation rate in the water column is set to a half-life of 1000 days.
- The degradation rate in the water column is set to the total system degradation rate calculated in Level P-1 and the degradation rate in the sediment is set to a half-life of 1000 days.

The case that provides the highest calculated concentrations in the water sediment study should be used in FOCUS surface water modelling with TOXSWA. Tests done to date (see Appendix 10) indicate that one of the cases when used as input to TOXSWA generally results in higher concentrations for both water and sediment. The exceptions observed to

date have been minor, so the default approach can still be conservative, and this evaluation can prevent the need to assess both cases with TOXSWA for all PEC_{sw} calculations

In some situations the Fsed test may be too strict, so that fitting with TOXSWA as described in Section 10.3.4 should be considered as an alternative when the Fsed test is not passed. TOXSWA fitting is an alternative to all three default parameter approaches.

When an acceptable fit has been found (no default parameter approaches used) in Level P-II, DegT50/90 for the whole system, DT50/90 from the water column and DT50/90 from the sediment may be calculated in Level P-II as a check to see if the results are similar to the results obtained at Level P-I. DegT50/90 in the whole system is simply calculated by summing the amount in the water column and the sediment and finding when 50% and 90% has degraded. DT50/90 in the water column calculated in a similar way, except that only the amount in the water column is used. And DT50/90 in the sediment is calculated in a similar way, but with time zero taken as the time at which the residues reach a peak in the sediment.

As noted earlier the flow chart for Level P-II requires the assessment of whether the degradation parameters are significantly greater than zero, particularly to demonstrate that degradation occurs in the water column and/or the sediment compartments. Such a test can be conducted if the degradation rate parameter is greater than zero. However, when the other degradation rate parameter is zero, then the implementation of such a test is problematic due to forcing all of the degradation to occur in one compartment. Therefore, this must be checked to see if this is consistent with other environmental fate data.

10.3.4 Alternative approach using TOXSWA

As an alternative to the procedures shown in Fig. 10-3, TOXSWA may be used to fit the water sediment data. TOXSWA describes the exchange between water and sediment itself assuming a uniform water concentration and Fick's law for diffusion in the sediment. Adriaanse *et al*. (2002) developed some initial guidance for describing water-sediment studies with TOXSWA. The criteria of passing the Fsed test, including non-zero back transfer, and degradation rate coefficients significantly above zero, do not need to be considered then because TOXSWA describes the exchange between water and sediment as mechanistically as possible given current knowledge. Moreover, TOXSWA (and thus the same concept for water-sediment exchange) is also used within the FOCUS Step 3 scenarios. However, the criterion of consistency with environmental fate data has to be checked because the fitting procedure is based on an iterative optimisation procedure which may have non-unique solutions.

When the TOXSWA solution is not consistent with environmental fate data, the third default approach described in Section 10.3.3 is used to determine the default degradation rates used in PEC_{sw} calculations.

Appendix 12 gives further guidance on how to fit TOXSWA to water-sediment systems including one example.

10.3.5 Application of Levels P-I and P-II

Three example parent compounds are used here to illustrate the application of levels P-I and P-II for non-volatile compounds (named Compounds 1, 3 and 6). In order to estimate persistence and modelling endpoint at level P-I, the data for the whole system, the water column and the sediment (after the peak height) were entered and three of the four recommended kinetic models were run[11]. For Compound 1, no modification to the fitting was required to fit the models to the data. For Compound 3, however, the value of M_0 for the water column was constrained to that obtained using water and sediment data, since it was much lower than this using a free fit. For Compound 6, an outlier (day 28 low recovery of 14%) was removed due to its large influence over the fitting and which reduced the differences between the kinetics models. The final results for three of these models are shown graphically in Figure 10-4A to C for the whole system (wc+sed), the water column (wc) and the sediment (sed).

[11] An attempt was made to run DFOP in another package. However, difficulties were experienced with fitting, in particular since the results were so similar to that for SFO kinetics.

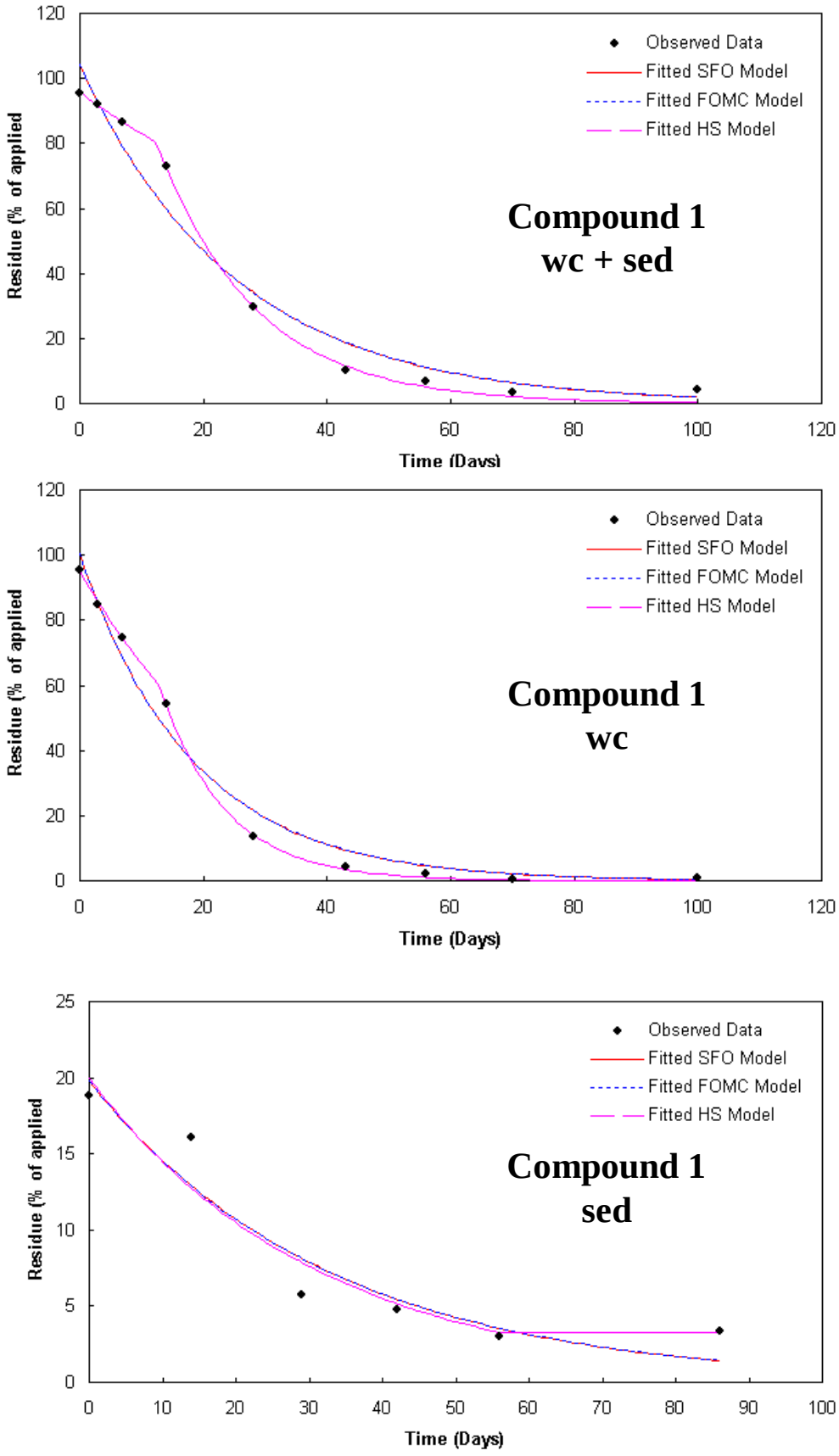


Figure 10-4A. Kinetic fits (level P-1) obtained for Compound 1 for the whole system (wc+sed), the water column (wc) and the sediment (sed).

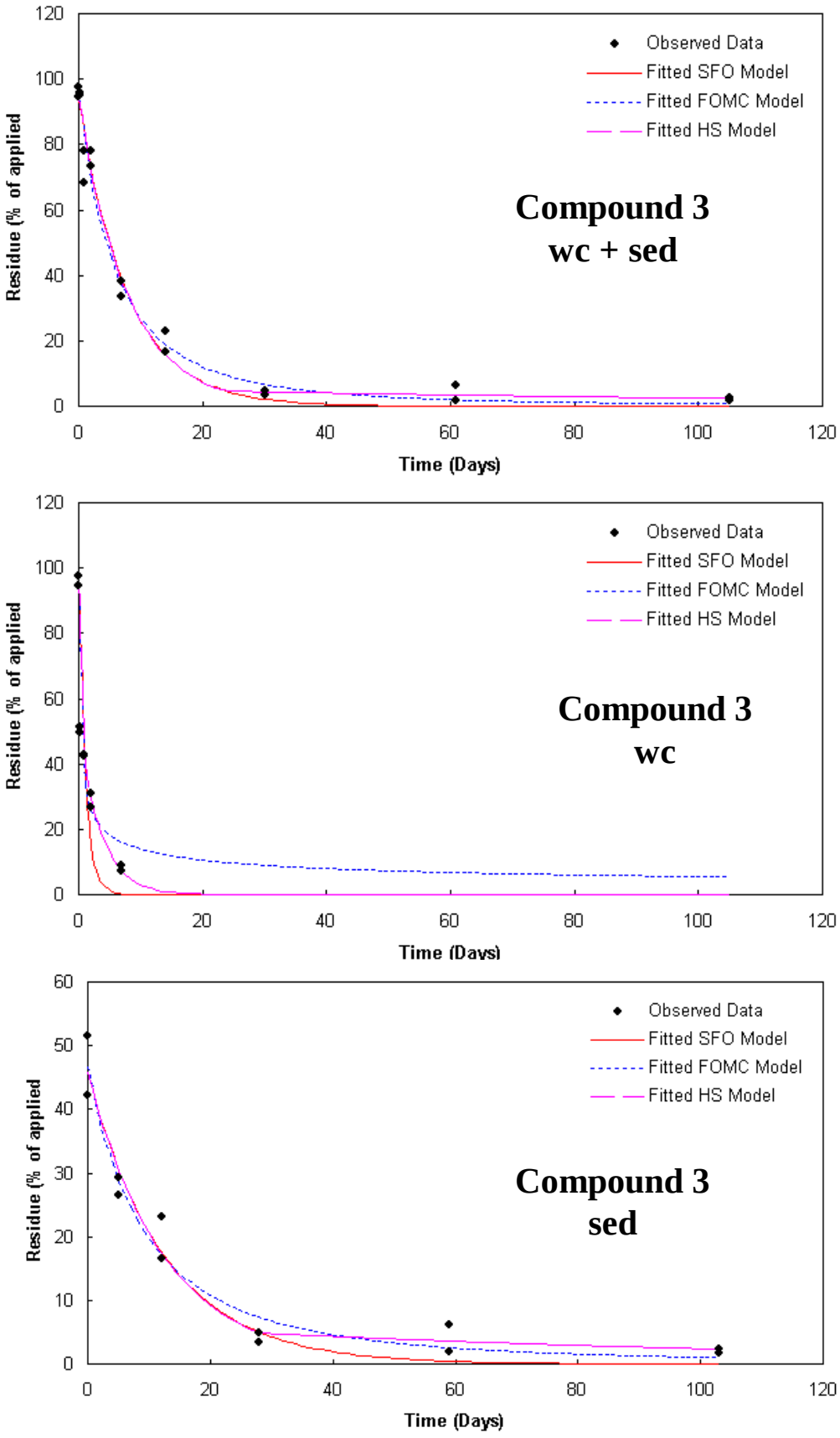


Figure 10-4B. Kinetic fits (level P-1) obtained for Compound 3 for the whole system (wc+sed), the water column (wc) and the sediment (sed).

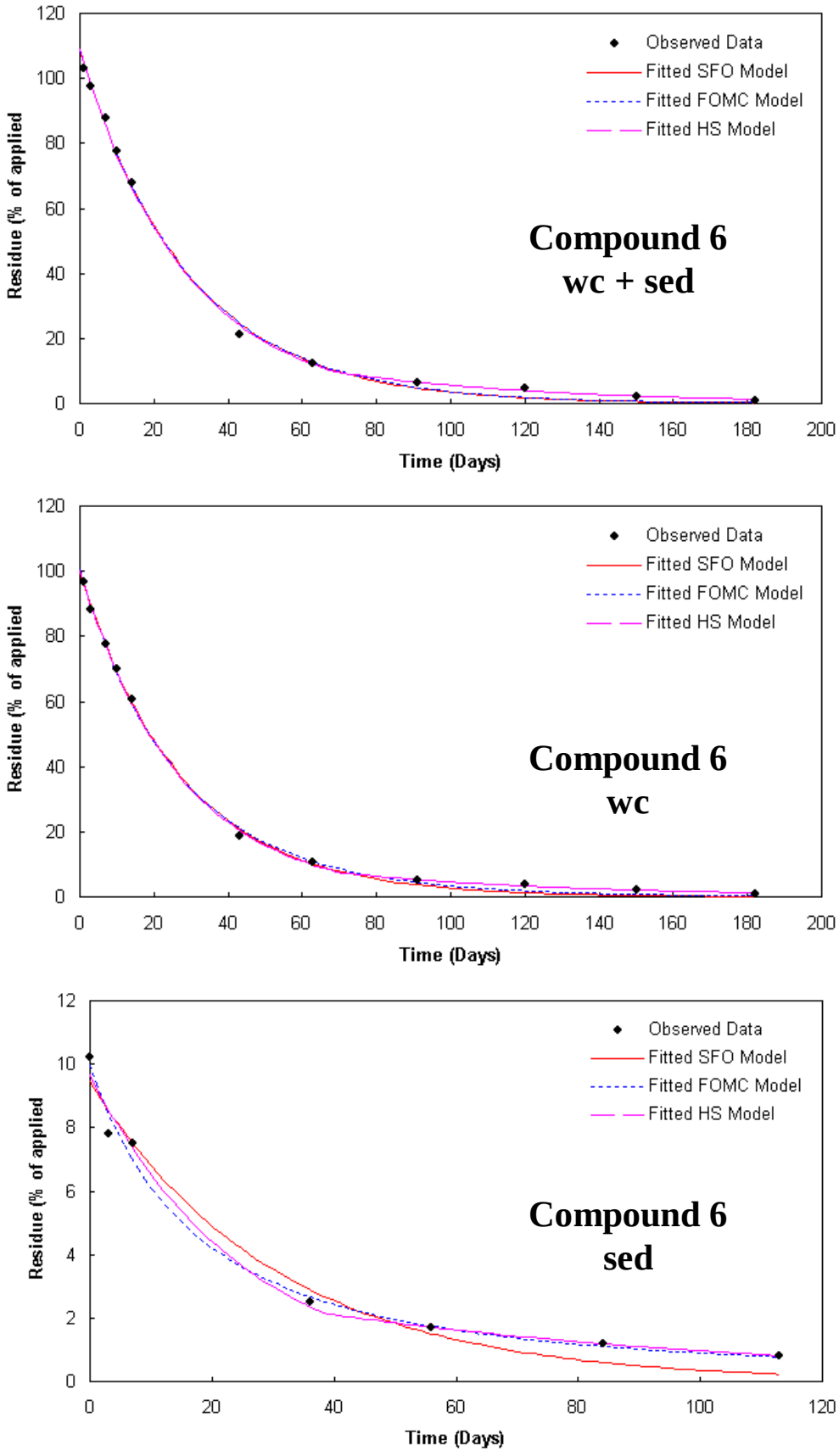


Figure 10-4C. Kinetic fits (level P-1) obtained for Compound 6 for the whole system (wc+sed), the water column (wc) and the sediment (sed).

The values for the DT50 are also shown in Table 10-6 along with the calculated error percentage at which the χ^2 test passes (Values in bold are for χ^2 values that exceeded the threshold value of 15%, although a visual inspection may show that these fits may be considered to be acceptable). A visual comparison of the three kinetic models shows that in general they produce similar fits to the data with two exceptions. The first exception is for Compound 1 (system and water column data) in which disappearance appears to increase after day 14 when residues reached their peak in sediment, due to the more rapid dissipation after the breakpoint in the fit of hockey-stick kinetics to the data for both whole system and water column data. This may indicate that transfer to sediment was a key rate-determining step in degradation in the system.

The second exception is over the later data points indicating a disappearance pattern that is relatively slower than could occur by SFO kinetics, which applied to sediment data for Compound 1 and to system, water column and sediment data for Compounds 3 and 6.

The net result at Level P-1 for all three compounds is that SFO kinetics generally appeared to give acceptable fits to the data based on the χ^2 values. The exceptions were Compound 1 for the sediment and Compound 3 for the water column. For Compound 1, this is mainly due to random scatter in the data and so it may be acceptable, particularly as similar results occur for all three types of kinetics. For Compound 3, the persistence endpoint for the water column would be estimated using HS kinetics since it provided an acceptable best-fit. The modelling endpoint for disappearance from the water column would be estimated using the FOMC fit, if an acceptable fit was obtained. However, the fit is not visually acceptable since it does not capture the DT90, so in this case the modelling endpoint should be estimated using HS kinetics which gives a visually acceptable fit resulting in a half-life of 0.80 days as DT90 / 3.32.

Table 10-6. Kinetic results obtained in three example cases, including the χ^2 error values plus the theoretical and fitted *Fsed* values.

Level	Compound	Compartment	Modification	DT50 in days (χ^2 error value)		
				SFO	FOMC	HS
P-I	1	wc+sed	None	17.4 (12.5)	17.3 (13.3)	20.6 (3.2)
		wc	None	12.6 (10.8)	12.6 (11.5)	15.3 (1.7)
		sed	None	22.6 (**17.6**)	22.5 (**19.4**)	21.5 (**17.4**)
	3	wc+sed	None	5.47 (7.7)	4.77 (7.1)	5.42 (8.1)
		wc	Fix M_0	0.794 (**21.9**)	0.388 (10.1)	0.447(2.5)
		sed	None	8.74 (10.5)	7.74 (9.6)	8.65 (9.1)
	6	wc+sed	Remove outlier	20.1 (3.4)	20.1 (3.6)	19.8 (3.0)
		wc	Remove outlier	19.1 (2.8)	18.6 (2.7)	18.7 (1.9)
		sed	Remove outlier	21.1 (9.4)	15.2 (6.5)	17.7 (7.7)

Level	Compound	Compartment	Modification during Initial Fit*	DegT50 in days (χ^2 error value)*	*Fsed*	
				SFO	Modelled	Theoretical
P-II	1	wc	None	27.9 (11.4)	1.00	0.48-0.91
		sed		9.54 (**16.7**)		
	3	wc	None	3.02 (**16.4**)	0.46	0.96-0.99
		sed		∞ (**25.5**)		
	6	wc	Remove outlier Fix M_0 =100	∞ (3.1)	0.44	0.28-0.57
		sed		2.16 (9.0)		

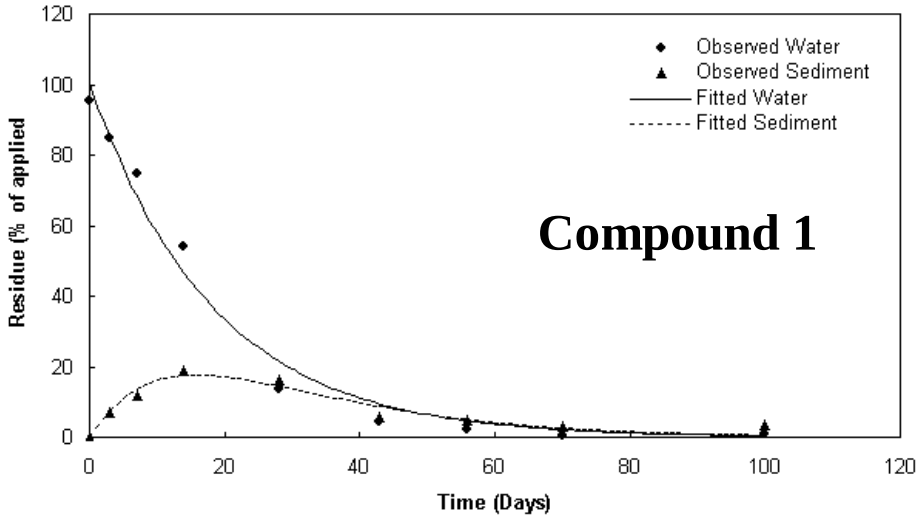
* These values are shown since these fits are used to calculate the Fsed and χ^2 values, but these are not the final kinetic results from the kinetic analysis. See the accompanying text for the results to be used in FOCUS SW modelling.

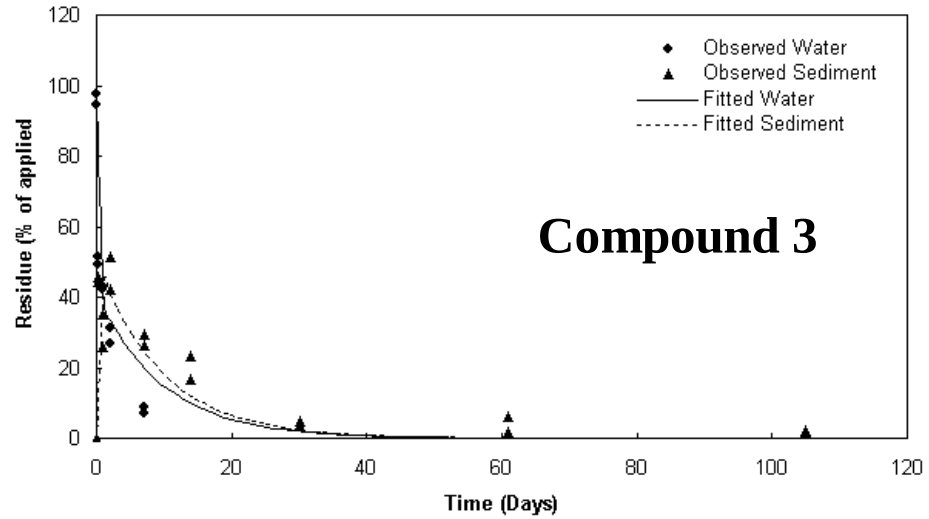
Next, in order to estimate the persistence and modelling endpoints using the flow chart in Figure 10-3, the data were entered and the water-sediment model in Box 10-2 was run. Fsed values were calculated from the fitted transfer rates and the theoretical ranges were derived as described in Appendix 9. The final fits of the model to the water column and sediment data for the three compounds are shown in Table 10-6 and graphically in Figure 10-5. The derivation of these final fits is now discussed in detail.

For Compound 1, the initial fit appeared to be statistically and visually acceptable; however, this fit was achieved with zero back transfer from the sediment to the water column. The consequence of this is that the degradation rate in sediment is likely to be somewhat too fast and that in the water column somewhat too slow. For use against study triggers, the recommendation here is thus not to use the Level P-II sediment DegT50 (9.5 days) but to use the Level P-I sediment DT50 (23 days) instead. And for use in FOCUS SW modelling, the recommendation is to use the Level P-II DegT50 value for the water column and the Level P-I system DegT50 value for the sediment to ensure that the degradation rate is not overestimated in sediment.

For Compound 3, the initial fit did not appear to be statistically and visually acceptable, particularly for the sediment. Attempts were made by stepwise modification of the fitting. This did not improve the fit noticeably, so a case-by-case decision would need to be made. In this case, for use against study triggers, the recommendation here is thus to not use the Level P-II sediment DegT50 (1000 days) but to use the Level P-I sediment DT50 (8.7 days) instead. Since the Fsed test failed and the initial Level P-II fit was considered not be consistent with the environmental fate data because it did not indicate in which compartment the degradation would be more rapid, the recommendation for use in FOCUS SW modelling would be evaluate both cases of setting the degradation rate in one compartment to the Level P-1 system half-life and to 1000 days in the other compartment (note that in this case both the Fsed test was failed and the Level P-II fit was inconsistent with environmental fate data, but either is sufficient to require this approach). When this was done according to the procedures described in Section 10.3.3 for the third default approach (the results of this are shown in Appendix 10, Figure A10-5), there was little discernible difference between the two approaches. Since setting the water column to the Level P-1 system half-life and the sediment to a half-life of 1000 days was marginally worse, this default approach is recommended for deriving inputs for FOCUS SW modelling.

For Compound 6, the initial fit appeared to be statistically and visually acceptable. However, the apparent degradation rate in the water column was zero and the modelled Fsed value was 16 percent. Constraining the initial value to 100 percent of applied radioactivity (the free fit was 105 percent) increased the Fsed value to 44 percent, resulting in a passed Fsed test. Since initial fit was considered to be inconsistent with the environmental fate data, since it did not indicate in which compartment the degradation would be more rapid, the recommendation for use in FOCUS SW modelling would be evaluate both cases of setting the degradation rate in one compartment to the Level P-1 system half-life and to 1000 days in the other compartment. When this was done using the procedures described in Section 10.3.3 for the third default approach (the results of this are shown in Appendix 10, Figure A10-5), there was an obvious difference between the two approaches. Since setting the sediment to the Level P-1 system half-life and the water column to a half-life of 1000 days resulted in higher amounts in both compartments, particularly the water column, this default approach is recommended for deriving inputs for FOCUS SW modelling.





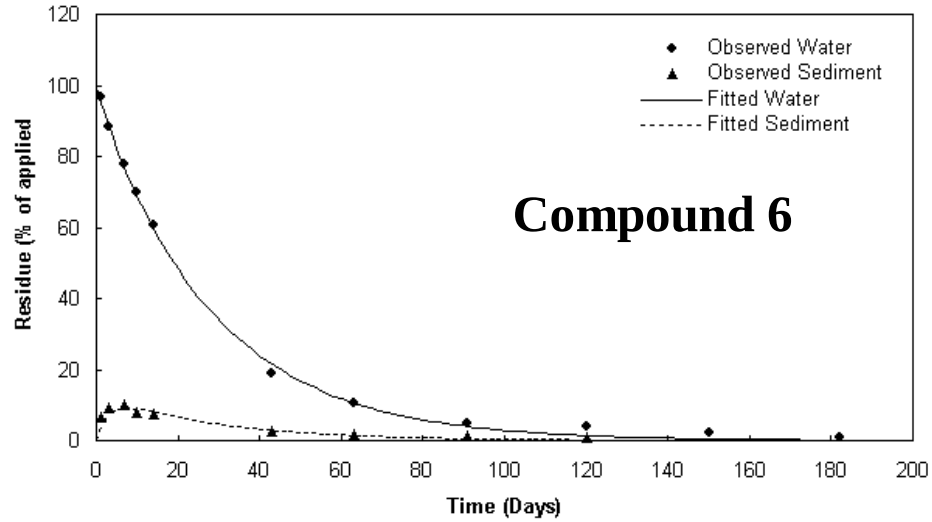


Figure 10-5. Water and sediment kinetic fits (Level P-II) for Compounds 1, 3 & 6

10.3.6 Resort for cases that require further consideration

In some cases further consideration will be required, if Level P-II fails to accurately describe the concentrations measured in water-sediment studies, or if TOXSWA fails to describe these concentrations. For example, this may be due to the compound exceeding its solubility, so it precipitates and then re-dissolves later on in the water-sediment study. Obtaining degradation rate parameters from such studies may be possible by tailoring the kinetics to include a precipitated phase. However, care should be taken to ensure that the use of tailored kinetics is scientifically justifiable.

10.4 Metabolite kinetics

10.4.1 Introduction

This section provides guidance on how to derive persistence and modelling endpoints for metabolites that are formed and degraded in water-sediment systems treated with the parent substance or another precursor of the metabolite. In cases when the metabolite itself is applied to the system as parent compound, refer to the guidance for parent kinetics. The work group decided to use compartmental approaches rather than more detailed mechanistic approaches for determining metabolite kinetics, to integrate the estimation of persistence and modelling endpoints as much as possible and make the overall approach consistent. Two levels were proposed: Level M-I for one-compartmental (system, water or sediment) approaches and Level M-II for two-compartmental (water and sediment) approaches. Owing to the difficulties experienced in estimating disappearance times for parent compounds in two-compartment systems, only a recommended approach for Level M-II can be given at this point. For substances that require further consideration beyond that outlined here, refinements or alternatives can be considered on a case-by-case basis. This is because the first two levels (M-I and M-II) should adequately cover the majority of cases, so alternatives/refinements should only be used as a last resort.

Within this overall approach, the recommended methods of fitting kinetics to data are similar to those for kinetics in other test systems such as soil systems. Hence, with respect to data entry, selection of fitting routine, selection of constraints, data exclusion, and types of kinetics considered, the general recommendations in Chapters 7 and 8 also apply here. For example, metabolites which appear at a nominal time zero in a water-sediment study should

be included as parent material, since this is normally a little time after application of parent and it is a simpler correction than trying to estimate the exact time of sampling.

Likewise, the methods used to make kinetic decisions, such as graphical and statistical evaluation of the goodness of fit are similar to those already discussed for kinetics in other test systems (see Chapters 7 and 8), while specific recommendations for water-sediment systems, e.g. regarding the number of parameters to be considered in the χ^2 test, are summarized in Section 10.2. In addition, details of what needs to be reported and where in the study report / raw data are discussed in Chapter 12, particularly with regard to the logic of the kinetic approach taken and the recording of the approach taken.

Given that the fate of metabolites formed in water-sediment studies is even more complex than that of parent compounds, disappearance times in these studies should be estimated only if required. First, such estimates may not be required to assess the relevance of certain metabolites, e.g. minor metabolites if they do not exceed certain levels, or if their potential ecotoxicological risks are implicitly covered by higher-tier ecotoxicity studies on the parent compound. Secondly, water-sediment or other aquatic studies are sometimes conducted using metabolites as parent substance, i.e. the substance is applied to the water column. If such studies are available, the parent scheme (Level P-I and P-II) should be used instead to estimate metabolite kinetics. However, justification is needed that such studies are the most appropriate ones from which to estimate the kinetics endpoints. Third, in certain cases metabolite formation is completed very rapidly after the application of parent substance, e.g. parent ester compounds that breakdown within days to acid metabolites that break down more slowly. In such cases, it is justifiable to add parent and metabolite data because degradation of the metabolite is the rate-determining step, so the parent scheme (Level P-I and P-II) can be used instead to estimate metabolite kinetics. However, while parent and metabolite may be combined to generate kinetic endpoints, the two substances may have very different toxicity and require separate risk assessments.

10.4.2 Level M-I

At Level M-I, both persistence and modelling endpoints are estimated using one-compartmental approaches that represent the:

- *Dissipation* rates from the whole system, the water column or the sediment as the decline phase from the peak metabolite level as shown in Box 10-3
- *Degradation* (plus *formation*) rates in the whole water-sediment system after an application to the system at time zero as shown in Box 10-4

Dissipation rates from the whole system, the water column or the sediment as the decline phase from the peak metabolite level may be used as persistence and modelling endpoints. In addition, degradation rates from the whole system may also be used as modelling and persistence endpoints. A summary of the different recommended uses of dissipation and degradation rates as decline DT50/90 and DegT50/90 from fitting one-compartmental models at Level I is provided earlier in Table 10-2A for persistence endpoints, and Table 10-2B for SFO modelling endpoints using FOCUS SW modelling as an illustrative example. The decline DT50/90 for the water column and sediment compartments at Level I provided earlier in Table 10-2A may also be used for simple spreadsheet calculations from decline that are not restricted to SFO kinetics.

Persistence Endpoints. These can be defined as based either on the *degradation rate of the metabolite* in a given compartment (after formation/entry into it), or on the *duration of metabolite exposure* in the compartment (assessed as the dissipation rate from decline of the metabolite after it reaches its peak amount). The user needs to decide which of these endpoints is the most relevant for the specific trigger considered and metabolite of interest.

Metabolite degradation rates can be obtained for the whole system using one-compartment approaches described in the next section with data for parent and metabolites. The degradation rates for metabolites in the water column and sediment, however, can only be obtained with a complex two-compartment approach. The system degradation rate may nevertheless serve as a conservative estimate of the degradation in the compartment where most of the degradation occurs.

Metabolite dissipation rates (DT50/90) are more appropriate to use to describe the *duration of the exposure* in the compartment of interest. However, when estimating the decline rate from the water or sediment data is not possible, e.g. because there are too few data points, or because of data scattering, then the system decline DT50/90 may in some cases be used instead as a conservative estimate of the dissipation in the compartment. In such cases, the conservative nature of the estimate needs to be justified, using information such as compound properties and behaviour in a weight of evidence argument.

Modelling Endpoints. Apart from the FOCUS SW modelling, PEC values for surface water (PEC_{sw}) and sediment (PEC_{sed}) are usually calculated using simple spreadsheets or tools, e.g. Excel or ModelMaker, for simple worst-case scenarios. For example, initial PEC_{sw} and PEC_{sed} for metabolites are often derived from the application rate of the parent compound and the maximum observed level of the metabolite in the corresponding compartment. In

such cases, PEC values at later time points should be calculated based on the decline data, and should not be limited to first-order kinetics. Hence, the modelling endpoints used for these types of PEC calculation should be based on the kinetic model that best fits the experimental decline data from the maximum observed.

For FOCUS SW modelling, however, SFO endpoints for the whole system are required at Step 1, and for the water column and sediment separately at Step 2 and Step 3. Since at Step 1 and 2 calculations for the metabolite are performed based on its maximum observed level in the compartments (system at Step 1 and separate water column and sediment at Step 2), decline half-lives are the appropriate endpoints at these Steps. However, since at Step 2 partitioning is already calculated based on the substance Koc, the decline half-lives from the water column and sediment compartments, which do not distinguish dissipation by transfer from degradation, are not appropriate, and the whole system decline half-life should be used for both compartments instead, following the recommendation of the FOCUS Surface Water Scenarios Workgroup[12]. At Step 3, degradation half-lives are required.[13] While the actual degradation rates in the water column and sediment cannot be derived with one-compartment models (and can be difficult to obtain with two-compartment models due to the complexity of the processes involved), degradation or dissipation endpoints for the total system may in some cases be used as conservative estimates for water or sediment degradation. Actual degradation rates for metabolites in the whole system can be derived from the one-compartmental approach described in the next section, using data for parent and metabolites together. As a conservative alternative, the dissipation endpoints for the metabolites can be estimated from the decline data in the system. The degradation rate for the total system may only be used as conservative estimate for the compartment where most of the degradation is assumed to occur, in combination with a conservative default value such as 1000 days for the other compartment. The worst-case nature of the modelling endpoint used must always be discussed based on all available data for the substance of interest, including distribution between water and sediment, sorption to soil and sediment, and weight of evidence from hydrolysis, anaerobic aquatic, anaerobic soil and other studies.

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[12] The actual recommendation of the surface water workgroup was to use degradation half-life in total system. However, since at Step 2 the metabolite concentrations are calculated from its maximum observed levels in water and sediment, dissipation should be described with a decline rate from the maximum onwards rather than with a degradation rate (as the latter would overestimate the actual decline).

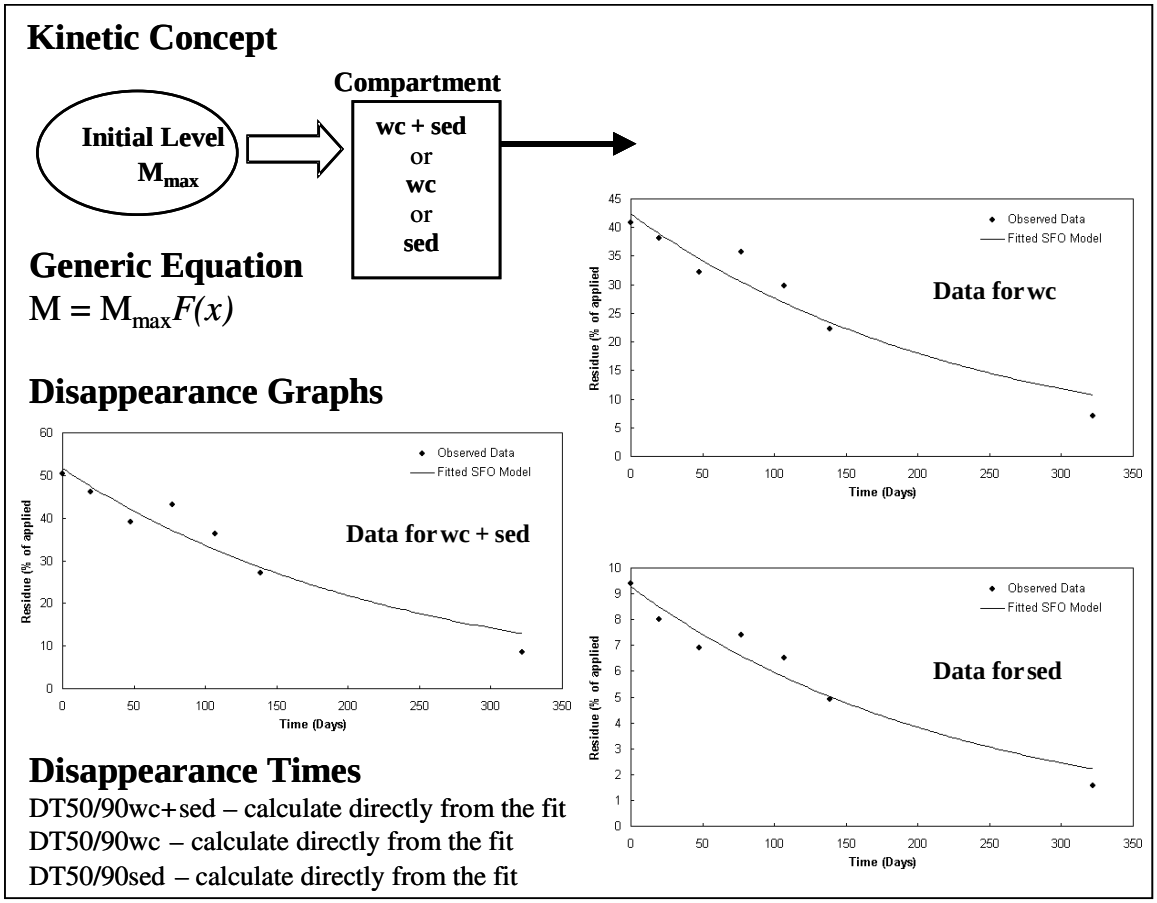
[13] Note: Current versions of the Step 3 FOCUS surface water models simulate the entry of metabolites formed in soil into the water bodies using MACRO and PRZM and the subsequent degradation of these metabolites in the water and sediment using TOXSWA. The formation of metabolite from parent substance in the water body is currently not considered in TOXSWA. Simple approximations are used instead to estimate PEC values for metabolites in water and sediment where this is a relevant process.

Guidance is provided below on how to derive the SFO modelling endpoints required for FOCUS SW.

10.4.2.1 Dissipation

For dissipation from the whole system, the water column or the sediment as DT50/90, estimation only requires kinetics to be fitted to the corresponding decline data for each compartment, starting from its maximum observed level in the compartment. Time zero is defined as the time the peak observed metabolite level is reached. As shown in Box 10-3, the dissipation endpoints are estimated using a generic equation describing the decline from the peak metabolite level (M_{max}), where the function $F(t)$ describes the rate of dissipation from that time on and is determined by the kinetic model employed, for example $F(t) = \exp(-kt)$ for SFO kinetics.

Three types of kinetics (SFO, FOMC and DFOP kinetics) are recommended. SFO kinetics are recommended as the default first choice and because FOCUS Surface Water modelling requires the use of SFO kinetics. FOMC kinetics are used to help evaluate whether the data depart appreciably from SFO kinetics, and DFOP kinetics are used because they have more flexibility in shape than FOMC kinetics due to having one more parameter. Hockey-stick kinetics are not recommended at this level for reasons outlined in Chapters 7 and 8.



Box 10-3. Metabolite Kinetics at Level M-I

Box 10-3 shows a generic kinetic equation describing disappearance from the compartment, where the function *F*(t) describes the rate of dissipation and is determined by the type of kinetics. For substitution of all the various types of kinetics into this generic equation, please see Chapter 5. The kinetic endpoints (DT50/90 values) can be calculated directly from the fits of these equations as described in Chapters 7 and 8.

Box 10-3 also shows an example of disappearance patterns from the whole water-sediment system, plus from the water column and from the sediment for a metabolite (a breakdown product of Compound 6), with the corresponding fit with SFO kinetics to the data for each compartment (FOMC and DFOP kinetics are not shown because they are virtually identical). The graphs indicate that dissipation from the whole system, the water column and sediment were all similar (DT50≈160 days). For further details of this example, including plot of residuals and statistical indices for the goodness of fit, see Section 10.4.3.

The recommended procedures to estimate the *persistence endpoints* from the decline of the metabolite from its maximum are outlined in Figure 10-6. The procedures essentially operate in the same way as those described in Chapters 7 and 8, so they are not repeated here.

These procedures need to be run three times, to cover dissipation from the whole system, the water column and the sediment degradation. Next, the recommended procedures to estimate the *modelling endpoints* from the metabolite decline in total system are outlined in Figure 10-7 for level M-I. Again, the procedures essentially operate in the same way to those described in Chapters 7 and 8, so they are not repeated here. Two sets of procedures are used because best fit kinetics are needed to estimate persistence endpoints, while SFO kinetics fits are required to estimate modelling endpoints (including how to derive a half-life when SFO kinetics do not provide the best fit to the data). An example of how to use these procedures is given in Section 10.4.3.

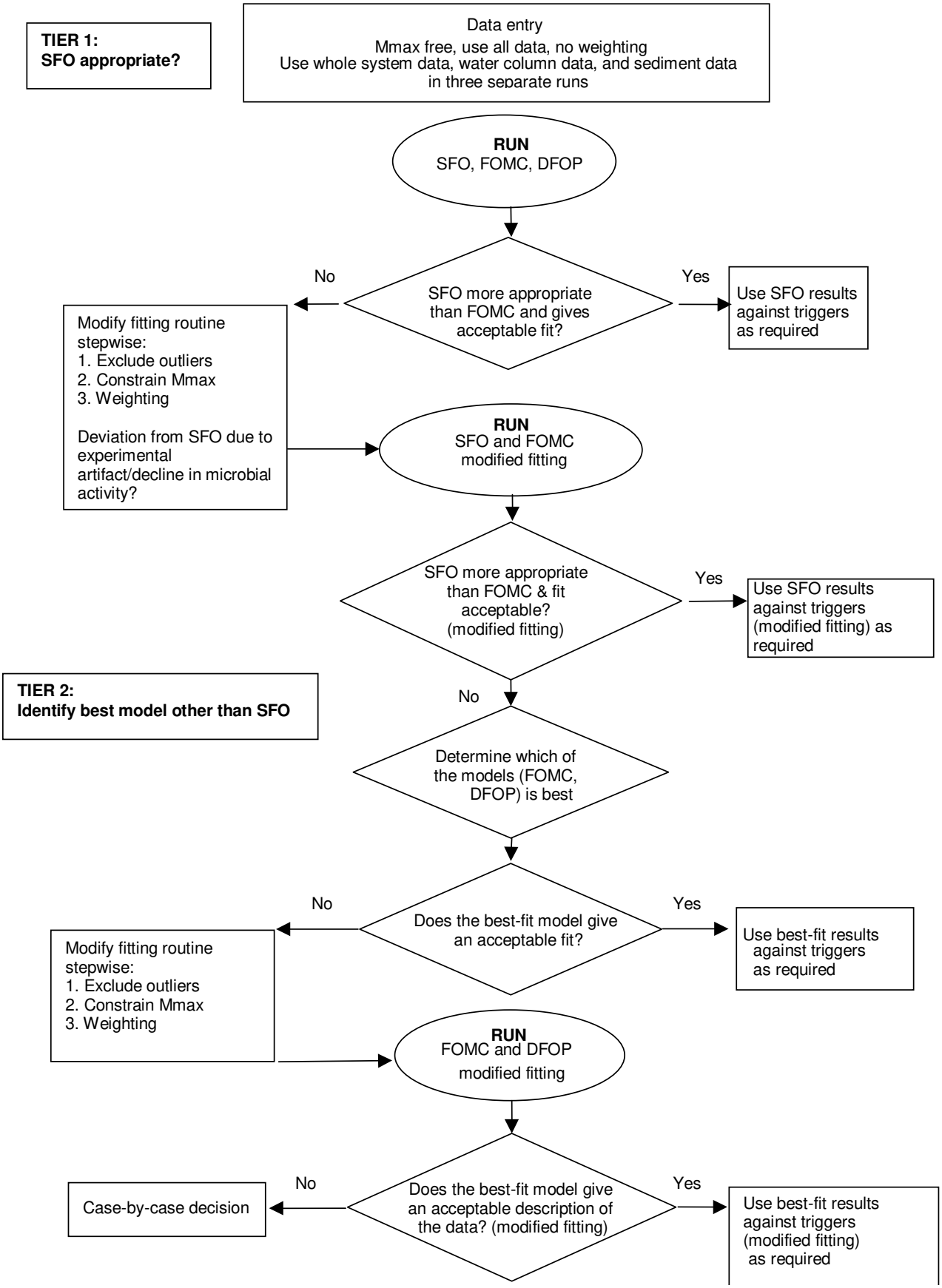


Figure 10-6. Recommended procedure at Level M-I to estimate persistence endpoints based on metabolite decline

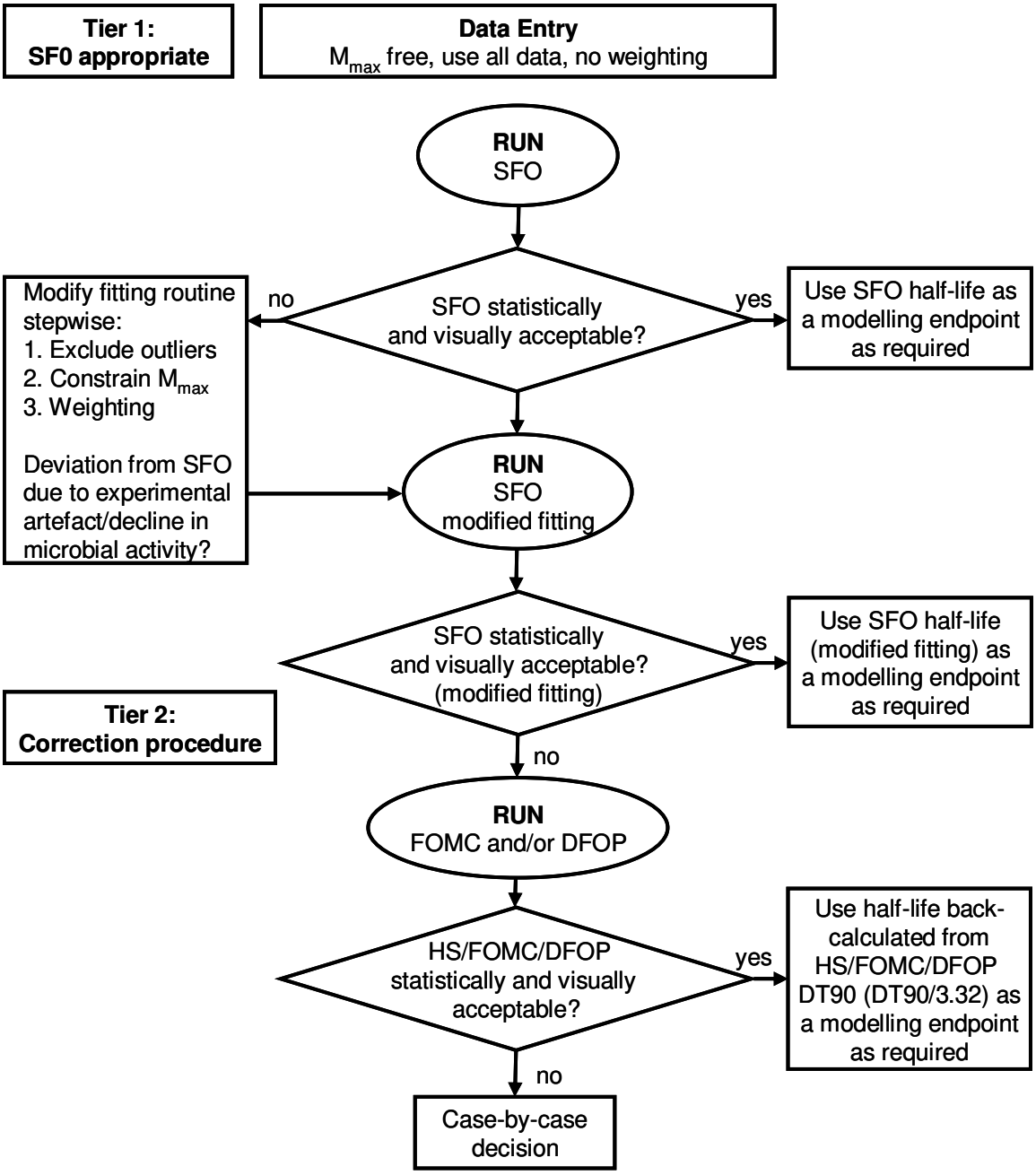


Figure 10-7. Recommended procedure at Level M-I to estimate modelling endpoints based on metabolite decline.

10.4.2.2 Degradation

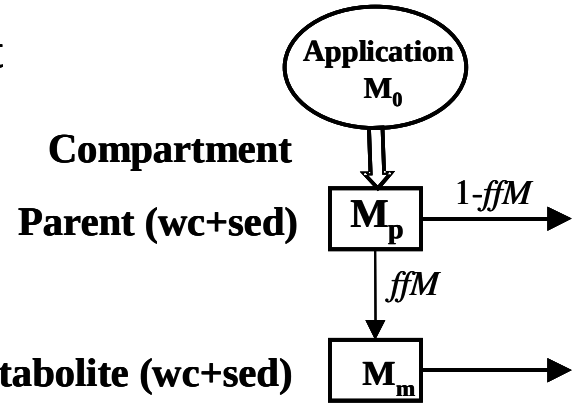
Estimating degradation in the whole water-sediment system requires fitting the whole system data to a kinetic model. As with parent substances, this is only valid for non-volatile compounds that only undergo losses by degradation. It may also be used for slightly volatile compounds if volatile losses can be accounted for by trapping, identification and quantification of volatiles (see Appendix 11 for details of the correction procedures, though a justification for their use must be made). The procedure for determining the formation and

degradation endpoints for metabolites in the total system is identical to the procedure described in Chapter 8 for other one-compartmental systems such as soil. The procedure can be used to evaluate the kinetics of several metabolites at once, as long as the metabolic pathway is known and the data supports the fitting, as discussed in Chapter 8.

A simple example of metabolite degradation kinetics is shown in Box 10-4 for a single metabolite formed by the breakdown of parent substance (a breakdown product of Compound 6). In Box 10-4, the breakdown of a fraction (*ffM*) of the parent, M_p[14], applied at a rate of M_0, results in the formation of the metabolite, M_m, while the remaining fraction of parent (1- *ffM*) degrades via another pathway that does not need to be specified explicitly, e.g. the formation of non-extractable residues, another metabolite etc. A specific sink term has thus not been included, since using sink data is generally not recommended (cf. Chapter 8).

[14] The term parent here is used in its broadest sense: it is only meant to denote the test substance applied, so it may also refer to the metabolite of a pesticide.

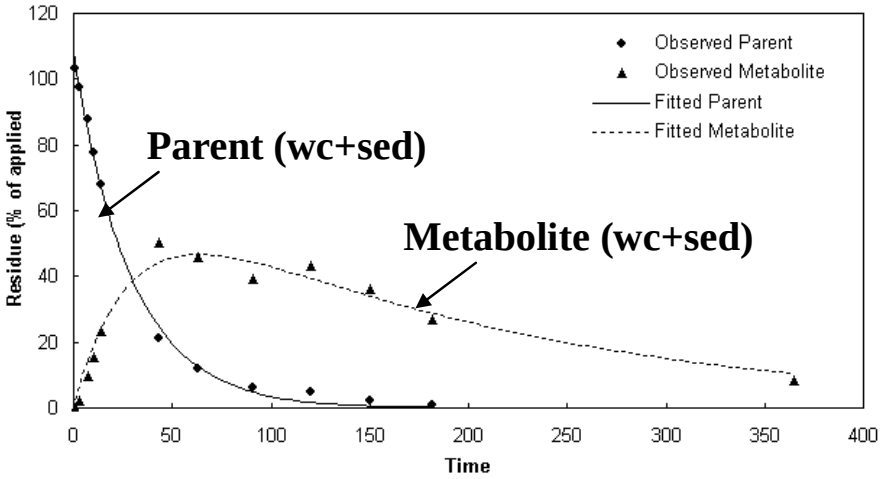
Kinetic Concept



Generic Equations

$$M_P = M_0 \, F_P(t) \qquad M_m(t) = -ffM \int_0^t M_0 \, [dF_P(t_i) / dt_i] \, F_m(t - t_i) \, dt_i$$

Disappearance Graph



Disappearance Times

DegT50/90wc+sed – calculate directly from the fit

Box 10-4. An example of metabolite degradation kinetics at Level M-I.

To describe the kinetic concept in Box 10-4, two generic kinetic equations are used to cover parent and metabolite behaviour[15], where the generic functions $F_P(t)$ and $F_m(t)$ describe the rate of parent and metabolite degradation, respectively. SFO, FOMC, DFOP, or HS kinetics can be substituted into the equation for parent, but only SFO, DFOP, or FOMC kinetics for

[15] The equation for metabolite behaviour is given as an integral equation rather than a differential equation, because it is generic and enables several types of kinetics to be substituted into it. For special cases, e.g. for SFO kinetics for parent and metabolite, a differential equation can be used instead, as given in Chapter 8.

the metabolite. Box 10-4 also gives an example graph of fitting kinetics to parent and metabolite data for the whole water-sediment system, which shows the fit of the degradation of the parent, and formation and degradation of the metabolite.

The recommended procedures to estimate *persistence endpoints* based on inherent degradation in the system are outlined in Figure 10-8 for level M-I. The procedures essentially operate in the same way as those described in Chapter 8, so they are not repeated here. These procedures only need to be run once. Next, the recommended procedures to estimate the *modelling endpoints* are outlined in Figure 10-9 for level M-I. Again, the procedures essentially operate in the same way to those described in Chapter 8, so they are not repeated here. Two sets of procedures are used because best-fit kinetics are needed to estimate persistence endpoints, while SFO kinetics fits are required to estimate modelling endpoints (including how to derive a half-life when SFO kinetics do not provide the best fit to the data). An example of how to use these procedures is given in Section 10.4.3.

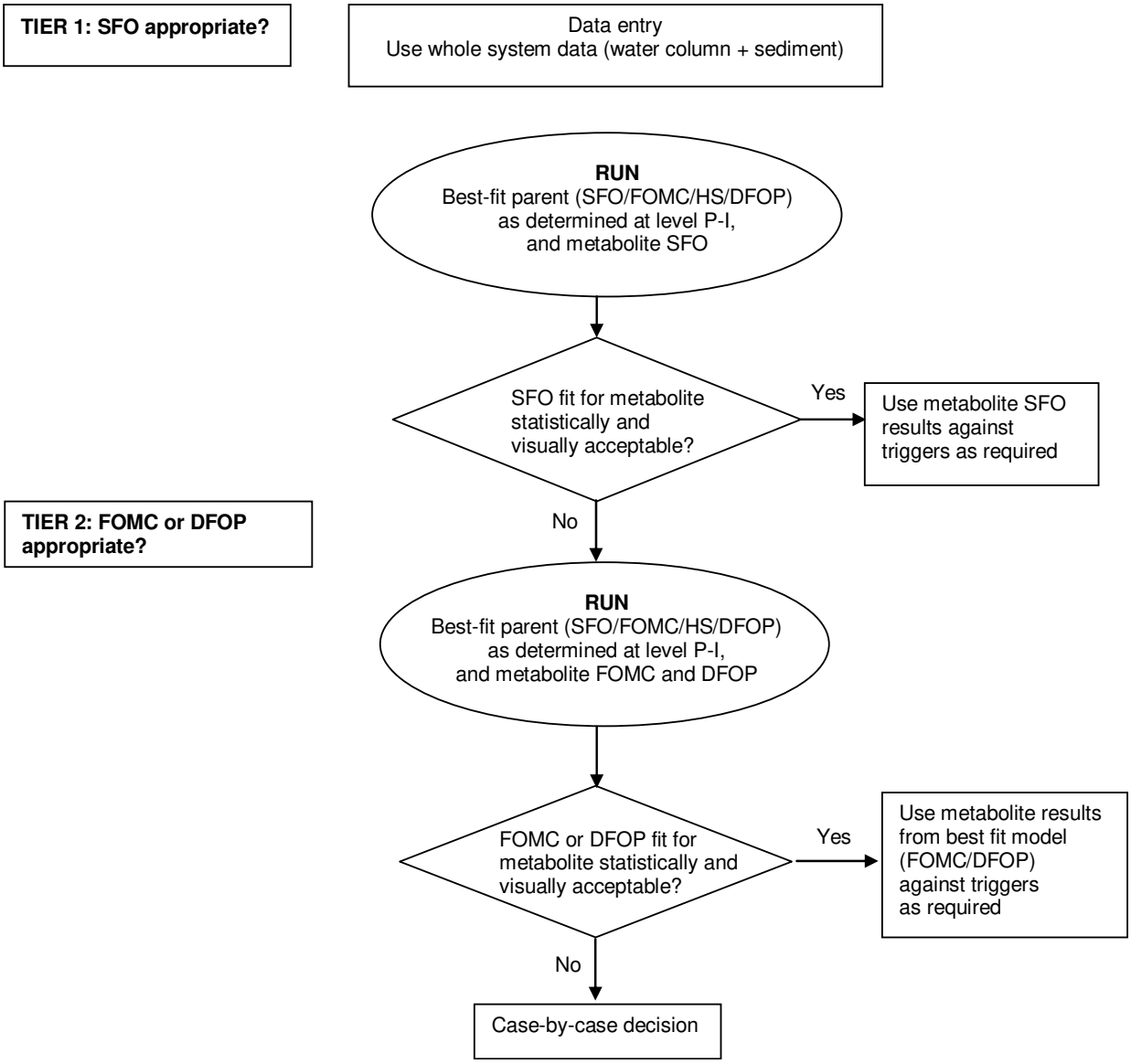


Figure 10-8. Recommended procedure at Level M-I to estimate persistence endpoints based on metabolite formation and degradation

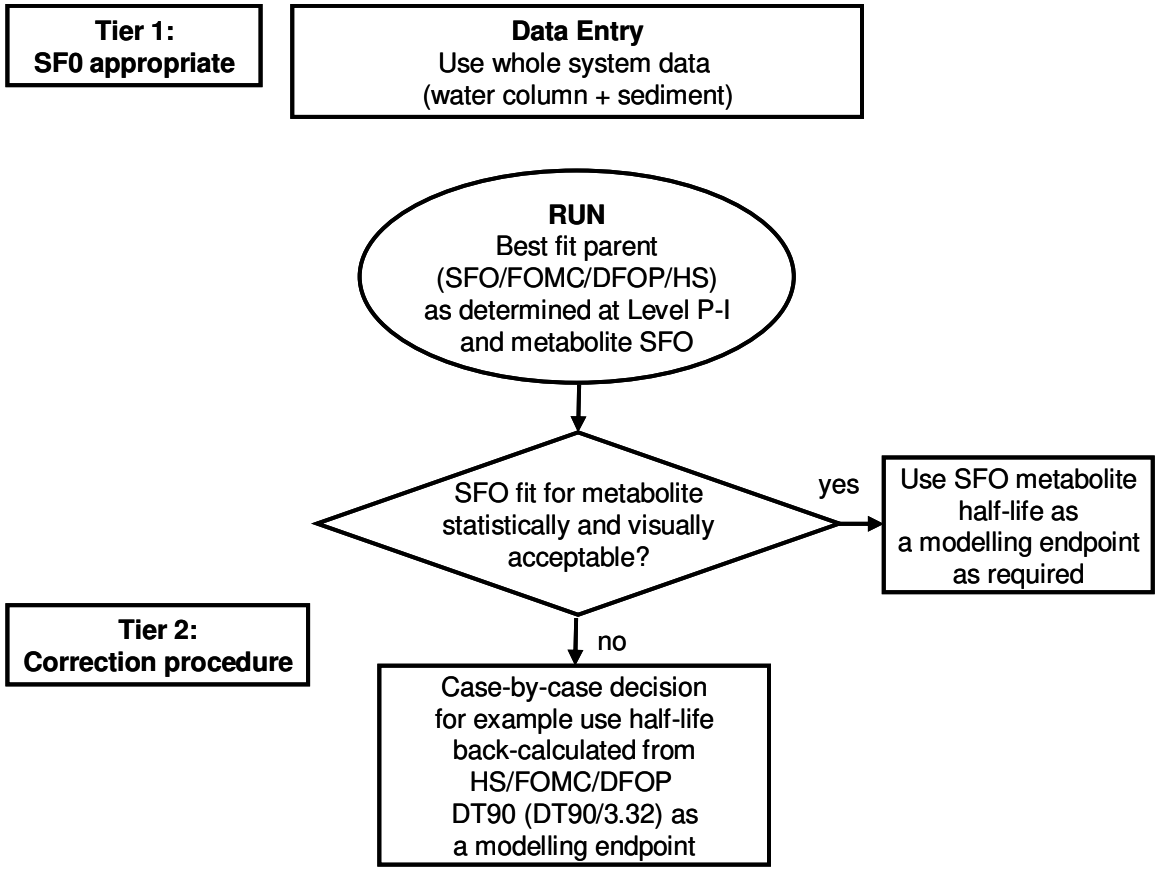


Figure 10-9. Recommended procedure at level M-I to estimate modelling endpoints based on metabolite formation and degradation.

10.4.3 Application of Level M-I

One example of the application at Level M-I is given here for a non-volatile metabolite (a metabolite forming by the breakdown of Compound 6). In order to estimate the persistence and modelling endpoints by *dissipation* at Level M-I, the decline data after the peak height for the whole system, the water column and the sediment were all entered and two of the three default kinetic models (SFO and FOMC) were run without any data modification. Since FOMC kinetics gave almost identical fits to SFO kinetics, and the error associated to the FOMC shape and location parameters alpha and beta was too high, indicating that the estimates were not reliable, the SFO model was deemed the best-fit model in all cases and only the results for SFO kinetics are presented here. In addition, to estimate the persistence and modelling endpoints by *degradation* at Level M-I, the data for the whole system were entered and the SFO kinetics model for metabolites run. The description of the decline data to derive the dissipation endpoints for each compartment is shown Figure 10-10, while the description of the degradation of the parent and formation and degradation of the metabolite in the total system to derive degradation endpoints is shown in Figure 10-11.

The dissipation and degradation half-lives are shown in Table 10-7 and 10-8 along with the parameter results and calculated error percentage at which the χ^2 test is passed for each fit. In all cases, based on graphical and statistical evaluation (random distribution of residuals, low χ^2 error and t-test for the rate constant parameters passing at 10 % error level) SFO appeared to give acceptable fits to the data for both decline DT50 values and degradation half-lives, so these values would be used as the persistence and modelling endpoints as required. For use against study triggers, the recommendation is to use the system DegT50/90 or decline DT50/90, water decline DT50/90 and sediment decline DT50/90 against system, water column and sediment triggers, respectively. Water and sediment decline half-lives should be used in PEC calculations for the metabolite with simple spreadsheets or tools. For FOCUS modelling, the system decline half-lives may be used at FOCUS Step 1 and at FOCUS Step 2 for both compartments, while the use of the system dissipation half-life or system degradation half-life for either water or sediment compartment at Step 3 would need to be discussed based on other available information on the degradation and transfer of this substance. For example, assuming that the metabolite has a low Koc and that other information indicates that most of the degradation would occur in the water column and that little degradation is expected to occur in the sediment (dissipation resulting from back-transfer to the water column), the system degradation half-life would be used at Step 3, while a default half-life of 1000 days would be used for the sediment. If it is not clear where the degradation occurs, and conservative estimates cannot be obtained from other studies such as hydrolysis for degradation in water, another very conservative option would be to use worst-case default values of 1000 days for both compartments. Otherwise, two-compartment fitting approaches at Level M-II should be attempted, as discussed in the next section.

Table 10-7. Kinetic results obtained at level M-I with the SFO model for decline of the metabolite in the example case.

Endpoint or Statistic	Compartment		
	System (wc+sed)	Water (wc)	Sediment (sed)
M_{max} (as % AR)	51.7±2.8	42.4±2.4	9.27±0.47
k_m (1/d)	0.0043±0.0007	0.0043±0.0007	0.0044±0.0007
χ^2 error metabolite	7.7	7.9	7.1
DT50 metabolite	161	163	157
DT90 metabolite	536	540	522

Table 10-8. Kinetic results obtained at level M-I with the SFO model for degradation of the metabolite in the example case.

Endpoint or Statistic	System (wc+sed) compartment
M_0 (% AR)	108±2
k_p (1/d)	0.0336±0.0019
ffM (as a fraction)	0.6197±0.0411
k_m (1/d)	0.0056±0.0007
χ^2 error parent	3.5
χ^2 error metabolite	10.4
DT50 parent	20.6
DT90 parent	68.5
DT50 metabolite	123
DT90 metabolite	408

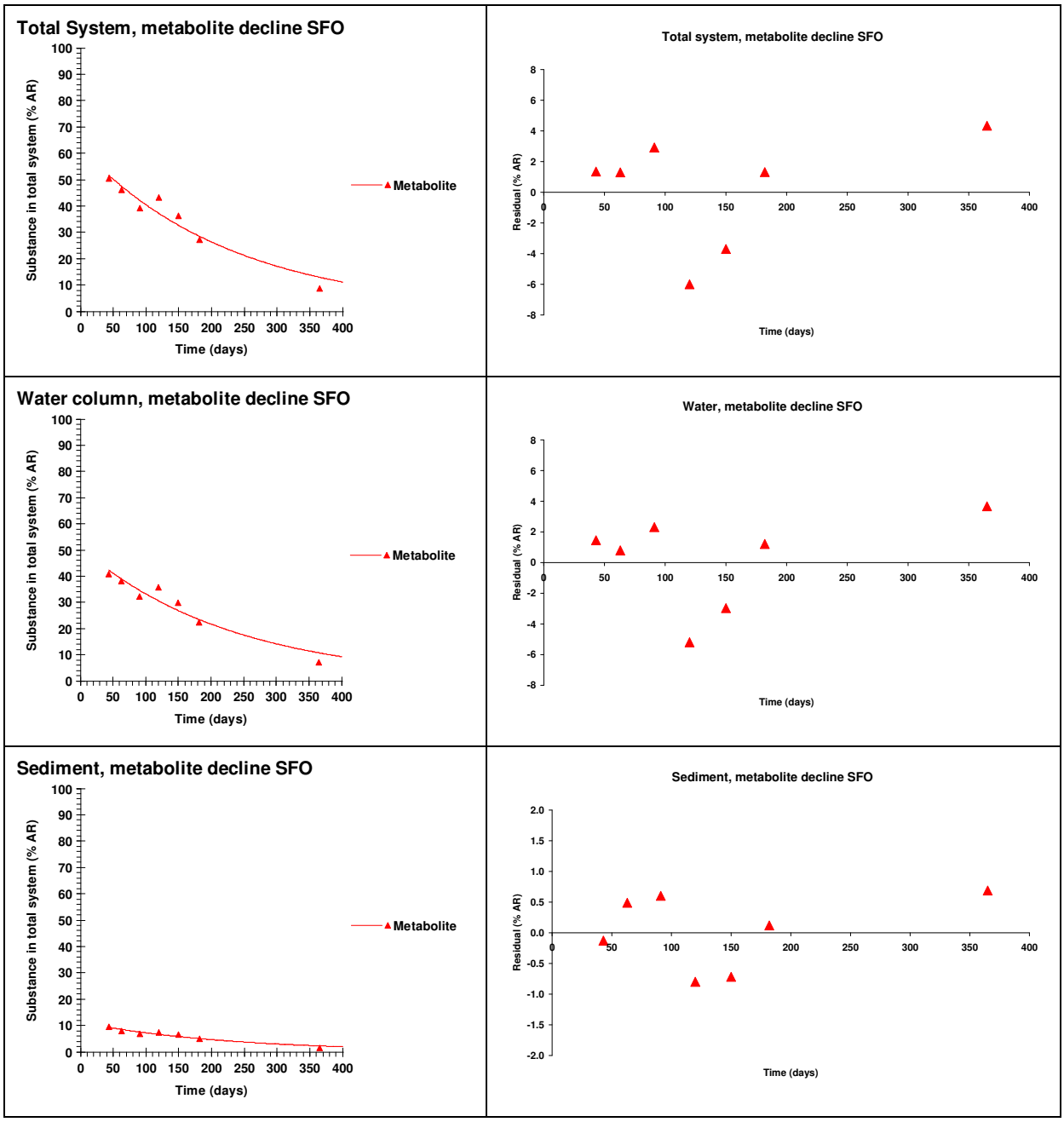


Figure 10-10. Results of level M-I for degradation endpoints from the metabolite decline in the example case.

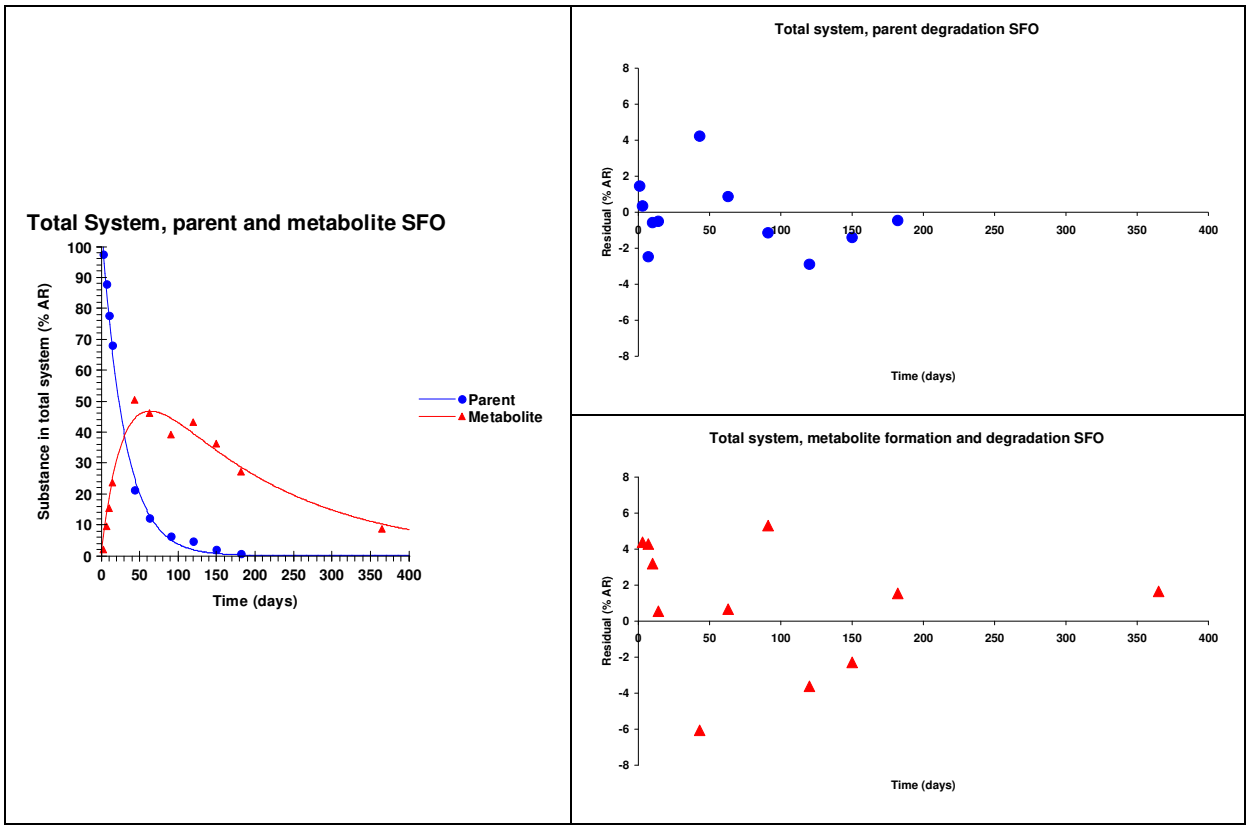


Figure 10-11. Results of level M-I for degradation endpoints in the example case.

10.4.4 Level M-II

At Level M-II, both persistence and modelling endpoints can be estimated using two-compartment approaches, comprising water and sediment compartments. However, as already mentioned, due to the complexities experienced with resolving Level P-II, only an outline of recommendations can be given of how this may be developed in future. An initial attempt was made to simplify transfer and degradation kinetics at this level, by representing:

- Parent degradation only at the whole system level
- Metabolite degradation separately in the water column and sediment
- Metabolite transfer between the water column and sediment as an instantaneous equilibrium process

While this attempt resulted in visually acceptable fits, it was nevertheless rejected due to concern that transfer between the water column and sediment cannot really be simplified to an instantaneous equilibrium process without potentially introducing significant non-quantified bias. Hence, the general recommendations of how it may be developed in future (if required) is that Level M-II kinetics should be an extension of Level P-II kinetics using:

- The minimum parent and metabolite data required, e.g. not to use sink data, to represent the kinetics of dissipation

- First-order kinetics to represent metabolite degradation in the water column and sediment and transfer between the water column and the sediment; and
- Formation fractions to describe the fraction of parent or precursor that degrades to form the metabolite in the water column and the sediment

In addition, when Level M-II kinetics are fitted to data, attempts should be made to constrain parameter values in various ways, to lessen the likelihood of obtaining physically implausible values, such as:

- Fitting the kinetics sequentially, i.e. the parent kinetics before the metabolite, rather than simultaneously
- Setting the formation fraction in the water column and the sediment to be the same, e.g. that at level M-I for the whole system

10.5 References

Adriaanse, P.I., Leistra, M., Vink, J.P.M., Brouwer, W.W.M., Tas, J.W., Linders, J.B.H.J., Pol, J.W., 2002. Estimating transformation rates of pesticides to be used in the TOXSWA model from water sediment studies. Alterra Report 23, Alterra, Wageningen, 130 pp.

11 APPLICATION OF KINETIC ENDPOINTS IN REGULATORY ASSESSMENTS

11.1 Reporting of kinetic endpoints

Results of kinetic analyses of environmental fate studies should be reported according to the guidelines reported in Chapter 12. Such analyses may be reported within the experimental study or in a separate report dealing only with the kinetic analysis. Usually the approach is to include relatively simple analyses within the study report while more complicated analyses are issued in a separate report.

The calculation of average values used as trigger values and in environmental modelling will usually not be the subject of a separate report. Simple averaging of study results used for triggers will normally be presented in the dossier and the derivation of input parameters used in PEC calculations should be described in the modelling report. The discussion on derivation of average parameters should include a list of all study results, including actual and normalised values.

11.2 Averaging of kinetic parameters

In the European registration process, different methods are used to determine kinetic parameters for use in assessing environmental exposure when multiple values are available. In some cases a worst case value is used, while in other cases a more central value, such as the mean or median is desired. For example, as discussed later in this section, PEC calculations for FOCUS ground and surface water calculations use average values of chemical properties such as degradation rates and sorption parameters. Values used to calculate PEC_s and for use as triggers are, in current standard practice, not averaged. This report does not intend to provide guidance on whether averages or single values should be used in the various aspects of the European registration process. Instead the reader should consult other guidance documents, some of which are referenced in Chapters 3 and 4.

In some circumstances using averages from different experiments is not appropriate. For example, averaging is not recommended when degradation is a strong function of the properties of the experimental media. Examples where averages should not be used include results of:

- hydrolysis studies conducted at different pH values

- soil degradation studies when degradation is a strong function of soil properties (such as pH for compounds that are partially ionised in the range of normal soil pH)
- water-sediment studies when degradation is a strong function of pH or organic matter nature and content
- field dissipation studies when degradation is a strong function of climatic conditions (other than what would be accounted for in the normalisation process discussed in Chapter 9), agricultural practice or soil properties (such as pH, soil structure and nature and content of organic matter).

In some of these cases, averaging of subsets of studies may be useful (for example, soils of similar pH if the degradation is a strong function of pH).

When averaging is not appropriate, one option would be to perform conservative assessments (such as using individual or worst-case values), which could demonstrate acceptable levels of exposure. Such an approach could be conducted in a stepwise manner to avoid unnecessary calculations (for example, if simple calculations with worst-case assumptions showed acceptable levels of exposure, more realistic calculations would not be needed).

Guidance requiring a mean value may be ambiguous, because different values are obtained if the degradation rates (rate constants) are averaged or the corresponding half-lives (or first-order DT50 values) are averaged. Averaging degradation rates results in greater weight being placed on the higher (faster) degradation rates while averaging the corresponding half-lives results in greater weight being placed on the higher half-lives (slower degradation). One approach which results in giving the same result whether degradation rates or half-lives are averaged is to use the geometric mean rather than the arithmetic mean.

As an example, consider the average of four half-lives: 10, 20, 30, and 100 days with corresponding rate constants of 0.06931, 0.03466, 0.02311, and 0.00691 $days^{-1}$. The arithmetic mean of the half-lives is 40 days and the arithmetic mean of the rate constants is 0.0335 $days^{-1}$, which corresponds to a half-life 20.7 days. The geometric mean of both the half-lives and the rate constants results in a half-life of 27.8 days.

The work group recommends that the geometric mean be used when averages of degradation rates are desired. This has the advantage that averages of half-lives and rate constants are equal. When several entire SFO degradation curves are averaged, the curve corresponding to the geometric mean of the half-lives represents the best SFO fit to the averaged points.

The recommendation to use a geometric mean applies only to degradation rates, half-lives, and trigger values. Averages of other kinetic parameters such as formation fractions and fractions of starting material applied to compartments in the DFOP model should be arithmetic means.

11.2.1 Use of DT50 and DT90 values as regulatory triggers

As discussed in Chapter 4, DT50 and DT90 values from environmental fate studies are often used to trigger further fate or ecotoxicology studies and/or used as the basis for regulatory decision making. Usually DT50 and DT90 values from individual laboratory and field studies conducted according to guideline conditions are directly compared to trigger values and not normalised to a set of reference conditions. When an average value of the DT50 or DT90 is desired, the calculation is straightforward since the nature of the kinetics does not need to be considered (however, the DT50 and DT90 estimates for a specific study need to be calculated with the same model). As mentioned in the previous section, averages should be the geometric mean of all of the values.

When determining averages of DT50 or DT90 values for comparison to trigger values, laboratory values should be corrected to a consistent temperature (usually 20°C). Except for aerobic soil metabolism studies, there is no need to correct laboratory studies to standard moisture conditions. Current EU practice is that triggers based on field study results to refer to the non-normalised values from relevant and reliable studies. Average values for field studies should be based on values obtained by normalising the results to account for differences in temperature and soil moisture. In the EU registration process, the reference conditions for normalisation should be 20°C and a pF value of 2.

11.2.2 Kinetic descriptions for use in models for calculating Ground and Surface Water PEC values

The determination of average soil degradation rates for use in soil and ground and surface water calculations is similar to that for trigger values when degradation rates follow first-order kinetics. However, since the simulation models generally assume first-order kinetics for degradation processes, determining the appropriate average values is more difficult when degradation in one or more of the soils is described by bi-phasic kinetics. The averaging of values for bi-phasic models is more difficult because instead of having one rate constant parameter, there are several parameters involved in bi-phasic kinetics that cannot be averaged individually (except in the case when all soils are described by DFOP kinetics).

11.2.2.1 Soil

If the degradation kinetics are adequately described by first-order kinetics, then the averaging process occurs as outlined in the report of the FOCUS Groundwater Scenarios Workgroup (FOCUS, 2000). A summary of this guidance is: If rates of parent degradation are based on four or more soils and the metabolite degradation is based on three or more soils then using an average is appropriate (a geometric mean is recommended by the kinetics work group). If the degradation rate is based on fewer soils, then the highest value should be used. When degradation rates in a large number of additional soils are available then the use of a median value may be most appropriate. Before averaging all values must be corrected to a consistent temperature and moisture content. In the absence of any measured data on the effect of temperature or moisture, the default values for the relevant parameters should be used, as described in Section 5.5 of the FOCUS Groundwater Scenarios report.

If the results of a laboratory study show appreciable bi-phasic kinetics, then the cause of the slow down in degradation rate needs to be investigated. When this slowdown can be attributed to the decline in the microbial activity, as indicated by a relatively constant degradation in the field during the degradation of the first 90-95 percent of the compound, the degradation rate can then be represented by a first-order fit to the first portion of the laboratory study or the results of the field study (if other loss mechanisms such as leaching and volatilisation are relatively unimportant in the field study). A representation of the degradation rate by a first-order fit to the first portion of the degradation curve observed in the laboratory study can also be applied if a decline of the microbial activity has actually been measured in the laboratory study.

When the cause of the slow down in degradation is due to non-availability of compounds to degradation due to binding to soil, this can be addressed with the recommended procedures described in Chapter 7.

If the results of a field study show appreciable bi-phasic kinetics, then the cause of the slow down also needs to be investigated. However, this investigation is different than for laboratory studies.

One potential cause of bi-phasic kinetics in the field is the presence of two different reaction mechanisms, for example, rapid photolysis and soil degradation. In this case the kinetics could be simulated by using the photolysis degradation rate in the soil near the surface and the non-photolytic degradation rate in deeper layers. Another cause could be volatilisation from the soil during the period following application. Volatilisation losses may be able to be

separated from degradation by using the volatilisation routines in some of environmental models.

The next step would be to normalise the data to standard temperature and moisture, as described in Chapter 9. If the bi-phasic pattern remains (and photolysis and volatilisation are not important), then most likely the bi-phasic degradation is the result of increasing sorption resulting in decreased availability of residues, which can be addressed with the procedures described in Chapter 7.

The approach used to calculate an average degradation rate for parent when one or more of the degradation rates is clearly bi-phasic depends on the results of the kinetic analysis of the individual studies (as discussed in Chapter 7). If kinetics from all of the studies fall into any of the following four categories if parent only, or the first two if there are metabolites being simulated, then the average can be represented by the average (geometric mean) of the half-lives determined in the kinetic analyses.

- Single first-order kinetics gives the best fit of the data
- Single first-order kinetics provides an adequate fit of the data
- The DT90 was reached in the experimental study so that the average degradation rate corresponds to the half-life obtained by dividing the DT90 value obtained from the Gustafson-Holden model by 3.32
- The half-life corresponds to the degradation rate for the second phase of decline (k_2) from the hockey-stick model

If the data do not permit calculation of an average value by the previously described procedures, then the next step will require higher-tier testing using either PEARL or bi-exponential models. If using the bi-exponential models (either DFOP or FOTC) the usual approach would be to obtain means of each of the three parameters (for DFOP the arithmetic mean of the fraction in the rapidly degrading compartment and the geometric means of the degradation rate in both compartments; for FOTC the geometric means would be obtained for the degradation rate in the rapidly degrading compartment, the transformation rate from the rapidly degrading compartment to the slowly degrading compartment, and the degradation rate in the slowly degrading compartment).

When averaging does not seem appropriate due to mechanistic differences in studies, then the registrant should proceed with the most scientifically defensible approach for the specific case. If the degradation rate is similar among all laboratory studies (when normalised to standard temperature and moisture) or among all normalised field studies, one potential

approach could be to use all of the data in a single regression to determine average parameters. However, such an approach is not appropriate when degradation rates are not about the same in all of the studies. Another approach would be to perform conservative assessments, which could demonstrate acceptable levels of exposure. For run-off and drainage simulations, representing the degradation over the first 30-60 days may be sufficient (run-off also is not very sensitive to degradation rate). For ground water assessments involving strongly sorbed compounds, simulations using conservative assumptions of the degradation rate may be sufficient to show no risk from leaching. Another approach could be to perform calculations using the individual study results that are most relevant to the conditions being simulated.

When increasing sorption is responsible for decreasing degradation, the increasing sorption should also be considered in PEC calculations. An example is shown in Appendix 4.

11.2.2.2 Water

For hydrolysis and aqueous photolysis studies conducted under sterile conditions, degradation in most cases follows first-order kinetics. In these studies, averaging of different values is not performed since the degradation half-life is often a single value, usually depending on pH and/or light intensity. When multiple values at the same pH or light intensity are available from studies of equivalent quality, the values can be averaged after normalisation to a reference temperature. The calculation of degradation rates in other water systems (for example, surface water without sediments) is similar to that described for soil. Often degradation rates are adequately described by first-order kinetics so the calculation of average values is straightforward. Before averaging, the results from different studies should be corrected to a consistent temperature. Results from comparable field studies are rarely available. The PEC surface water models also do not have provision for using bi-phasic kinetics for water degradation rates. Therefore, when single first-order kinetics do not give an adequate fit, such occurrences need to be handled with the most scientifically defensible approach for the specific case. Some of the suggestions for soil (such as using all of the data in a single regression or performing simulations based on conservative assumptions) may be useful for developing such an approach.

11.2.2.3 Surface water and sediment studies

In the report from the FOCUS Surface Water Workgroup the following recommendation is provided: "Generally, information on two different water-sediment systems is available in the dossier. It is recommended to calculate the average of these two values and to use this value in the models STEPS 1 and 2 in FOCUS and TOXSWA in FOCUS." TOXSWA requires a

true degradation (not dissipation) rate in water as an input variable and procedures for calculation are provided in Chapter 10.

These values are normally obtained from one study conducted with two different water-sediments systems at a constant temperature; thus there should be no need to normalise results to a standard temperature. If results from studies performed at different temperatures are used as input to the models, then the results should be normalised to one single temperature (normally 20°C) before averaging (geometric mean).

As mentioned previously, averaging results of different degradation experiments is not recommended when degradation rates are a strong function of properties of the water-sediment system, such as pH and organic matter content.

For parent compounds, calculation of the average dissipation rate is straightforward at Level P-I if single first-order model adequately describes the data, and at Level P-II since SFO is the only kinetic model considered at that level (as outlined in Figures 10-4 and 10-5). When the dissipation rates obtained at Level P-I from one or more water-sediment system is clearly bi-phasic, calculation of an average is still possible if the kinetics from all systems fall into any of the following four categories.

- Single first-order kinetics give the best fit of the data
- Single first-order kinetics provide an adequate fit of the data
- The DT90 was reached in the experimental study so that the degradation rate corresponds to a half-life calculated by dividing the DT90 value obtained from the Gustafson-Holden or hockey-stick model by 3.32
- The half-life corresponds to the degradation rate for the second phase of decline (k_2) from the hockey-stick model

If an accurate description of degradation is dependent on bi-phasic degradation then obtaining a meaningful average description of the degradation rate may be difficult. The registrant should proceed with the most scientifically defensible approach for the specific case. One example might be if the degradation rate is similar among the different water-sediment systems tested, when normalised to standard temperature, one approach would be to use all of the data in a single regression to determine average parameters. However, such an approach is not appropriate when appreciable differences exist from system to system. Another possibility would be to perform simulations using conservative assumptions that may be sufficient to show low risk for aquatic organisms. For example, simulations could be done

for the individual kinetic descriptions for each individual water-sediment system, starting with the system that produces the most conservative PEC values.

11.2.2.4 Special considerations for metabolites

As with parent, the objective of the kinetic description for metabolites is to provide the best estimate of metabolite formation and degradation. The starting point for such a determination for metabolites is the best estimate of the degradation of the parent or predecessor metabolite. This understanding of the objective is important because of the complex relationship between degradation of parent or predecessor metabolite and the formation of the metabolite. For example, more rapid degradation of parent results in higher maximum values for the metabolite but such a change could result in either a higher or lower PEC due to the influence of the scenario (for example, the time of application versus the occurrence of rainfall events).

When the differences in formation fraction and degradation rates among the studies are minor, then averaging the values is probably the most appropriate approach. When there are important differences among the various studies, then averaging of formation fractions and degradation rates will usually be appropriate unless they are strong functions of soil properties. Alternate approaches to using an average value in PEC calculations should reflect the most scientifically defensible approach for the specific case. Such approaches could include using conservative assumptions to show no risk and performing the PEC calculations with the study results that are the most relevant to the conditions being simulated.

Sometimes data on a metabolite may be available from two sources, studies in which parent is applied and studies in which the specific metabolite is applied. If the data are of equivalent quality, then the best approach is probably to average all of the values. If the data are not of equivalent quality, then the most scientifically defensible approach for the specific case should be followed.

Normally due to the higher uncertainty of the kinetic analysis for metabolites, using bi-phasic kinetics for metabolites will not be appropriate. However, bi-phasic kinetics for metabolites should be used when indicated by the kinetic analysis described in Chapter 8.

The approach of calculating ground and surface water PEC values for both parent and metabolite in a single or sequential model run is preferred when feasible, assuming a conservative formation fraction when this parameter is not available. When this is not

possible due to model limitations or lack of information, calculations are performed as part of a simulation with only the metabolite.

For surface water simulations involving only a single metabolite, the starting value should correspond to the maximum fraction of the amount of substance (peak at the end of the formation phase) and the degradation rate should be the decline rate obtained from a regression of the metabolite data after the peak at the end of the formation phase.

For ground water simulations involving only a single metabolite, the actual degradation rate should be used if available. The starting amount should correspond to the application rate corrected for the differences in molecular weight and the formation fraction. If the formation fraction is not known, a formation rate of 100 percent or other conservative estimate can be assumed. However, the starting amount will be larger than the maximum fraction of the amount of substance. If the actual degradation rate is not known, the value of the decline rate obtained from a regression of the metabolite data after the peak at the end of the formation phase can be substituted as a conservative estimate. In this circumstance the starting amount should correspond to the maximum fraction of the amount of substance. When the parameters are conservatively estimated as described in this paragraph, the approach of applying the metabolite at a single time usually provides a conservative estimate. However, timing of rainfall events can result in different results (either higher or lower concentrations) than if the metabolite had been formed over a period of time.

11.3 Use of degradation rates from field studies

As stated in the final report of the FOCUS Groundwater Scenarios Work, the choice of either field or laboratory studies to determine degradation rates for use in estimates of movement to water needs to be justified. The procedure for conducting laboratory studies usually results in all losses of parent and metabolites being attributable to degradation. However sometimes degradation decreases with time as soil microbial activity diminishes, so degradation rates may be slower than observed in the field, especially for metabolites. Also field conditions are usually more dynamic than in laboratory studies due to cycling of temperature, movement of water due to precipitation and evapotranspiration, and tillage; all of which may enhance degradation. Field studies represent measurements representative of actual use conditions, but sometimes losses occur for reasons other than degradation. Well conducted field studies often eliminate most of these concerns for many compounds, but the potential losses from processes other than degradation in soil must always be evaluated. Plant uptake is one pathway for which losses are difficult to separate from degradation. Although the effect of plant uptake is small for most compounds, if field dissipation data were generated in studies

in which crops or other vegetation were present, the uptake routines in models must be turned off to avoid the potential for double accounting of losses. An exception would be when the determination of degradation rates was with inverse modelling in which uptake was considered.

When the quality of field studies has been determined to be acceptable for the estimation of degradation rates, usually the most appropriate approach is to normalise the degradation to standard conditions, preferably using one of the quantitative procedures described in Chapter 9. Average values of degradation rates from field studies for comparison to trigger values should be determined using the guidance provided in Sections 11.2.1. Average values for soil degradation rates to be used in models for PEC calculations should be determined using the guidance provided in Sections 11.2.2.1.

In those cases where the data are not sufficient for the quantitative procedures described in Chapter 9, average values of soil temperature and moisture can be substituted as reference conditions for a single site as described in Section 9.3. If results from multiple sites are available then the field DT50 values from each of the sites are **normalised to 20°C** using the representative average temperature for the individual field study following the recommendations from Section 9.3 with regard to period for averaging etc. Conservative estimates (e.g. 100% FC) for soil moisture conditions during the field study can be used as a reference moisture content. When these reference conditions are used along with the average kinetic expressions, then the temperature and moisture correction routines should be activated. This approach allows the dissipation rates or corresponding half-lives from different field study sites to be averaged (geometric mean) and used as a model input value, because all trials are normalised to identical reference conditions. However, sometimes using the result from a single trial may be more appropriate, such as when degradation rates are strong functions of soil properties.

As mentioned previously, normalisation of field degradation rates or the corresponding half-lives will be the preferred option in most cases. However, in the following three situations using field degradation rates without normalisation (and turning off the temperature and moisture corrections) may still be an appropriate approach.

- When the normalisation procedure increases the variability in the DegT50 values for an unknown reason.
- When there is little variability in the results originating from a wide range of soils or climatic conditions throughout Europe.

- When actual data are available for similar conditions to those being simulated in the model.

However, using field degradation rates without normalisation is not recommended for leaching assessments if the DegT50 value was derived over the spring and summer period only and the compound persists into the autumn and winter. Degradation rates in spring and summer will be higher compared to autumn and winter.

11.4 Calculation of soil PEC values

The calculation of PEC values for soil for parent compounds has been described in the final report of the Soil Modelling Work Group of FOCUS (FOCUS, 1996). The intent of this discussion is not to change any of the recommendations of the previous work group, but instead to provide more information on the calculation of PEC values using kinetic models other than first order. The Soil Modelling Workgroup did anticipate the use of such kinetic models but did not provide any details for the individual equations.

Previous guidance documents have not addressed whether an average or a single degradation rate should be used for calculation of PEC_s. The current practice has been to use the longest relevant half-life to ensure a conservative approach. Use of field data is preferred if available so that loss processes such as plant uptake and volatilisation are considered. If no field data are available, the current practice is to use the relevant laboratory study with the slowest degradation. Minor deviations from this normal practice, such as using the 90^{th} percentile value when there are numerous field study results available, are approaches that have also been used. There is also no guidance on metabolites. If a worst-case approach is desired, one approach would be to use the kinetic model developed from the soil study in which the metabolites concentrations were the highest or the most persistent.

The guidance in the following section provides analytical solutions for PEC_s calculations. Especially as the system increases in complexity (multiple applications and metabolites), the approach usually taken is a numerical approach where the concentrations are calculated using the kinetic rates and application times and the maximum concentrations are determined directly from the curves of concentration as a function of time. The time weighted average values are determined by a numerical integration, sometimes using a moving time-frame approach. A higher tier approach outlined in the FOCUS (1996) is to use leaching

models to simulate the concentrations in soil. The desired concentrations can then be determined at desired times by determining the amounts in the desired soil layer and using a moving frame approach to determine time weighted averages.

11.4.1 Calculation of soil PEC values for parent following a single application

The calculation of the initial PEC concentration ($PEC_{S,0}$) in mg/kg immediately following a single application is independent of kinetics and the recommendation in the FOCUS Soil report is provided here for information:

$$PEC_{S,0} = A \times (1- f_{int}) \ / \ (100 \times depth \times bd) \qquad (11\text{-}1)$$

Where A = application rate (g/ha)
f_{int} = fraction intercepted by crop canopy
depth = mixing depth (cm)
bd = dry soil bulk density (g/cm^3)

As reported in the FOCUS soil report, the PEC_s is calculated by assuming a bulk density of 1.5 g/cm^3, and a mixing depth of 5cm for applications to the soil surface or 20 cm where incorporation is involved. Unless better information is available the fraction intercepted is assumed to be 0 for applications to bare soil, or up to 0.5 for applications when a crop is present. Since the issuance of the FOCUS soil report, information on interception has been presented in FOCUS (2002). Using these assumptions the concentration in soil immediately after a single application (mg/kg) becomes:

$$PEC_{S,0} = A (1- f_{int}) / 750 \quad \text{assuming no incorporation} \qquad (11\text{-}2)$$

$$= A (1- f_{int}) \ / 3000 \quad \text{assuming incorporation} \qquad (11\text{-}3)$$

The concentration of a parent compound at time t ($PEC_{S,t}$) following a single application at time zero is:

$$PEC_{S,t} = PEC_{S,0} \, F(t) \qquad (11\text{-}4)$$

Where $F(t)$ is the fractional amount remaining in the soil at time t after application

In order to calculate $PEC_{S,t}$ values, Equation 11-4 needs some form of kinetics to be substituted into it for the generic term $F(t)$. For the kinetic models used in this report, this results in the following equations (using the variable names defined in Chapter 5 for each model):

Single first-order kinetics:

$$PEC_{S,t} = PEC_{S,0} \exp[-kt] \qquad (11\text{-}5)$$

Gustafson-Holden (FOMC):

$$PEC_{S,t} = PEC_{S,0} [t/\beta + 1]^{-\alpha} \quad (11\text{-}6)$$

Hockey-stick kinetics:

$$PEC_{S,t} = PEC_{S,0} \exp[-k_1 t] \quad \text{if } t < t_b \quad (11\text{-}7)$$

$$PEC_{S,t} = PEC_{S,0} \exp[-k_1 t_b] \exp[k_2(t - t_b)] \quad \text{if } t > t_b \quad (11\text{-}8)$$

Bi-exponential (DFOP) (FOTC is not shown because DFOP and FOTC give equivalent results and FOTC must be solved numerically):

$$PEC_{S,t} = PEC_{S,0} \{g \exp[-k_1 t] + (1-g)\exp[-k_2 t]\} \quad (11\text{-}9)$$

Logistic:

$$PEC_{S,t} = PEC_{S,0} \{a_{max}/(a_{max} - a_0 + a_0 \exp[r\, t])\}^{(amax/r)} \quad (11\text{-}10)$$

The highest time weighted averages for parent occur immediately after application. Therefore, the highest time weighted average for a period of t days occurs between the application and t days afterwards. Hence, the time-weighted average concentration ($PEC_{S,twa}$) for a time of t days can be expressed with the following integral

$$PEC_{S,twa} = (PEC_{S,0} / t) \int_0^t F(t)\, dt \quad (11\text{-}11)$$

Equation 11.11 can be integrated to produce the following equations:

Single first-order kinetics:

$$PEC_{S,twa} = PEC_{S,0} (1 - e^{-kt}) / kt \quad (11\text{-}12)$$

Gustafson-Holden (FMOC):

$$PEC_{S,twa} = PEC_{S,0} \frac{\beta}{t(1-\alpha)} [(t/\beta + 1)^{1-\alpha} - 1] \quad (11\text{-}13)$$

Hockey-stick kinetics:

$$PEC_{S,twa} = (PEC_{S,0}/k_1 t) [1 - \exp(-k_1 t)] \quad \text{for t less than or equal to } t_b \quad (11\text{-}14)$$

$$PEC_{S,twa} = (PEC_{S,0}/t) \{(1/k_1)[1 - \exp(-k_1 t_b)] + (\exp(-k_1 t_b)/k_2)[1 - \exp(-k_2(t - t_b))]\} \quad \text{for } t > t_b \quad (11\text{-}15)$$

Bi-exponential (DFOP):

$$PEC_{S,twa} = (PEC_{S,0}/t)\ \{(g/k_1)[1 - \exp(-k_1 t)] + [(1-g)/k_2][1 - \exp(-k_2 t)]\} \quad (11\text{-}16)$$

11.4.2 Calculation of soil PEC values for parent following multiple applications

For multiple applications, the simple approach is to use the equations presented in the previous section with the initial application rate equal to the total amount applied in a year. If this does not provide an acceptable exposure, then the following approach can be used for those equations not considering a lag phase. Approaches for those compounds with a lag phase must be considered on a case by case basis.

The overall soil PEC at a specified time will be the sum of the concentrations resulting from N applications:

$$PEC_{S,t} = \sum_{J=1}^{N} PEC_{S,j,t} \quad (11\text{-}17)$$

However, Equation 11-4 must be modified to take into account that all of the applications do not occur at time zero. Therefore, the soil PEC for application number j at a time t_j ($PEC_{S,j,t}$) becomes

$$PEC_{S,j,t} = PEC_{S,j,0}\ F(t - t_j) \quad (11\text{-}18)$$

Where t_j is the time of the application

$PEC_{S,j,0}$ is the contribution of the application number j to the overall soil PEC immediately after application j

Equations 11-5 through 11-10 then, for application j, become:

Single first-order kinetics:

$$PEC_{S,j,t} = PEC_{S,j,0} \exp[-k(t - t_j)] \quad (11\text{-}19)$$

Gustafson-Holden (FMOC):

$$PEC_{S,j,t} = PEC_{S,j,0}\ [(t - t_j)/\beta + 1]^{-\alpha} \quad (11\text{-}20)$$

Hockey-stick kinetics:

$$PEC_{S,j,t} = PEC_{S,j,0} \exp[-k_1(t - t_j)] \quad \text{if } t < (t_j + t_b) \quad (11\text{-}21)$$

$$PEC_{S,j,t} = PEC_{S,j,0} \exp[-k_1 t_b] \exp[-k_2(t - (t_j + t_b))] \quad \text{if } t > (t_j + t_b) \quad (11\text{-}22)$$

Bi-exponential (DFOP):

$$PEC_{S,j,t} = PEC_{S,j,0} \{g \exp[-k_1(t - t_j)] + (1-g) \exp[-k_2(t - t_j)]\} \quad (11\text{-}23)$$

The time weighted average for multiple applications is also the sum of the time weighted averages for the individual applications.

$$PEC_{S,twa} = \sum_{J=1}^{N} PEC_{S,j,twa} \quad (11\text{-}24)$$

If the same amount is applied at each application and the spacing between applications is uniform or decreasing, then the time period for averaging will start immediately after the last application (assuming no effect of environmental conditions on degradation). If not, the appropriate time period will have to be determined based on the specific case. When the period of maximum average concentration is not readily apparent, results of several different periods may need to be presented to demonstrate that the chosen period does represent the maximum time weighted average concentration. Another approach would be to generate the concentrations analytically or numerically as a function of time. Then the time-weighted average concentrations can be calculated numerically, sometimes using a moving time-frame approach.

For an ending time of t_f and an averaging period of Δt, Equation 11-24 becomes

$$PEC_{S,j,twa} = (PEC_{S,j,0} / \Delta t) \left[\int_{t_j}^{t_f} F(t - t_j)\, dt - \int_{t_j}^{t_f - \Delta t} F(t - t_j)\, dt \right] \quad (11\text{-}25)$$

For single first-order kinetics, this reduces to

$$PEC_{S,j,twa} = PEC_{S,j,0} \{\exp[k\,(t_f - \Delta t - t_j)] - \exp(k\,(t_f - t_j))\} / (k\,\Delta t) \quad (11\text{-}26)$$

For relatively simple cases, analytical solutions are available. For example, USES (Uniform System for the Evaluation of Substances) (RIVM et al., 2002) is a software package used to assist in pesticide registration in the Netherlands. The package calculates a TWA-concentration for specific cases with multiple applications (fixed time interval between applications, identical dosage, no influence of environmental conditions on the transformation rate).

11.4.3 Calculation of soil PEC values for metabolites

The principles established for parent in the previous two sections can be applied to metabolites. However, analytical solutions for many cases may not be available. Also the time where the maximum concentration occurs is not generally obvious. Therefore, often the most practical way to calculate soil PEC values is by using the kinetic models (including parent and all predecessor metabolites) to generate a concentration profile of the metabolite as a function of time. Then the maximum concentration can be read directly from this output and numerical integration can be used to determine time weighted average PEC values using a moving time-frame approach for the desired time intervals. The start of the highest time weighted average period is not necessarily at the peak concentration.

The calculation of soil PEC must consider the ratios of the molecular weights for parent compound to the various metabolites. Usually results of laboratory studies are expressed as percent of applied radioactivity (i.e. parent equivalents). Results of field studies are reported in a number of ways (including soil concentrations, mass per area, and percent of applied). When concentrations or mass units are used, the calculation of soil PEC values must consider whether they are reported as actual values or as the equivalent amount of parent.

The approach of calculating soil PEC values in a single or sequential model run beginning with parent is preferred when feasible. When this is not possible due to model limitations or lack of information, calculations are performed as part of a simulation with only the metabolite. The amount applied at each application should correspond to the maximum amount observed and the degradation rate should be the value obtained from a regression of the metabolite data after the maximum amount observed.

11.5 Uncertainties of risk assessment procedures resulting from uncertainties in kinetic endpoints

As described in Chapter 4, kinetic endpoints are required for use in risk assessments and these uses can be divided into two categories:

- triggers for higher-tier experiments for risk assessment concerning soil persistence, soil organisms and aquatic organisms (see the list in Section 4.1)
- parameters for calculating predicted environmental concentrations in soil (PEC_S), groundwater (PEC_{GW}), surface water (PEC_{SW}) and sediment (PEC_{SED}).

As discussed in Chapter 4, the kinetic endpoints in the risk assessment procedure are described by the following characteristics:

- type of kinetic parameter: degradation rate or formation fraction
- substance: parent or metabolite
- relevant compartment: soil, water, or water-sediment
- source of data (study type): laboratory or field.

As can be derived from the previous chapters, uncertainties in these kinetic endpoints depend upon:

- the selection of the soils and water-sediments (e.g. see discussion on effects of soil properties in the second paragraph of Section 11.2)
- the design, performance and interpretation of each study (e.g. see discussion of experimental artefacts in Section 6.1.7)
- the kinetic analysis of data of individual studies (see Chapters 6 to 10)
- the averaging or selection of kinetic parameters from a number of studies for further use in the risk assessment (see Section 11.2)

Knowledge of the influence of the listed uncertainties in the different kinetic endpoints on the uncertainty in the end result of the risk assessment procedures for soil persistence, soil organisms, aquatic organisms and ground water would be useful. The effects of errors in the trigger values on the end result are very difficult to assess because usually no scientific justification of these trigger values can be found in the underlying guidance documents. FOCUS (2000) discussed uncertainties in PEC_{GW} for FOCUS ground water scenarios (in its Chapter 6) and stated that in general simulated leaching is very sensitive to substance parameters (see its Section 6.4.5) but no details on sensitivity to kinetic parameters were provided. FOCUS (2003) discussed uncertainties in PEC_{SW} for FOCUS Step 3 surface water scenarios (in its Chapter 8) but did not include uncertainty resulting from kinetic parameters into its considerations (note that this PEC_{SW} is only the exposure part and thus not the end result of the aquatic risk assessment). In general a distinct propagation of the listed uncertainties to the uncertainty in the end results of the different risk assessments is expected, but quantifying this without performing additional research is difficult. There is currently also no agreed methodology for ‘uncertainty propagation’ in the EU pesticide risk assessment procedure. Therefore, the work group recommends research be conducted to develop such methodology.

11.6 References

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RIVM, VROM, VWS. 2002. Uniform System for the Evaluation of Substances 4.0 (USES 4.0). Edited by J.B.H.J. Linders, M.G.J. Rikken J. Bakker and P. van der Poel National Institute of Public Health and the Environment (RIVM), Ministry of Housing, Spatial Planning and the Environment (VROM), Ministry of Health, Welfare and Sport (VWS), The Netherlands. RIVM report 601450012.

12 GUIDELINES FOR REPORTING OF KINETIC ANALYSES

The intent of this work group is not to prescribe how a kinetic analysis should be inserted into a registration dossier, but only to indicate what needs to be reported in such an analysis, whether part of another report or contained in its own separate report. However, the nature of the report will determine some of the background information that needs to be included.

Reporting of kinetic analyses has two aspects. As kinetic analyses have many similarities with modelling, the report should follow the fundamental principle of good modelling practice that enough information should be provided to allow independent duplication of the results. Secondly the report should contain the statistical assessments of the kinetic models used (see Chapter 7 and Appendix 3).

Good modelling practices have been discussed in more detail in the FOCUS leaching report (Boesten *et al*., 1995) based on the information provided in Estes and Coody (1993) and Görlitz *et al*. (1993). Although the discussion in these three documents is directed towards environmental modelling, the principles are also applicable to kinetic analyses. A more recent paper (Erzgräber *et al*., 2002) deals specifically with kinetic analyses, and also contains an example of a kinetic report.

As mentioned earlier the fundamental principle of good modelling practice is to provide enough information to allow independent duplication of the results. Likewise, for the kinetic analyses enough information should be provided to allow independent duplication of the results and verification with alternative software packages.

The flow charts (see Chapters 7, 8, and 10) sometimes require a number of simulations to be made to determine which kinetic model best fits a specific data set. Good Modelling Practices requires that the report includes the relevant statistical measures and diagrams used to make the **decisions** in these intermediate steps, following the guidelines given below.

Data

The reporting of the kinetic analyses should include a listing of all original values to be used in the analyses. When the kinetic analysis is separated from the experimental report, a reference to the study report along with a short summary of the study should be included. If some of these data points are discarded in later analyses, these should be noted in the data

table. When data points are discarded as part of the kinetic analysis described in the flowsheets, the rationale for discarding the data points should be included in the report. Besides any information from the study that might help to explain outliers or deviations, this would include the statistical analyses, if any, performed to demonstrate the data point(s) being (an) outlier(s) and a justification of the use of the statistical routine. The discussion on the data should also include any other actions taken as part of the data handling issues discussed in Chapter 6.

Kinetic analysis

The report should address four aspects of the kinetic analysis:

1. Software package(s). The name and exact version of the software package or software packages used. For the more generic packages, this includes the listing of toolboxes, add-ins, sub-modules, etc. In order to facilitate independent duplication of results, kinetic analyses should be performed with a publicly available software package and preferably with a package commonly used for such analyses (such as the ones described in this document) whenever technically feasible.
2. Analyses. The report should provide an exact description of the kinetic models used in the regressions. In addition, software options that possibly influence the final results should be reported. This includes: range limits for the parameters (for example, when a parameter is limited to positive values), initial values and restrictions to the optimisation routine. When different sets of initial values are used (as described in Section 6.2), the report should include these values. Any simplification of the conceptual model during the stepwise process (for example, elimination of a flow to the sink) should be reported, including a proper justification.
3. Visual and statistical assessment of the results. The report should include figures comparing predicted and observed values as a function of time and residual plots. Optionally, 1:1 plots of predicted versus observed may also be included. Furthermore, the results of the χ^2-test and all other statistical endpoints used in the decision-making process (see Chapters 6 and 7) should be reported. Showing the results with one set of starting values is sufficient if the resulting kinetic model is essentially independent of the starting values.
4. Uncertainty of estimated parameters. The report should include estimated standard deviations or confidence intervals of all estimated parameters. This includes degradation rate constants and formation fractions but not DT50 and DT90 values, due to the complexity of obtaining appropriate confidence intervals (see Section 6.3.1.3)

If a parameter such as a DT50 or DT90 is extrapolated beyond the experimental period, this must be clearly stated in the report and also noted in any data summaries in the report. Most software packages optionally provide many of the necessary reporting requirements automatically. Therefore, many of the reporting requirements outlined in this section can be fulfilled by including the software generated report in the report describing the kinetic analysis. In addition the work group has prepared an Excel spreadsheet which provides the required statistics and graphs.

12.1 References

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13 SOFTWARE PACKAGES

13.1 Introduction

The number of software packages that can be used for parameter estimation is large. Establishing a complete overview of existing software was beyond the remit of the work group. Instead, the group followed a pragmatic approach and prepared an overview of software, known to be used by group members and / or by registration authorities. These packages are described in this report and used for estimating parameters for defined data sets. The results of this benchmarking are reported here.

13.2 Overview of packages and their functionality

Categorising software is doomed to failure, because development of each package mostly started because of a specific problem to be solved. For practical reasons the following categories were defined:

1. generic parameter estimation packages; packages developed around a parameter estimation problem;
2. general purpose packages; packages for which parameter estimation is only one out of many possible applications;
3. specific parameter estimation packages; packages developed for solving a specific type of problem, not intended to be used for other types of problem;
4. PEC-models; models that can be used for parameter estimation using inverse modelling techniques.

The PEC-models will only be listed, while the other packages will be described in some detail. However, for each of these categories a different approach is followed.

Table 13-1. Software packages considered by the work group.

ref. number	name	versions	no. of substances	remarks
1 generic parameter estimation packages				
1.1	Berkeley Madonna	8.0	>1	
1.2	Graphpad PRISM	2.0, 3.0, 4.0	1	
1.3	Kinetica	4.2	>1	
1.4	ModelMaker	3 and 4	>1	
1.5	ModelManager	1.1	>1	Excel for reporting
1.6	Statistica	6.0	>1	Metabolites: analytical equations required
1.7	Tablecurve	2D	1	
1.8	Topfit	2.0.0	>1	
2 general purpose packages				
2.1	ACSL		>1	
2.2	Excel	95, 97, 00, 02, 03	1	solver add-in required
2.3	Mathematica	4.2	> 1	
2.4	Matlab	7.0	> 1	
3 specific parameter estimation packages				
3.1	PEARL_NEQ	1	1	long term sorption
3.2	CODEWS	1	1	long term sorption
4 PEC-models[#]				
4.1	MACRO	4.4.2	> 1	
4.2	PEARL	2.2.2	> 1	
4.3	PELMO	3.3.2	> 1	
4.4	PRZM	2.4.1	> 1	
4.5	TOXSWA	1.1.1	1	

[#] Currently available version; check FOCUS website for latest release.

13.3 Benchmarking packages

Appendix 13 gives details of the generic parameter estimation packages and the general purpose packages. These descriptions may assist in selecting a package that meets the specific requirements of a data set.

13.3.1 Packages

13.3.1.1 Generic parameter estimation packages

Graphpad PRISM and Tablecurve are two packages that can handle a single substance in a single compartment. All kinetic models that are described in Chapter 5 can be implemented or chosen from the built-in models. The other generic parameter estimation packages can handle more substances and / or more compartments. Topfit is capable of handling SFO-kinetics only, for a sequential transformation scheme. The initial amount (or concentration) is a fixed value, not fitted by the package. Advanced users, however, can circumvent this. ModelManager contains a set of predefined transformation and transfer schemes; the user can choose out of a number of kinetic models. Berkeley Madonna and Kinetica allow the user to define his own transformation and transfer schemes; the equations however must be difference or differential equations in Berkeley Madonna. ModelMaker expands on the concept by allowing the use of the integrated equations, as well as solving sets of differential equations. Statistica is capable of estimating parameters for metabolites too, but only if the governing equations are in analytical form.

The general advantage of the generic parameter estimation packages is that the packages are dedicated to this area of problems. Examples, Help, Manuals and Tutorials are dedicated to the area of parameter estimation. The general disadvantage of such packages is that the user is limited in changing, customising or adapting the package.

13.3.1.2 General purpose packages

EXCEL is a general purpose spreadsheet and can be used for estimating the degradation parameters when the additional Solver Package is installed (add-in module of EXCEL that is included with the standard installation package). Without the Solver add-in, EXCEL is able to estimate parameters for only SFO kinetics, and only after log-transformation; this implies weighting of the data. Any function that can be written in analytical form can be used with Solver (for example, FOMC, HS, and DFOP for parent). More complex problems can be addressed using Visual Basic (version 97 or later), with the user writing his own code. This, however, requires quite some knowledge of this language.

ACSL, Mathematica and Matlab are general mathematics packages. In each package the user can define his transformation and transport scheme, usually in the form of sets of differential equations. Each of the packages has a library with (standard) solutions for solving the sets of equations. In the same way statistical modules can be invoked from the library to calculate and report required statistical endpoints.

The general advantage of these packages is that, in principle, the packages are very flexible. This is especially true for the general mathematics packages. A general disadvantage is that usually quite some knowledge of the package language is required.

13.4 Data sets

The purpose of this benchmarking exercise was to find out whether packages often used in pesticide registration are capable of handling kinetics and basic procedures outlined in this report. Modellers were asked to fit all parameters for each model, including the initial concentration or amount. For the data sets concerning parent and metabolite and the water-sediment systems, the analyses were restricted to SFO kinetics only.

Table 13-2 gives the data sets used in the software evaluation exercise. Data are either generated (Data sets A and B) using a model and assuming some variability, or taken from existing data sets (data sets C – F). Data set D stems from an experiment performed in duplicate; at each point in time the data are considered true replicates. Data set C is identical to Data set E with regard to the parent compound.

Note that in Data set E, there is metabolite present in the initial sample. Because those performing the simulation had not received the instructions on how to handle this situation, none of the corrections described in Chapters 6 and 10 were made. However, the results reported here are sufficient for the purpose of evaluating the various models.

Table 13-2. Data sets used for the software evaluation exercise.

Data set A		Data set B		Data set C		Data set E		
time (d)	P (%)	time (d)	c(%)	time (d)	P (%)	time (d)	P (%)	M (%)
0	101.24	0	98.62	0	85.10	0	85.10	1.10
3	99.27	3	81.43	1	57.90	1	57.90	20.00
7	90.11	7	53.18	3	29.90	3	29.90	34.00
14	72.19	14	34.89	7	14.60	7	14.60	40.20
30	29.71	30	10.09	14	9.70	14	9.70	35.20
62	5.98	62	1.50	28	6.60	28	6.60	27.60
90	1.54	90	0.33	63	4.00	63	4.00	14.90
118	0.39	118	0.08	91	3.90	91	3.90	12.50
				119	0.60	119	0.60	8.80

Data set D				
time (d)	P1 (%)	P2 (%)	M1 (%)	M2 (%)
0	99.46	102.04	0.00	0.00
1	93.50	92.50	4.84	5.64
3	63.23	68.99	12.91	12.96
7	52.32	55.13	22.97	24.47
14	27.27	26.64	41.69	33.21
21	11.50	11.64	44.37	46.44
35	2.85	2.91	41.22	37.95
50	0.69	0.63	41.19	40.01
75	0.05	0.06	40.09	33.85
100	<0.01	<0.01	31.04	33.13
120	<0.01	<0.01	25.15	33.31

Data set F			
time (d)	system	water	sediment
	P (%)	P (%)	P (%)
0	95.60	95.60	
3	91.90	84.70	7.20
7	86.50	74.60	11.90
14	72.90	54.10	18.80
28	29.60	13.50	16.10
43	10.00	4.30	5.70
56	6.80	2.00	4.80
70	3.50	0.50	3.00
100	4.20	0.80	3.40

13.5 Results

13.5.1 SFO kinetics, parent substance

Using SFO kinetics, the results of the packages are very close to each other for all data sets (see Tables 13-3a-f). Exceptions are from the package TOPFIT for which the initial amount is fixed by the package. Expert users can use a work around this feature of the package.

Table 13-3a. SFO fits for data set A.

package	M0	k	DegT50	DegT90
ACSL	109.20	0.0372	18.63	61.90
Excel	109.15	0.0372	18.62	61.87
Kinetica	109.11	0.0371	18.66	62.00
Madonna	109.20	0.0372	18.63	61.90
Mathematica	109.15	0.0372	18.62	61.87
MatLab	109.15	0.0372	18.63	61.87
ModelMaker	109.10	0.0371	18.68	62.06
ModelManager	109.15	0.0372	18.62	61.86
PRISM	109.20	0.0372	18.63	61.90
Statistica	109.15	0.0372	18.63	61.90
Tablecurve 2D	109.15	0.0372	18.62	61.87
Topfit	100.00*	0.0329	21.07	69.99

* fixed to 100

Table 13-3b. SFO fits for data set B.

package	M0	k	DegT50	DegT90
ACSL	99.20	0.0782	8.86	29.44
Excel	99.17	0.0782	8.87	29.46
Kinetica	99.17	0.0781	8.87	29.47
Madonna	99.18	0.0782	8.87	29.46
Mathematica	99.17	0.0782	8.87	29.46
MatLab	99.17	0.0782	8.89	29.46
ModelMaker	99.20	0.0780	8.89	29.52
ModelManager	99.17	0.0782	8.87	29.46
PRISM	99.17	0.0782	8.87	29.46
Statistica	99.17	0.0782	8.87	29.46
Tablecurve 2D	99.17	0.0782	8.87	29.46
Topfit	100.00*	0.0791	8.76	29.11

* fixed to 100

Table 13-3c. SFO fits for data set C.

package	M0	k	DegT50	DegT90
ACSL	82.50	0.3062	2.26	7.52
Kinetica	82.40	0.3043	2.28	7.57
Madonna	82.49	0.3060	2.27	7.52
Mathematica	82.49	0.3060	2.26	7.52
MatLab	82.49	0.3060	2.27	7.52
ModelMaker	82.49	0.3054	2.27	7.54
PRISM	82.49	0.3061	2.26	7.52
Tablecurve 2D	82.49	0.3061	2.26	7.52

Table 13-3d. SFO fits for data set D.

package	M0	k	DegT50	DegT90
ACSL	99.64	0.0989	7.01	23.29
Madonna	99.45	0.0979	7.08	23.52
MatLab	98.31	0.0989	7.00	23.28
PRISM	99.44	0.0979	7.08	23.51
Tablecurve 2D	99.44	0.0979	7.08	23.51

Table 13-3e. SFO fits for data set F (system).

package	M0	k	DegT50	DegT90
Kinetica	104.42	0.0398	17.40	57.80
Madonna	104.49	0.0399	17.35	57.64
Mathematica	104.48	0.0399	17.35	57.64
MatLab	104.48	0.0400	17.35	57.63
ModelMaker	104.50	0.0398	17.42	57.85
ModelManager	104.47	0.0399	17.35	57.64
PRISM	104.50	0.0400	17.35	57.64

Table 13-3f. SFO fits for data set F (water).

package	M0	k	DegT50	DegT90
ACSL	100.54	0.0551	12.58	41.80
Kinetica	100.88	0.0554	12.51	41.57
Madonna	100.54	0.0550	12.59	41.83
Mathematica	100.55	0.0551	12.58	41.80
MatLab	100.55	0.0551	12.58	41.80
ModelMaker	100.50	0.0549	12.63	41.94
ModelManager	100.55	0.0551	12.58	41.80
PRISM	100.50	0.0551	12.58	41.80

13.5.2 Gustafson-Holden kinetics, parent substance.

Tables 13-4a-f give the results for the fits with the Gustafson Holden model for the same data sets (parent substance). The initial amounts and the DegT50 and DegT90 values are quite close to each other for all packages except some of the Kinetica analyses. Although there seems to be good correspondence for the DegT50 values for the packages, the underlying parameters deviate quite substantially from each other, except for data set C. The standard deviations for the α and β parameters are quite large. Obviously the curves are not typical Gustafson-Holden curves, which results in uncertain parameters. The Gustafson-Holden model has been derived to deal with bi-phasic kinetics; in case of SFO kinetics there is an infinite number of solutions to the FOMC equation, i.e. many combinations of the alpha and beta parameters give approximately the same line.

In ModelMaker one can use both the integrated and the differentiated form of the Gustafson-Holden model. The results are slightly different from each other, probably due to the choice of initial values. The differences are not important and are also not very different from the results of the other packages.

Table 13-4a. FOMC results for data set A.

package	M0	alpha	beta	DegT50	DegT90
ACSL	109.34	2.93E+03	7.80E+05	18.43	61.32
Excel	109.20	2.36E+06	6.33E+07	18.62	61.87
Kinetica	107.29	4.26E+05	9.64E+06	15.68	52.09
Madonna	109.18	2.08E+06	5.59E+07	18.60	61.79
Mathematica	109.15	1.07E+06	2.87E+07	18.62	61.87
MatLab	109.45	2.74E+01	7.18E+02	18.39	62.93
ModelMaker	109.20	2.54E+04	6.82E+05	18.62	61.87
Modelmaker[#]	109.16	2.99E+02	8.04E+03	18.66	62.15
ModelManager	109.17	5.15E+02	1.38E+04	18.61	61.93
PRISM	109.20	5.50E+05	1.48E+07	18.62	61.86
Statistica	109.20	1.25E+04	3.37E+05	18.62	61.87
Tablecurve 2D	109.14	-3.43E-04	-9.22E+02	18.62	61.90

[#] differentiated form

Table 13-4b. FOMC results for data set B.

package	M0	alpha	beta	DegT50	DegT90
ACSL	99.60	1.32E-03	1.61E+02	8.69	30.71
Excel	99.20	4.94E+06	6.32E+07	8.87	29.46
Kinetica	99.66	1.27E+01	1.55E+02	8.67	30.72
Madonna	99.66	1.28E+01	1.38E+01	8.69	30.76
Mathematica	99.67	1.28E+01	1.56E+02	8.68	30.75
MatLab	99.75	1.10E+01	1.33E+02	8.65	30.98
ModelMaker	99.70	1.28E+01	1.56E+02	8.69	30.76
ModelMaker[#]	99.67	1.25E+01	1.53E+02	8.72	30.95
ModelManager	99.67	1.28E+01	1.56E+02	8.68	30.75
PRISM	99.67	1.28E+01	1.56E+02	8.68	30.74
Statistica	99.66	1.28E+01	1.56E+02	8.68	30.76
Tablecurve	99.66	1.28E+01	1.56E+02	8.68	30.76

[#] differentiated form

Table 13-4c. FOMC results for data set C.

package	M0	alpha	beta	DegT50	DegT90
Kinetica	85.87	1.06	1.92	1.79	15.12
Madonna	85.88	1.05	1.92	1.79	15.14
Mathematica	85.87	1.05	1.92	1.79	15.15
MatLab	85.88	1.05	1.92	1.79	15.15
ModelMaker	85.88	1.04	1.89	1.79	15.39
PRISM	85.88	1.05	1.92	1.79	15.16
Tablecurve 2D	85.87	1.05	1.92	1.79	15.15

Table 13-4d. FOMC results for data set F (system).

package	M0	alpha	beta	DegT50	DegT90
Kinetica	103.94	2.21E+03	5.22E+04	16.41	54.55
Madonna	104.49	2.51E+06	6.27E+07	17.34	57.59
Mathematica	104.47	1.28E+06	3.19E+07	17.35	57.64
MatLab	104.68	3.84E+01	9.42E+02	17.17	58.26
ModelMaker	104.50	1.05E+02	2.63E+03	17.39	58.21
ModelManager	104.48	2.13E+03	5.32E+04	17.35	57.65
PRISM	104.50	8.38E+04	2.10E+07	17.35	57.65

Table 13-4e. FOMC results for data set F (water).

package	M0	alpha	beta	DegT50	DegT90
Kinetica	100.51	1.26E+03	2.27E+04	12.51	41.58
Madonna	100.55	3.76E+06	6.83E+07	12.59	41.81
Mathematica	100.55	1.86E+06	3.39E+07	12.58	41.80
MatLab	100.73	4.79E+01	8.55E +02	12.47	42.13
MMaker	100.60	1.20E+02	2.18E+03	12.60	42.15
ModelManager	100.55	2.14E+03	3.89E+04	12.58	41.81
PRISM	100.50	9.78E+04	1.77E+06	12.55	41.68

13.5.3 Bi-exponential kinetics, parent substance

Results of these fits are presented in Tables 13-5a-b. For data sets C – F too few packages were used and therefore no conclusion can be drawn for these data sets. The following is solely based on data sets A and B.

Two forms of the bi-exponential model exist (cf. Chapter 5) and although they are equivalent, different results are obtained. For ACSL and Berkeley Madonna analyses, the first form of the DFOP model (cf. Box 5-4) was used, the second form can be used as well. Tablecurve 2D has the first form built in, but the user may implement the second form as well. The results listed in the tables refer to the built-in routine. Statistica can use both forms (the first form was chosen for this exercise). Mathematica, ModelMaker, and PRISM use the second form of the DFOP model; in all three packages the other form can be implemented as well. For data set A, ACSL optimises to only one compartment, which converts the model to SFO. Berkeley Madonna, Mathematica, MatLab, ModelMaker, PRISM, and Tablecurve distinguish two compartments, but each compartment has virtually the same transformation rate. So, actually also these packages return a single compartment. The distribution of the initial amount over the two compartments is quite different. This can be explained again by the nearly identical transformation rates; any distribution would lead to a similar degradation curve. As a result of its ability to be described adequately by SFO kinetics, data set A seems not to be a good data set for checking the packages with respect to DFOP.

Data set B seems to be much more suitable for checking DFOP kinetics in the packages. Initial amounts and DegT50 and DegT90 values are similar for all tested packages Except for ACSL, the distribution over the two compartments and the transformation rates are similar.

Table 13-5a. DFOP results for data set A,

package	M0	f	k1	k2	DegT50	DegT90
ACSL	109.30	1.00	0.0376	0.0000	18.43	61.24
Madonna	109.15	0.54	0.0372	0.0372	18.62	61.87
Mathematica	109.15	0.58	0.0372	0.0372	18.62	61.87
MatLab	109.15	0.50	0.0372	0.0372	18.62	61.86
ModelMaker	109.10	0.07	0.0369	0.0371	18.70	62.10
PRISM	109.16	0.50	0.0372	0.0372	18.65	61.88
Tablecurve	109.14	0.79	0.0372	0.0373	18.63	61.86

Table 13-5b. DFOP results for data set B.

package	M0	f	k1	k2	DegT50	DegT90
ACSL	99.59	0.82	0.0890	0.0439	8.70	30.60
Madonna	99.65	0.67	0.0959	0.0526	8.64	30.34
Mathematica	99.65	0.67	0.0958	0.0525	8.68	30.79
MatLab	99.61	0.80	0.0903	0.0452	8.69	30.71
ModelMaker	99.70	0.68	0.0955	0.0517	8.70	30.90
PRISM	99.65	0.67	0.0958	0.0525	8.68	30.79
Statistica	99.65	0.67	0.0958	0.0526	8.64	30.74
Tablecurve 2D	99.65	0.67	0.0958	0.0526	8.68	30.79

13.5.4 Hockey-stick kinetics, parent substance

Results for the fitting of this model to the data sets are presented in Tables 13-6a-e. The package Tablecurve 2D seems to be incapable of fitting hockey-stick kinetics; this package was unsuccessful for all data sets. For data sets A and B all other packages (except ModelMaker for data set B) estimate similar initial amounts and DegT50 values. However, the underlying rate values and the breakpoints for EXCEL (2^{nd} fit), Kinetica and Mathematica are different from the other ones. These packages have a breakpoint after 5.11, 5.96 and 5.33 days for data set A, while all other have their breakpoint after 10.9 days. Although nearly the same DegT50 values are recorded, the DegT90 values differ quite substantially.

For data set C, ACSL and Kinetica give similar results, but Mathematica differs. The DegT50 values for the latter package is nearly the same, but the initial value clearly differs. This package estimates a negative value for the breakpoint, which is unrealistic. Mathematica is also the only package giving different results for data set F, both for the whole system as for the water only. Again the breakpoint is much earlier than for the other packages. Also here the largest difference can be observed for the DegT90.

Table 13-6a. Hockey-stick kinetics for data set A.

package	M0	Tb	k1	k2	DegT50	DegT90
Excel*	102.31	10.92	0.0167	0.0544	20.29	49.86
Excel*	100.75	5.11	0.0006	0.0456	20.22	55.49
Kinetica	101.24	5.96	0.0066	0.0462	20.13	54.99
Madonna	102.31	10.91	0.0167	0.0544	20.29	49.86
Mathematica	100.26	5.33	0.0462	0.0000	20.33	49.56
ModelMaker	102.30	10.90	0.0167	0.0543	20.31	49.95
ModelManager	102.31	10.91	0.0167	0.0545	20.29	49.85
PRISM	102.30	10.91	0.0167	0.0545	20.29	49.85
Statistica	102.31	10.92	0.0167	0.0544	20.31	49.89
Tablecurve	no fit					

* Different initial values were used; the package is obviously sensitive to this.

Table 13-6b. Hockey-stick kinetics for data set B.

package	M0	Tb	k1	k2	DegT50	DegT90
Excel*	99.33	26.00	0.0788	0.0592	8.79	30.27
Excel*	100.42	7.00	0.0848	0.0702	8.42	31.36
Kinetica	100.14	7.00	0.0833	0.0710	8.55	31.23
Madonna	100.19	7.00	0.0839	0.0704	8.50	31.37
Mathematica	98.62	26.26	0.0744		8.93	29.05
ModelMaker	no fit					
ModelManager	99.34	26.01	0.0789	0.0592	8.79	30.26
PRISM	99.20	35.03	0.0783	0.0538	8.86	29.42
Statistica	99.33	26.00	0.0789	0.0592	8.79	30.26
Tablecurve 2D	no fit					

* Different initial values were used; the package is obviously sensitive to this.

Table 13-6c. Hockey-stick kinetics for data set C.

package	M0	Tb	k1	k2	DegT50	DegT90
ACSL	84.50	5.10	0.3562	0.0247	1.95	24.76
Kinetica	84.50	5.16	0.3562	0.0225	1.95	25.84
Madonna	84.50	5.15	0.3562	0.0227	1.95	25.78
Mathematica	91.45	-0.33	0.3060	0.0000	1.93	7.10
ModelMaker	84.51	5.15	0.3555	0.0225	1.95	26.12
PRISM	84.50	5.15	0.3562	0.0227	1.95	25.77
Tablecurve	no fit					

Table 13-6d. Hockey-stick kinetics for data set F (system).

package	M0	Tb	k1	k2	DegT50	DegT90
ACSL	96.17	12.57	0.0151	0.0630	20.55	46.09
Kinetica	95.71	12.49	0.0143	0.0633	20.60	46.03
Madonna	95.71	12.48	0.0143	0.0635	20.59	45.95
Mathematica	93.75	6.46	0.0505	?	20.17	
ModelMaker	95.70	12.40	0.0142	0.0633	20.57	45.99
ModelManager	95.71	12.48	0.0143	0.0635	20.59	45.94
PRISM	95.71	12.48	0.0143	0.0635	20.59	45.94

Table 13-6e. Hockey-stick kinetics for data set F (water).

package	M0	Tb	k1	k2	DegT50	DegT90
Kinetica	95.17	12.85	0.04	0.0955	15.32	32.18
Madonna	95.16	12.86	0.0356	0.0955	15.33	32.18
Mathematica	95.60	1.96	0.06	?	14.76	37.83
ModelMaker	95.18	12.85	0.04	0.0951	15.33	32.25
ModelManager	95.17	12.86	0.04	0.0955	15.32	32.18
PRISM	95.17	12.86	0.04	0.0955	15.29	32.14

13.5.5 Results for parent and metabolite

Two data sets have been used to compare packages with respect to their capabilities of fitting parameters for parent – metabolite systems. This exercise was restricted to SFO kinetics for both parent and metabolite.

13.5.5.1 Dataset D

Five packages were used to estimate kinetic parameters for this data set and results are presented in Table 13-7. All packages (Berkeley Madonna, Kinetica, Mathematica, MatLab, and ModelMaker) assumed that parent flowed to both the metabolite and the sink compartment and the metabolites flowed to the sink compartment. Fitted initial amounts and transformation rates for the parent (and thus the DegT50) were rather similar for all packages. Mathematica estimated a slightly higher formation fraction for the metabolite and a somewhat smaller DegT50, compared to the other four packages using the same transformation scheme.

Table 13-7. Optimised parameter values for data set D (parent + metabolite).

Parameter	Definition	Kinetica	Madonna	Mathematica	MatLab	ModelMaker
M0	total size of compartment	99.59	99.77	98.21	99.55	99.59
k12	rate coefficient parent->metabolite	0.0507	0.098[$]	0.0541	0.0508	0.0506
k13	rate coefficient parent->rest	0.0480		0.0443	0.0478	0.0478
k23	rate coefficient metabolite->rest	0.0052	0.0053	0.0062	0.0053	0.0053
f12	formation fraction metabolite	0.51	0.51	0.55	0.5148	0.51
DegT50_p	half life parent	7.03	7.05	7.04	7.03	7.04
DegT50_m	half life metabolite	132.84	130.39	111.21	131.61	130.78

[$] K12 obtained with Madonna should be compared to k12 + k13 of the other packages.

13.5.5.2 Dataset E

Four packages were used to estimate parameters for data set E (parent + metabolite) and results are reported in Table 13-8. Mathematica, MatLab, and Kinetica give very similar results, while the results of Berkeley Madonna are different.

Table 13-8. Optimised parameter values for data set e (parent + metabolite).

Parameter	Definition	Kinetica	Madonna	MatLab	Mathematica
M0	total size of compartment	84.71	86.90	84.68	84.74
k12	rate coefficient parent->metabolite	0.3509	0.3885	0.1991	0.1991
k13	rate coefficient parent->rest			0.1524	0.1528
k23	rate coefficient metabolite->rest	0.0183	0.0176	0.0183	0.0182
f12	formation fraction metabolite	0.57	0.54	0.57	0.57
DegT50_p	half life parent	1.98	1.78	1.97	1.97
DegT50_m	half life metabolite	37.98	39.46	37.96	37.99

[$] K12 obtained with Kinetica and Madonna should be compared to k12 + k13 of MatLab and Mathematica.

13.5.6 Results for water-sediment systems

Four packages were used to estimate parameters for the water-sediment data set (level II approach without reversible partitioning), Kinetica, Berkeley Madonna, ModelMaker and ModelManager. As shown in Table 13-9, these packages gave similar results for this approach. For all packages, the DegT50 in the sediment is much shorter than the DegT50 in the water.

At level II with reversible partitioning, the Mathematica package was also used. All packages estimate very small values (< 5E-5 (Kinetica, Berkeley Madonna and ModelMaker), zero (ModelManager) or a negative value (Mathematica)) for the transfer rate for sediment to water. ModelManager returns exactly zero for the back-transfer of substance, so the results for level II with and without reversible partitioning are identical. For the Kinetica, Berkeley Madonna and ModelMaker packages, the results obtained at level II with reversible partitioning fall within the confidence limits of level II without reversible partitioning. This shows that inclusion of the back-transfer rate does not contribute to the understanding of this

substance in water-sediment systems. For unknown reasons, the results obtained with Mathematica differ substantially from the other results.

Table 13-9. Results for water-sediment system, level II assessments (without reversible partitioning)

Parameter	Definition	Kinetica	Madonna	ModelMaker	ModelManager
M	total size	100.51	100.57	100.60	100.52
kdeg_wat	rate coefficient water	0.0248	0.0249	0.0244	0.0251
kdeg_sed	rate coefficient sediment	0.0730	0.0726	0.0738	0.0722
trwater_sed	exchange water->sediment	0.0302	0.0302	0.0306	0.0299
DegT50_w	half-life in water	27.95	27.86	28.41	27.64
DegT50_s	half-life in sediment	9.50	9.54	9.39	9.60

Table 13-10. Results for water-sediment system, level II- assessments (with reversible partitioning)

Parameter	Definition	Kinetica	Madonna	Mathematica	ModelMaker	ModelManager
M	total size	100.80	101.14	99.31	100.50	100.52
kdeg_wat	rate coefficient water	0.0259	0.0264	0.0282	0.0245	0.0251
kdeg_sed	rate coefficient sediment	0.0695	0.0693	0.0197	0.0736	0.0722
trwater_sed	exchange water->sediment	0.0297	0.0289	0.0217	0.0305	0.0299
trsed_water	exchange sediment -> water	0.0000	0.0000	-0.00988	0.0000	0.0000
DegT50_w	half-life in water	26.80	26.30	24.59	28.29	27.64
DegT50_s	half-life in sediment	9.98	10.01	35.17	9.42	9.60

13.6 Conclusions

Only a very limited number of (potentially usable) packages have been reviewed.

The software packages that have been reviewed differ quite substantially in their capabilities. Some of the packages can be used to estimate parameters for all kinetic models, for both parent and metabolites and for water sediment systems. Other packages are more limited in their use; for instance they can be used for parent only or are limited in the models they can handle.

In general, the packages do what they promise and only a few bugs have been reported. Most packages can handle all kinetic models recommended in this report, when applied to a single substance / compartment. Differences found in this exercise are mostly attributable to the data sets or to settings of the packages used. For studies including parent and metabolite or water and sediment, fewer packages have been reviewed but the same general conclusion holds.

Based on the review of the packages, experience of work group members with these packages and Chapters 6 - 10, the following are limitations or points of concern:

Excel (for the purpose of parameter estimation only). Without the add-in Solver module or user-coded functions, this package is capable of fitting SFO kinetics only, and this only after log-transformation of the data. In general transformation of data is not recommended and is allowed only when justified after thorough analysis. Therefore, the Solver add-in, which comes as part of the standard installation package, should be used when kinetic fitting is done with Excel.

Topfit This package has limited possibilities and time zero amounts are fixed. The latter can be circumvented if the user is very familiar with the package. Furthermore, the package is not supported any longer by the developer.

Statistica This package needs analytical solutions for the formation and transformation of metabolites. So in practice the use will be rather limited for estimating parameters for metabolites.

ModelMaker Two versions (3.1 and 4.0) are available. Version 4.0 has less functionality than version 3.1. A bug in one of the statistic functions in version 3.1 has been reported.

Berkeley Madonna This package needs the description of kinetic models in the form of differential or difference equations. The package lacks statistical functionality, but

data transfer to other software – for instance EXCEL – is excellent so statistical analysis can be done with a separate package.

13.7 Recommendations

In general the guidelines of Chapters 6 – 11 should be followed quite strictly, deviations should occur only when this can be justified.

Most packages have quite some flexibility with regard to data handling, weighting, parameter restrictions, settings of the objective function, etc. Results might be rather sensitive to the settings. In general limiting parameters to physically / chemically realistic values is allowable; all limitations should be justified in the report.

Initial values of all parameters might have effect on the estimated results. Therefore, at least two sets of contrasting initial values should be used and the resulting estimates should be checked to see if they are identical. If not, the optimisation possibly stopped at a local minimum.

Although most packages have tutorials, examples, demos, and a comprehensive manual, courses should be organised to train people from both industry and evaluating authorities in the use of software packages and the application of the guidelines given in this report.

A software package that includes the kinetic models and statistical analyses recommended by the work group would be quite useful. If developed, such a package might be capable of covering around 90% of all possible situations.

APPENDIX 1: EXISTING GUIDANCE ON EXPERIMENTAL LABORATORY DEGRADATION STUDIES

	SETAC	OECD	US-EPA
Soil			
Number	1 plus 3 for degradation rate(s)	1 plus 3 for degradation rate(s)	At least 1
Property requirements	Agricultural soil, approximately 2-5 % OM, pH 5.5-7.5, 10-25% clay	Representative sandy loam, silt loam, loam or loamy sand (FAO, USDA classification), pH 5.5-8.0, OC 0.5-2.5% and microbial mass of at least 1% of total organic carbon (OECD, 1995)	Representative sandy loam or silt loam
Characterisation requirements	Texture, pH, % organic matter, cation exchange capacity, water holding capacity, microbial activity e.g. biomass	Texture, pH, % organic matter, cation exchange capacity, water retention characteristic, bulk density, microbial activity/ biomass (e.g. substrate-induced respiration)	General soil characteristics
Origin	Agricultural soil	Representative soil	Field representative for compound use
Sampling	Freshly sampled	Freshly sampled	Not specified
Storage	ISO 10381-6 (1993)	ISO 10381-6 (1993)	Not specified
Previous exposure	No adverse effects on soil micro-organisms	No treatment with a.s. or structural analogs within previous 4 years	Not specified

	SETAC	OECD	US-EPA
Application			
Test system	Sufficient air exchange, must allow measurement of volatile components	Flow-through or biometer	Flow-through or biometer
Application rate	Maximum label rate, sufficient for metabolite identification	Maximum label rate, sufficient for metabolite identification, no unrealistic suppression of soil micro-organisms	Sufficient for following parent decline and metabolite identification
Test substance	Preferably ^{14}C radiolabelled	Preferably ^{14}C radiolabelled	Preferably ^{14}C radiolabelled
Allowed vehicle	Water, acetone	As solid, water, acetone, organic solvents, may not suppress soil micro-organisms	Not specified
Incubation conditions			
Duration	Until pattern of decline of a.s. and formation and decline of degradation products established, should not exceed 120 days, may last 6-12 months, however decrease of soil micro-organism activity after 4 month should be accounted for in interpretation of results	Should not exceed 120 days, may last 6-12 months with additional biomass measurement at end of study	1 year or when pattern of decline of a.s. and formation and decline of degradation products are established, whatever comes first
Temperature	20 ± 2°C, experiment at 10°or 30°C or calculation	20 ± 2°C, 10 ± 2°C for use in cold climates	18-30°C
Soil moisture	40-50% of maximum water holding capacity	pF 2.0-2-5	75% of 1/3 bar
Light regime	dark	dark	dark

	SETAC	OECD	US-EPA
Sampling			
Minimum sampling dates	Minimum of 8, for non-linear analysis more	Minimum of 6	Minimum of 12
Number of replicates	Single	Two	Sufficient samples to allow interpretable results
Measurements			
Soil microbial activity	At start of study	At start of study using e.g. SIR	Not specified
Mass balance	Yes, between 90-110% of applied mass for 14C-labelled test substance, 70-110% for non-labelled test substance.	Yes, between 90-110% of applied mass	Yes
Extraction methods	Exhaustive extraction		Must allow rate, type and degree of metabolism of the a.s. and its major degradates
Analysis and metabolite identification	Analysis of a.s. and identification of metabolites >10% AR, attempt to characterise metabolites approaching 10% AR, characterisation of bound residues, identification of volatile components > 10% AR	Analysis of a.s. and transformation products, quantification of non-extractable radioactivity, analysis of volatile components Limit of detection (LOD) for a.s. and transformation products at least 0.01 mg kg^{-1} or 1% of applied dose, whichever is lower. Specification of limit of quantification (LOQ) required	Analysis of a.s. and major degradates, identification of metabolites occurring at > 0.01 mg kg^{-1} soil
Results			
Metabolic pathway	Yes	Yes	Yes
DT50 / DT90	Yes	Yes, for simple-first-order r^2>0.7	Yes

APPENDIX 2: MICHAELIS-MENTEN KINETICS

Michaelis-Menten kinetics are useful for describing reactions that are more linear than first order and can be used as an alternative kinetic model where degradation is between zero order (straight line) and first-order. As this type of degradation pattern is not common in environmental fate studies, Michaelis-Menten kinetics were not considered as a standard model. Another drawback of this model is that the endpoints depend on the initial concentration of the pesticide.

The rate of degradation is describes by the following equation

$$dM/dt = -V_m\, M/(K_m+M) \qquad \text{(A2-1)}$$

where V_m = maximum rate of degradation
M = pesticide concentration
K_m = Michaelis constant

Most pesticides are degraded by microorganisms involving enzymes. The simplest enzymatic law derived in 1913 by Michaelis and Menten is based on the reaction scheme

$$[E]+[M] \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} [EM] \xrightarrow{k_2} [E]+[P] \qquad \text{(A2-2)}$$

where [E] = concentration of free enzyme
[EM] = concentration of enzyme substrate complex
[P] = product concentration
[M] = pesticide concentration
k_1, k_{-1}, k_2 = microscopic kinetic constants

From the reaction scheme the following “enzymatic law”, i.e. a relationship between the reaction velocity and substrate concentration is obtained

$$V(M) = \frac{V_m M}{M+K_m} \qquad \text{(A2-3)}$$

where V_m = maximum rate of degradation
K_m = Michaelis constant (half saturation constant)

The macroscopic constants V_m and K_m are related to the microscopic constants via

$$V_{max} = k_2[E_{tot}] \quad \text{and} \quad K_m = \frac{k_2 + k_{-1}}{k_1} \tag{A2-4}$$

where $[E_{tot}]$ = total enzyme concentration

The differential equation for enzymatic degradation is thus given by

$$\frac{dM}{dt} = -\frac{V_m M}{M + K_m} \tag{A2-5}$$

According to the initial substrate concentration M_0, three cases can be distinguished:

1. $M_0 << K_m$

In this case, M is negligible in the denominator of equation A2-5, yielding a pseudo first order reaction

$$\frac{dM}{dt} \approx -\frac{V_m}{K_m} M = -kM \quad \text{with} \quad k = \frac{V_m}{K_m} \tag{A2-6}$$

2. $M_0 >> K_m$

In this case, saturation is reached resulting in a zero order reaction

$$\frac{dM}{dt} \approx -V_m \tag{A2-7}$$

3. The intermediate case

In the intermediate case, i.e. M_0 in the order of magnitude of K_m, no analytical solutions in the form M(t) are available. However, it is still feasible to obtain the inverse relationship:

$$t(M) = \frac{M_0 - M}{V_m} - \frac{K_m}{V_m} \ln \frac{M}{M_0} \tag{A2-8}$$

Hence the DT50 value is given explicitly by:

$$DT50 = \frac{M_0}{2V_m} + \frac{K_m}{V_m}\ln 2 \qquad \text{(A2-9)}$$

Note that the DT50 value depends on the initial concentration!

In the limiting case 1, the DT50 value approaches the first order expression

$$DT50 = \frac{K_m}{V_{max}}\ln 2$$

In degradation studies, Michaelis-Menten degradation schemes are rarely seen because the initial concentrations are too small to reach saturation. Furthermore, in most cases, measurement errors do not allow to establish a zero order reaction rate. However, saturation effects may occur, if the pesticide is frequently applied.

Patterns of decline in pesticide concentration as predicted by Michaelis-Menten kinetics are shown in Figure A2-1.

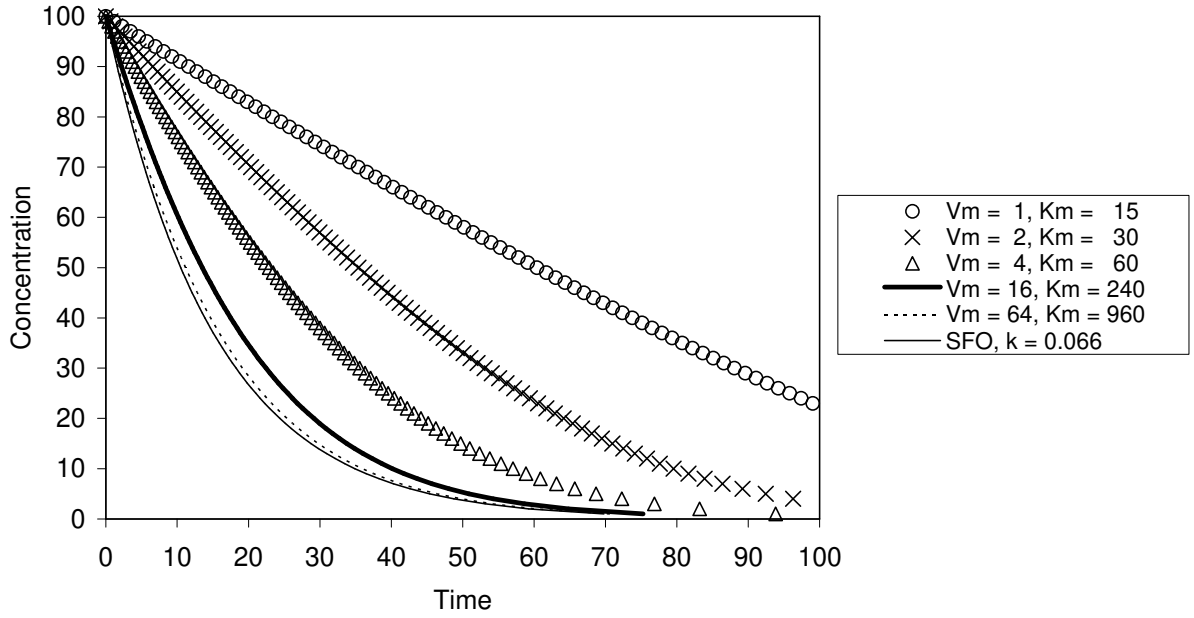


Figure A2-1. Patterns of decline of pesticide concentration as predicted by Michaelis-Menten kinetics. The decline curves have been calculated using a unique Vm/Km ratio (=0.066) and compared to the corresponding first-order kinetics with k=Vm/Km=0.066.

Equation (integrated form) **Differential equation**

Chemical equation: $E + M \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} EM \xrightarrow{k_2} E + P$

at the steady state (dEM/dt=0): $\frac{dM}{dt} = -\frac{V_m M}{K_m + M}$

$$t = \frac{K_m}{V_m} Ln \frac{M_0}{M} + \frac{M_0 - M}{V_m}$$

where

E : enzyme concentration

E_0: total enzyme concentration

M : substrate concentration

M_0: total chemical concentration applied at t=0

EM : enzyme-substrate complex concentration

P : product concentration

$k_{1,-1,2}$: rates constants for the formation and dissociation of the enzyme-substrate complex

V_m: maximum rate ($=k_2 * E_0$)

K_m: Michaelis constant

Parameters to be determined

M_0, V_m, K_m

Endpoints

$$DT_x = \frac{K_m}{V_m} Ln \frac{100}{100 - x} + \frac{x M_0}{V_m 100}$$

Box A2-1. The Michaelis-Menten model

APPENDIX 3: EXAMPLES OF KINETIC ANALYSES FOR PARENT COMPOUNDS

The following examples are presented to illustrate the use of the tools

- χ^2-test,
- visual evaluation of a plot of observed/fitted concentrations vs. time,
- visual evaluation of a plot of residuals up to the DT90,

to assess whether a SFO-fit can describe the measured data appropriately (see Figure 7-2).

In certain cases assessment of a bi-phasic fit taken from the exercise to derive regulatory trigger values can be useful (see Section 7.1.2.1.).

All examples assume that modified fitting routines like exclusion of outliers, constrain initial mass and weighting of data were already taken into account and that observed bi-phasic behaviour was not due to artefacts. For field studies, normalisation of soil residue data prior to kinetic analysis was assumed.

The following examples focus on the decision making process to decide SFO vs. bi-phasic. Once the proposed kinetic model for modelling endpoints was of bi-phasic nature the further differentiation as presented in Figure 7-2 is not presented.

Laboratory Data: Example 1

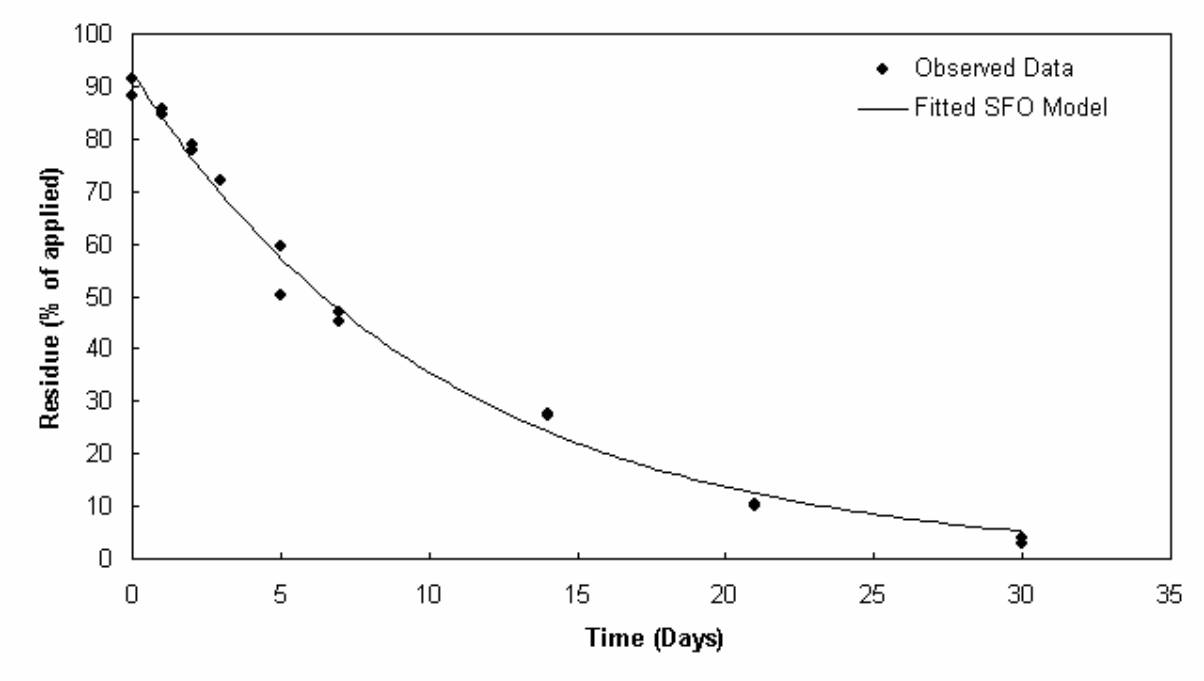
L1: SFO: Table observed vs. fitted (% applied):

Time (d)	Parent observed	Parent fitted SFO
0	88.3	92.471
0	91.4	92.471
1	85.6	84.039
1	84.5	84.039
2	78.9	76.376
2	77.6	76.376
3	72.0	69.412
3	71.9	69.412
5	50.3	57.330
5	59.4	57.330
7	47.0	47.352
7	45.1	47.352
14	27.7	24.247
14	27.3	24.247
21	10.0	12.416
21	10.4	12.416
30	2.9	5.251
30	4.0	5.251

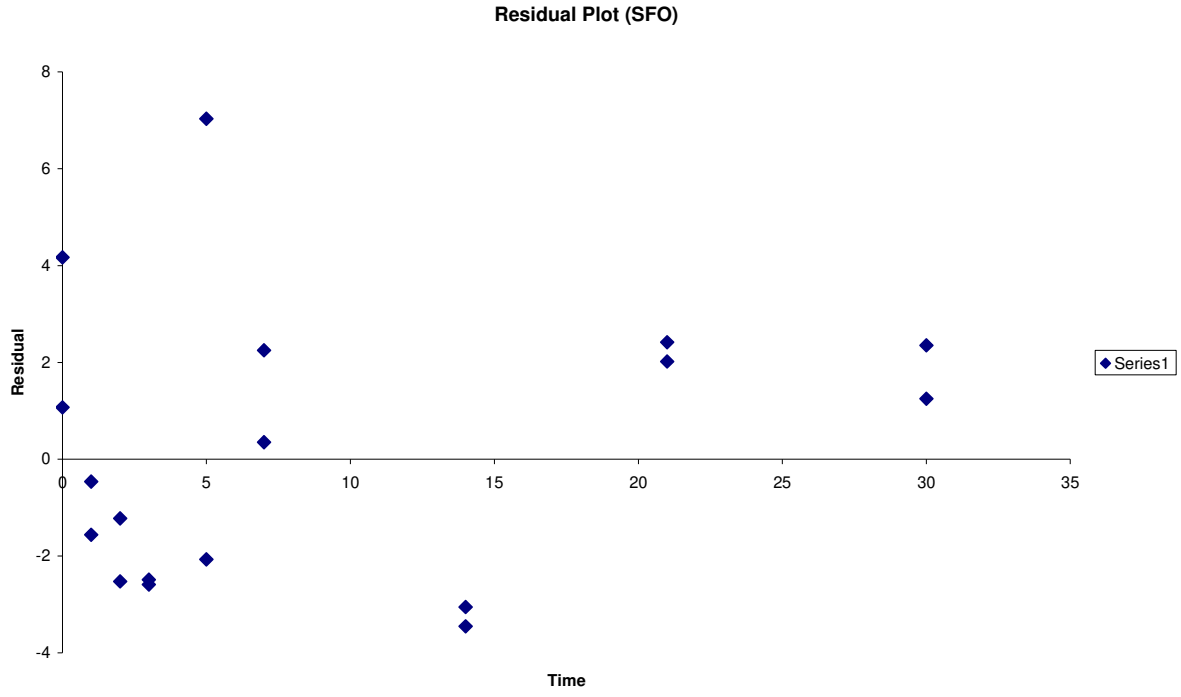
L1: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	7.3	24.1

L1: SFO: Graphs observed vs. fitted:



L1: SFO: Residual Graphs:



L1: SFO: Table χ^2-test results (using average value for duplicates):

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	4 %

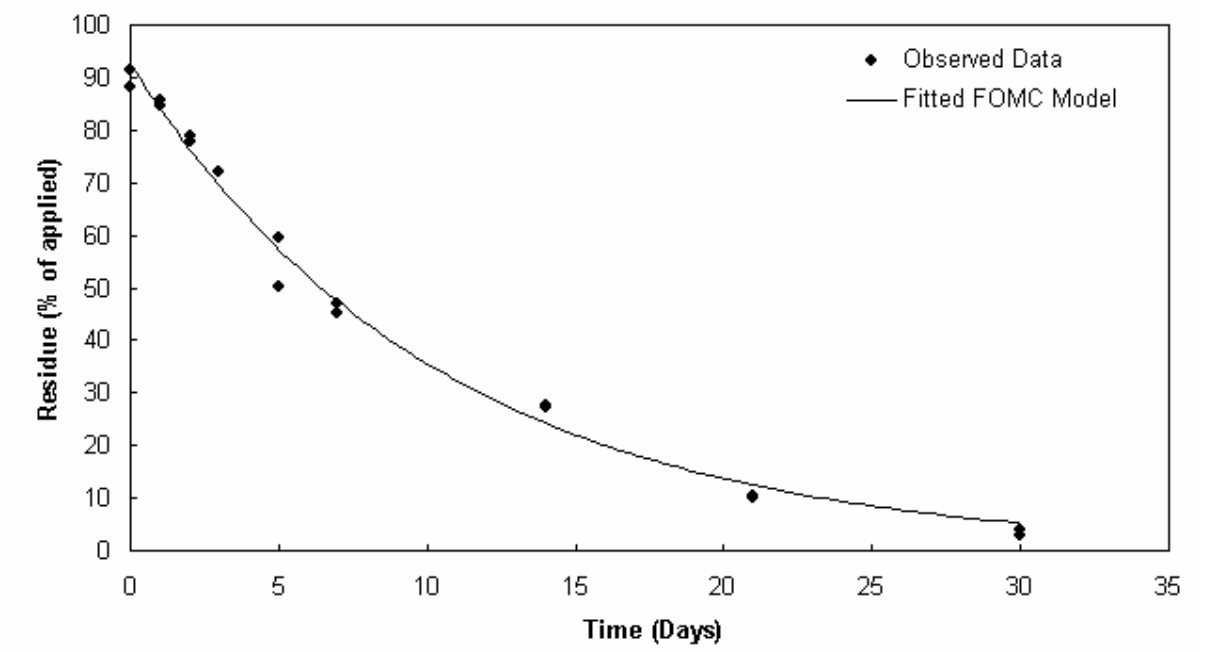
L1: Conclusions:

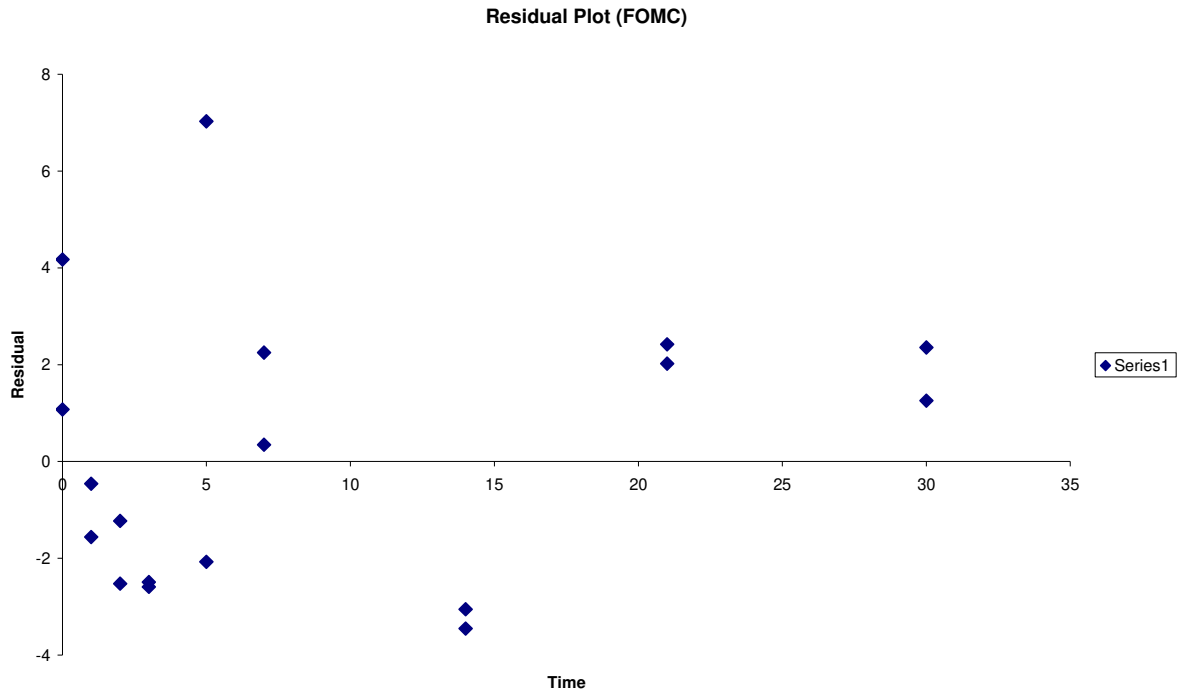
- χ^2 error value for SFO at 4%.
- No systematic error apparent in residual plot. Well behaved data-set, very limited scatter in the measured data.
- SFO appropriate for use in modelling.

Additional information on potential bi-phasic behaviour (see following section)

- No improvement of χ^2 statistics for FOMC (4%).
- No improvement of residual pattern for FOMC.

L1: Additional information: FOMC





Laboratory Data: Example 2

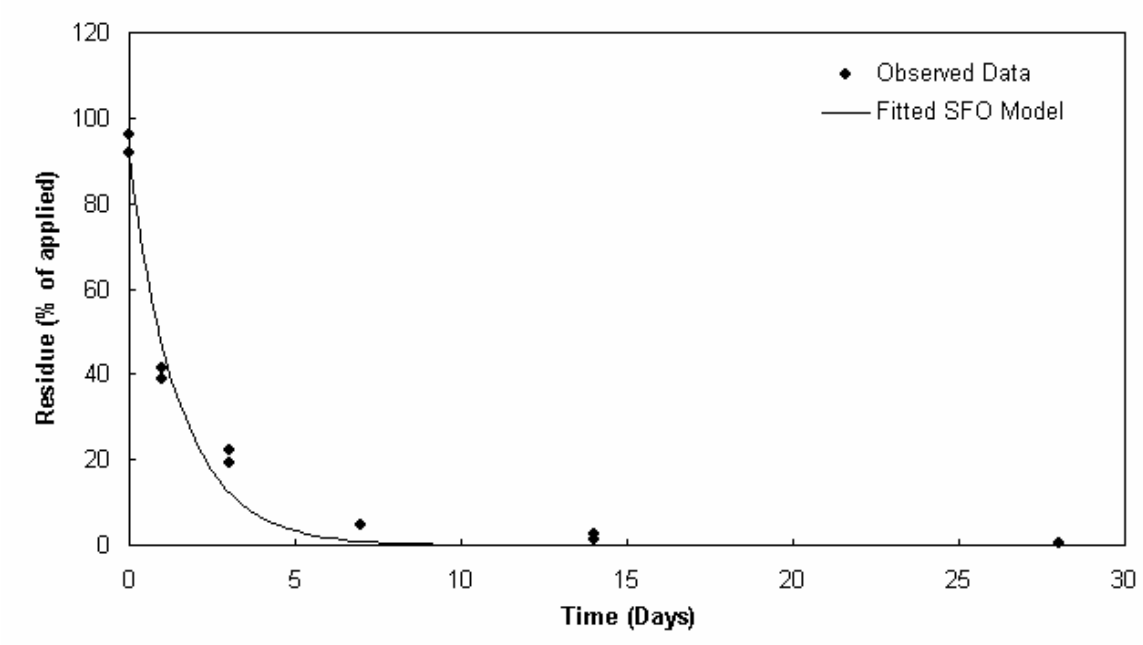
L2: SFO: Table observed vs. fitted (% applied):

Time (d)	Parent observed	Parent fitted SFO
0	96.1	91.466
0	91.8	91.466
1	41.4	47.139
1	38.7	47.139
3	19.3	12.521
3	22.3	12.521
7	4.6	0.883
7	4.6	0.883
14	2.6	0.009
14	1.2	0.009
28	0.3	0.000
28	0.6	0.000

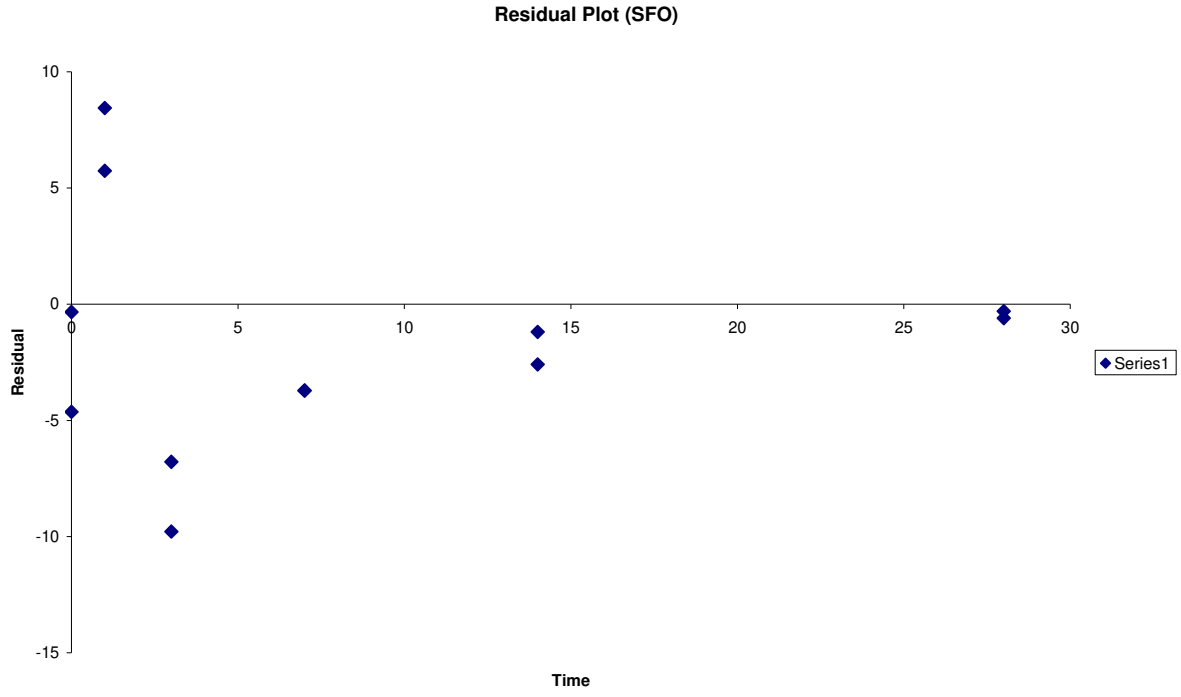
L2: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	1.0	3.5

L2: SFO: Graphs observed vs. fitted:



L2: SFO: Residual Graphs:



L2: SFO: Table χ^2-test results (using average value for duplicates):

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	15 %

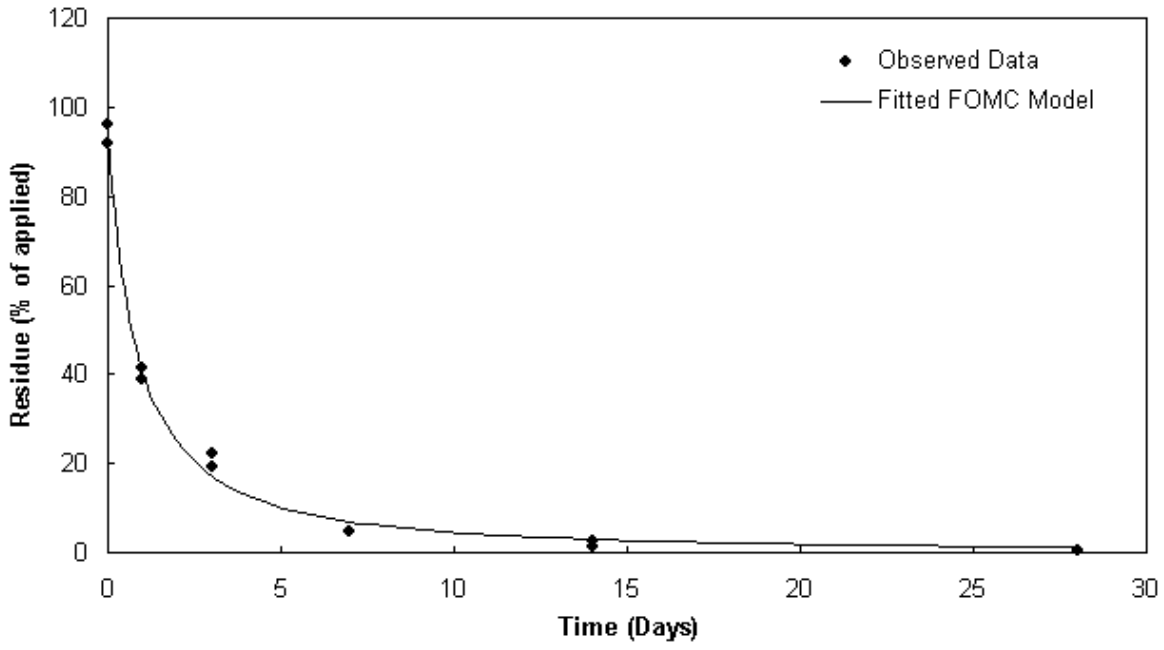
L2: Conclusions:

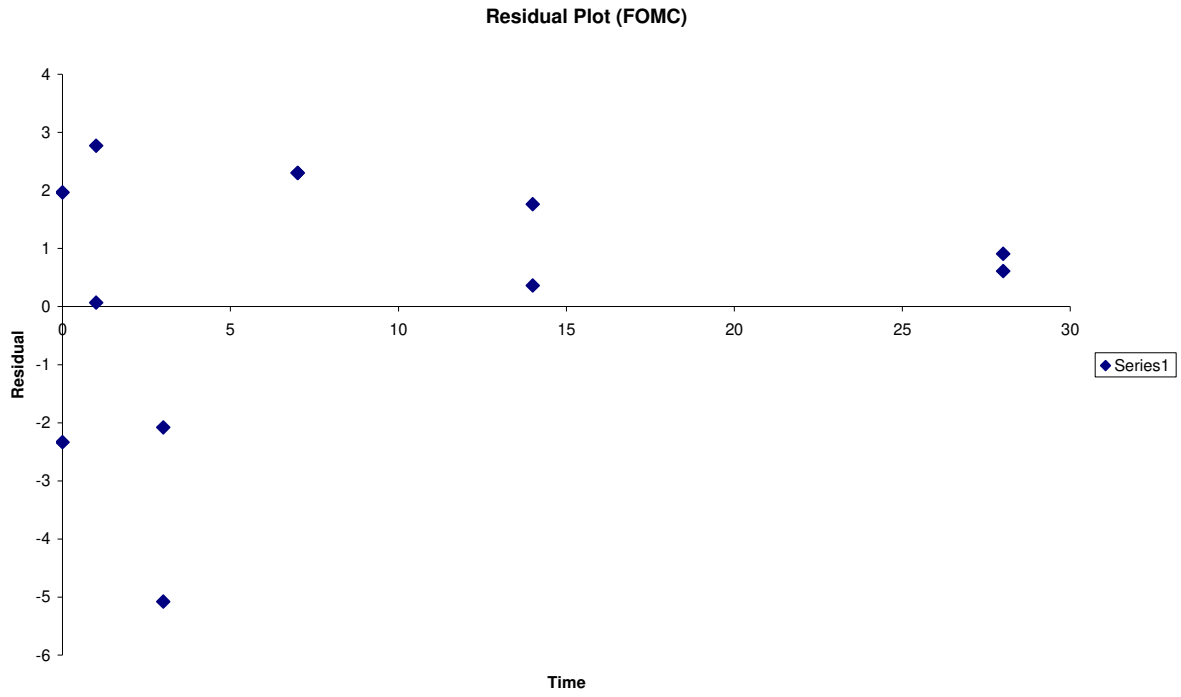
- χ^2 error value for SFO at 15%
- No apparent systematic error observed from residual plot up to measured DT90 (approx. at day 5), underestimation beyond that point.
- SFO appropriate for use in modelling.

Additional information on potential bi-phasic behaviour (see following section)

- Improvement of χ^2 statistics for FOMC (7%).
- No improvement of residual pattern for FOMC with regard to random nature up to measured DT90.

L2: Additional information: FOMC





Laboratory Data: Example 3

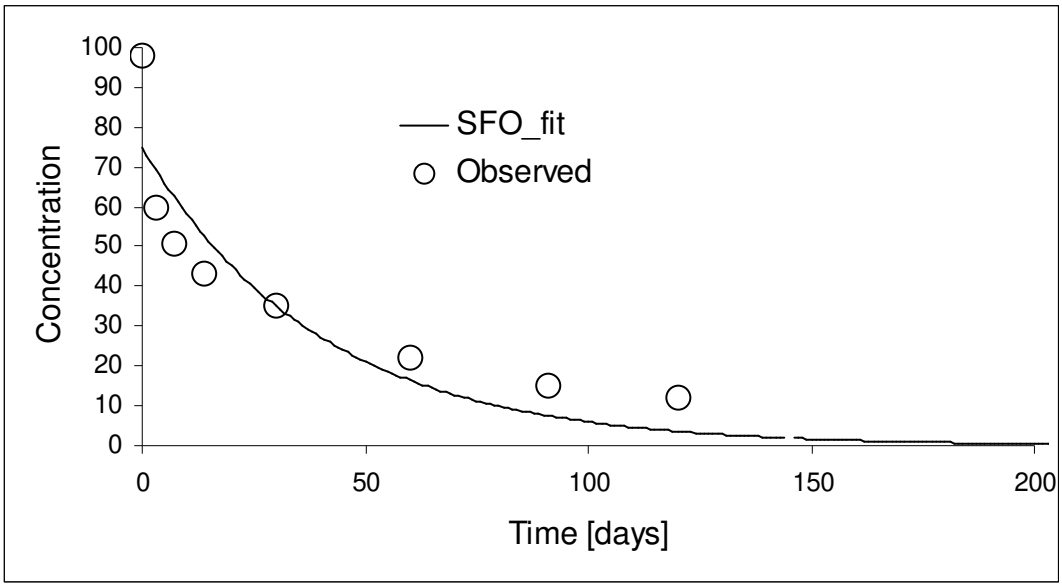
L3: SFO: Table observed vs. fitted (% applied):

Time (d)	Parent Observed observed	Parent fitted SFO
0	97.8	74.87
3	60	69.41
7	51	62.73
14	43	52.56
30	35	35.08
60	22	16.44
91	15	7.51
120	12	3.61

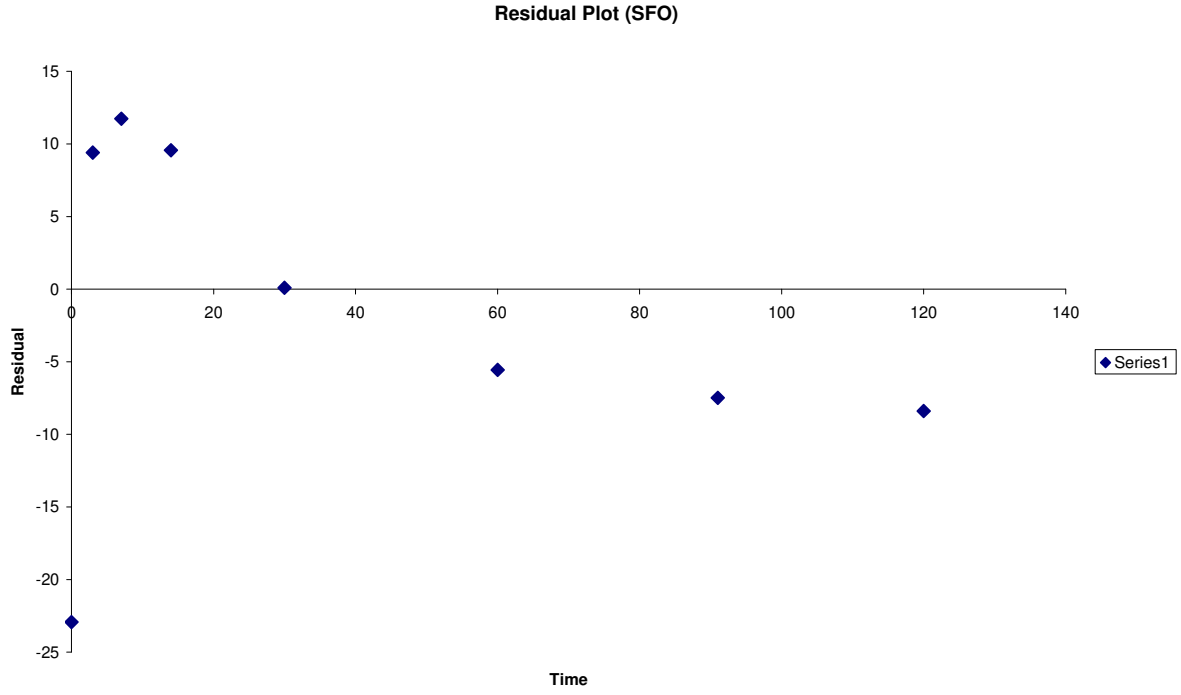
L3: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	27.4	91.1

L3: SFO: Graphs observed vs. fitted:



L3: SFO: Residual Graphs:



L3: SFO: Table χ^2-test results:

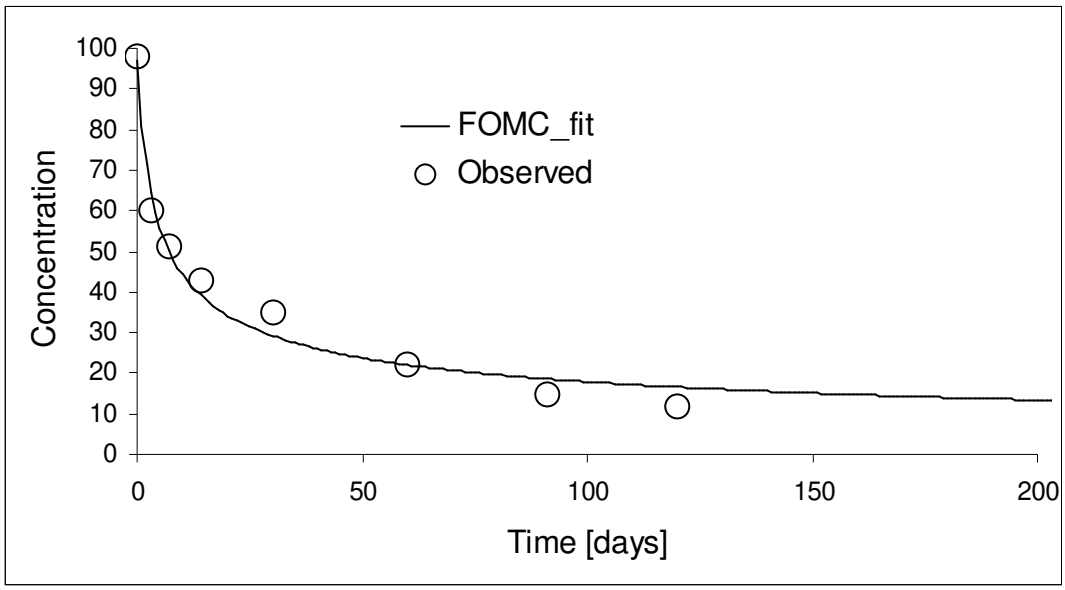
Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	22%

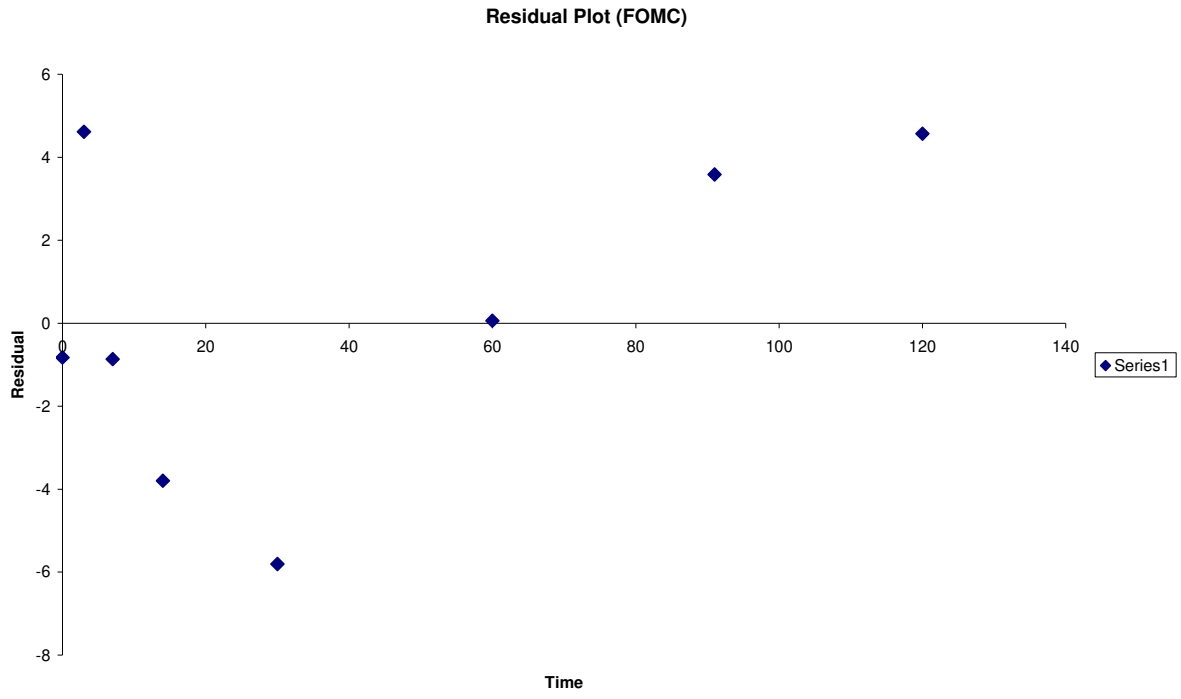
L3: Conclusions:

- χ^2 error value for SFO at 22%.
- SFO misses measured initial concentration.
- Residual graph of SFO indicates systematic deviation for later sampling dates in period up to DT90.
- Observed limited decrease after 30d sampling probably not due to decline in microbial activity.
- SFO considered **not** appropriate for modelling - > bi-phasic pattern (see following section)

- Improvement of χ^2 statistics for FOMC (8%).
- Better description of initial concentration with FOMC
- No improvement with regard to random nature of residuals, however overall smaller absolute deviations compared to SFO
- Use bi-phasic kinetics in modelling (e.g. FOMC: DT50= 7.7d; DT90= 431.1d)

L3: Additional information FOMC





Laboratory Data: Example 4

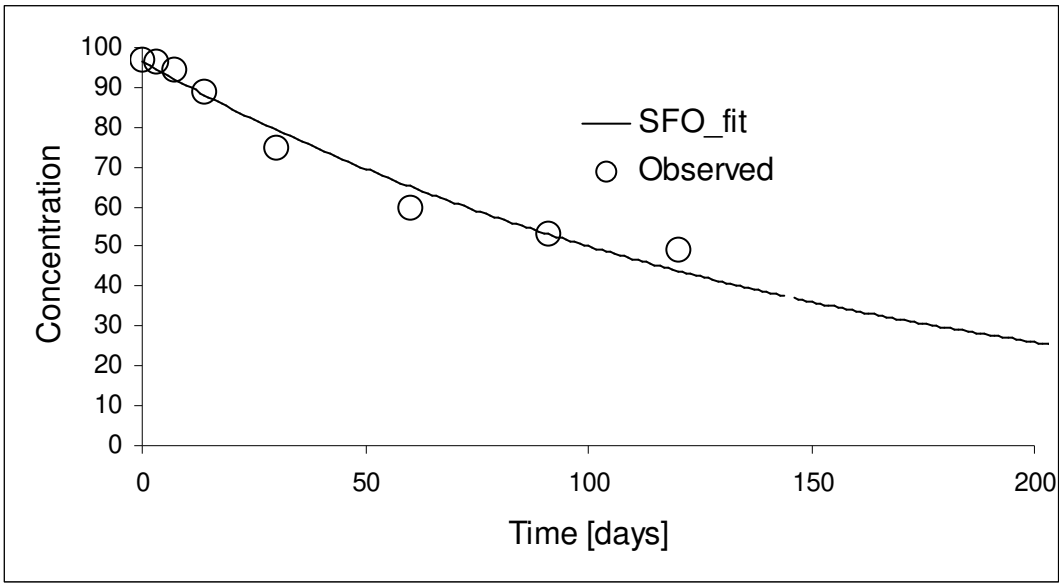
L4: SFO: Table observed vs. fitted (% applied):

Time (d)	Parent Observed observed	Parent fitted SFO
0	96.90	96.53
3	96.30	94.65
7	94.30	92.20
14	88.80	88.06
30	74.90	79.30
60	59.90	65.14
91	53.50	53.16
120	49.00	43.96

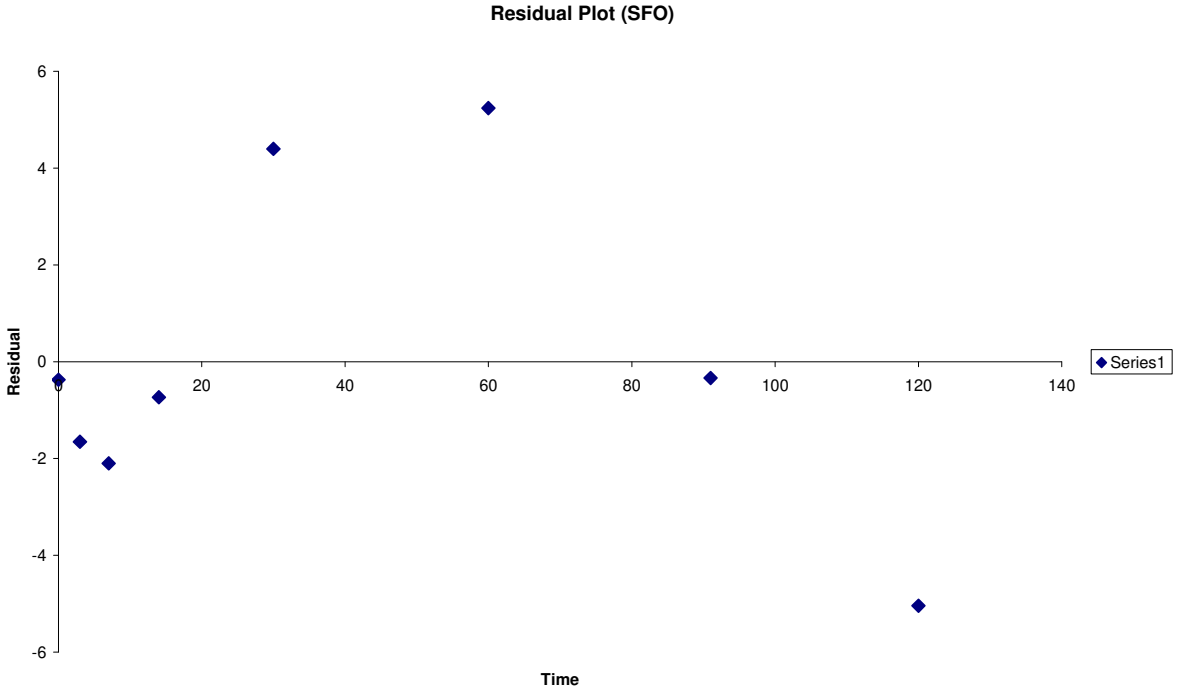
L4: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	105.8	351

L4: SFO: Graphs observed vs. fitted:



L4: SFO: Residual Graphs:



L4: SFO: Table χ^2-test results:

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	4%

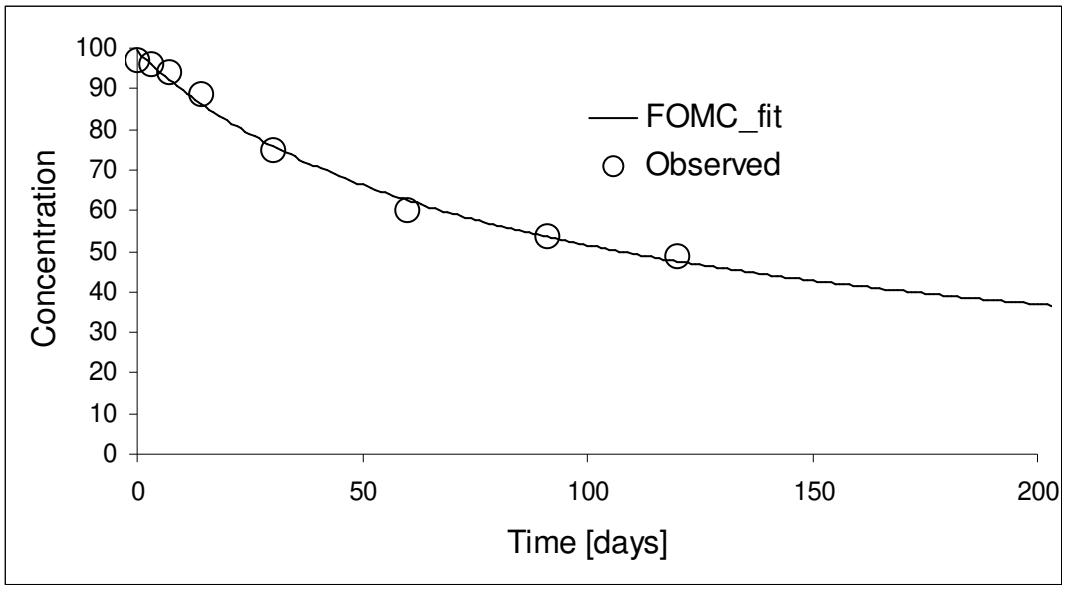
L4: Conclusions:

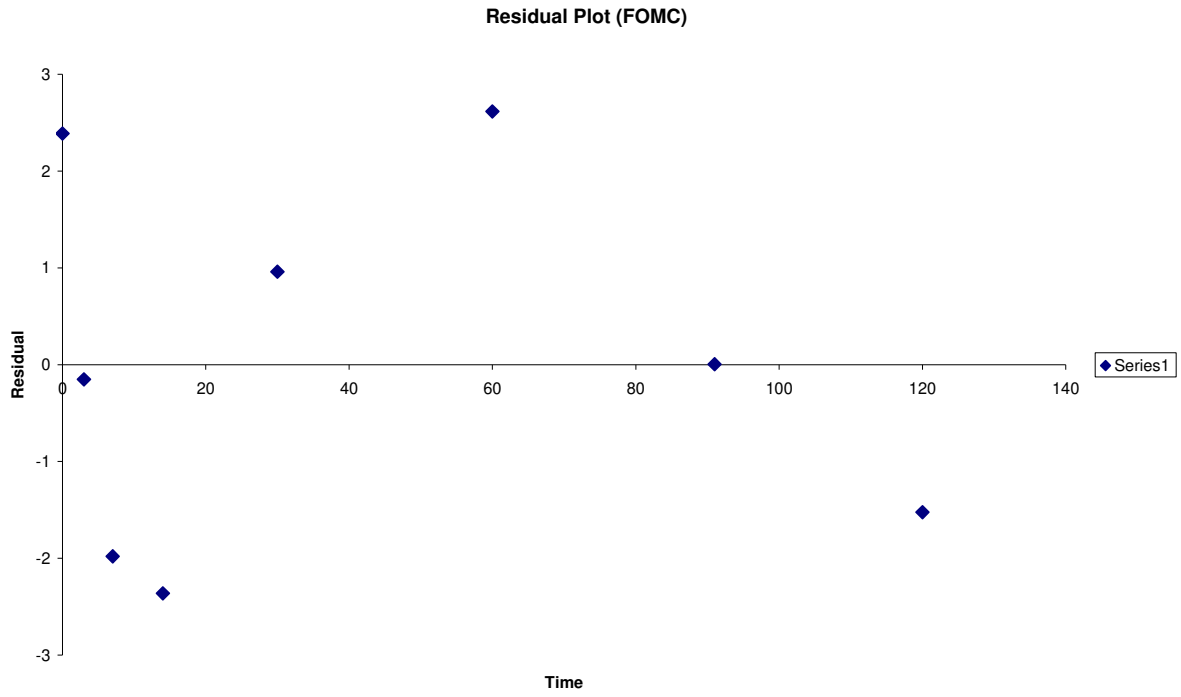
- χ^2 error value for SFO at 4%.
- Good description of initial concentration and early decline.
- Residual graph of SFO indicates random deviation.
- Last sampling point (120d) indicates slower decrease, a phenomenon often observed in laboratory studies.
- SFO considered appropriate for modelling, confirm against bi-phasic kinetics.

Additional information on potential bi-phasic behaviour (see following section)

- Slight improvement of χ^2 statistics for FOMC (2%).
- No improvement with regard to random nature of residuals, however overall smaller absolute deviations compared to SFO
- SFO still considered appropriate for modelling.

L4: Additional information: FOMC





Field Data: Example 1

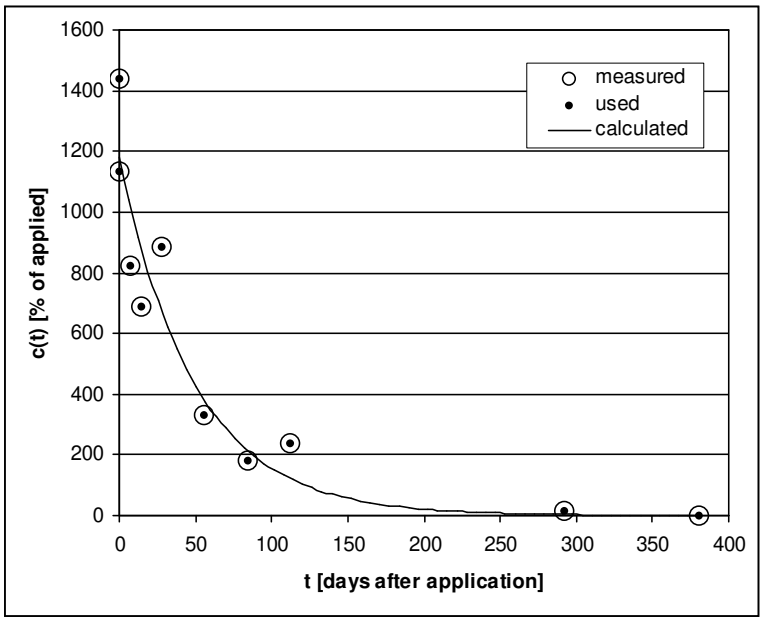
F1: SFO: Table observed vs. fitted (mg/kg):

Time (d)	Parent observed	Fitted parent SFO
0	1134	1180.7
0	1440	1180.7
7	825	1024.9
14	690	889.6
28	885	670.3
56	330	380.6
84	180	216.1
112	240	122.7
292	15	3.2
380	0	0.5

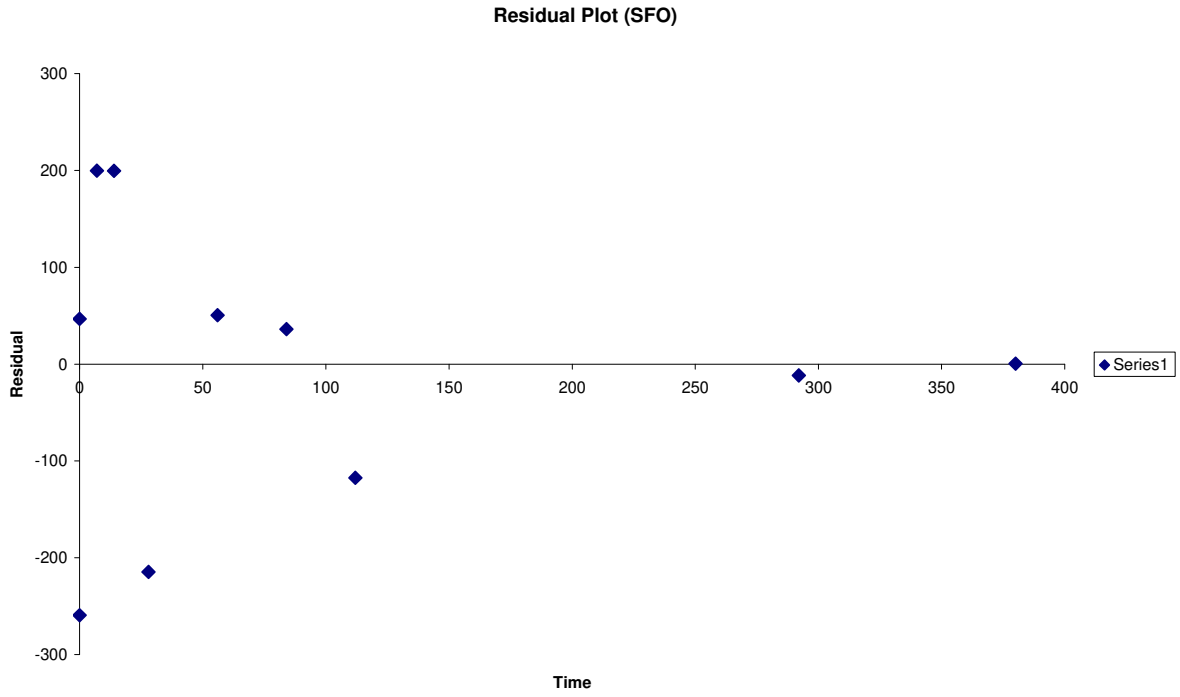
F1: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	34.3	113.9

F1: SFO: Graphs observed vs. fitted:



F1: SFO: Residual Graphs:



F1: SFO: Table χ^2-test results (using average value for duplicates):

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	22 %

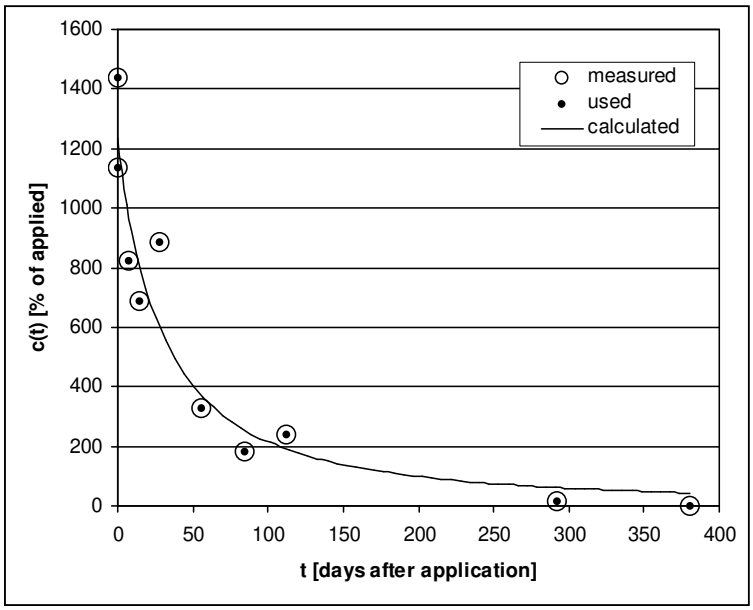
F1: Conclusions:

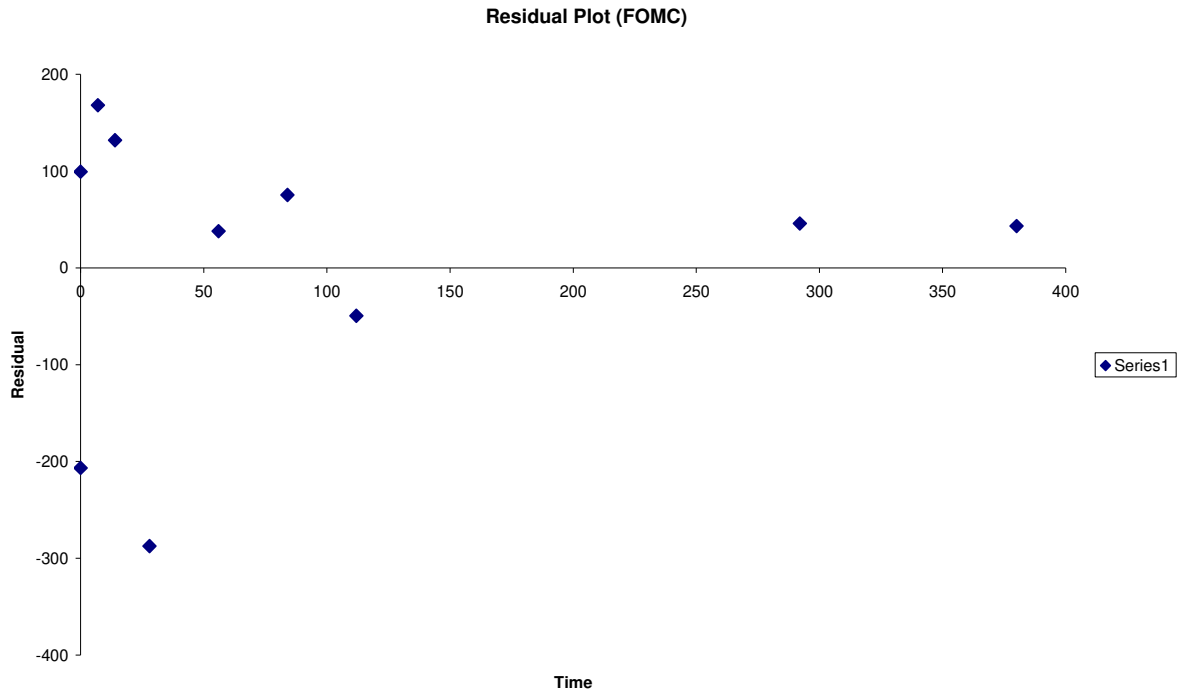
- χ^2 error value for SFO at 22%.
- Residuals plots indicate no systematic error of the SFO model, rather that the observed pattern is most likely due to scatter of early measurements.
- <u>SFO considered appropriate for modelling, confirm against bi-phasic kinetics.</u>

Additional information on potential bi-phasic behaviour (see following section)

- No improvement of χ^2 statistics for FOMC (22%).
- No improvement with regard to random nature of residuals.
- <u>SFO still considered appropriate for modelling.</u>

F1: Additional information: FOMC





Field Data: Example 2

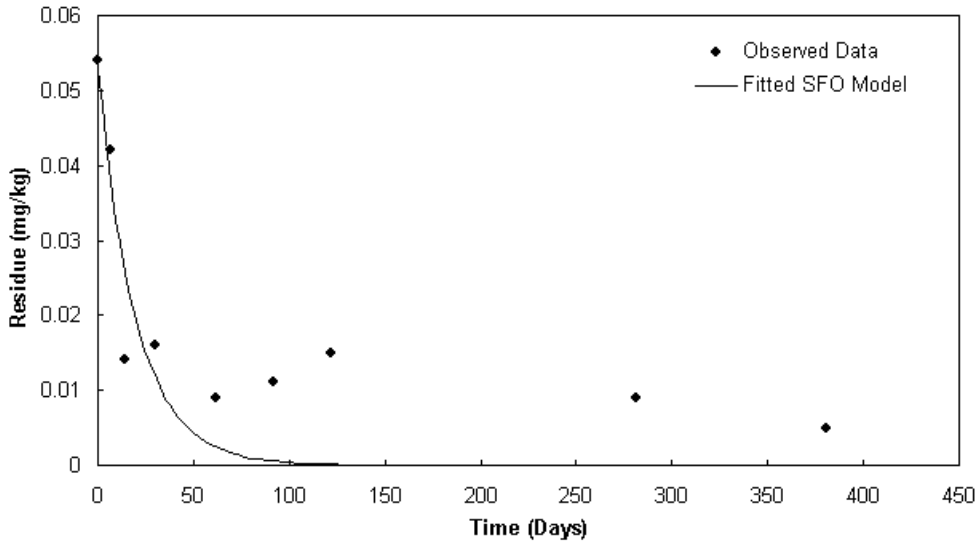
F2: SFO: Table observed vs. fitted (mg/kg):

Time (d)	Parent observed	Parent fitted SFO
0	0.054	0.053
7	0.042	0.037
14	0.014	0.026
30	0.016	0.012
62	0.009	0.002
92	0.011	0.001
122	0.015	0.000
281	0.009	0.000
381	0.005	0.000

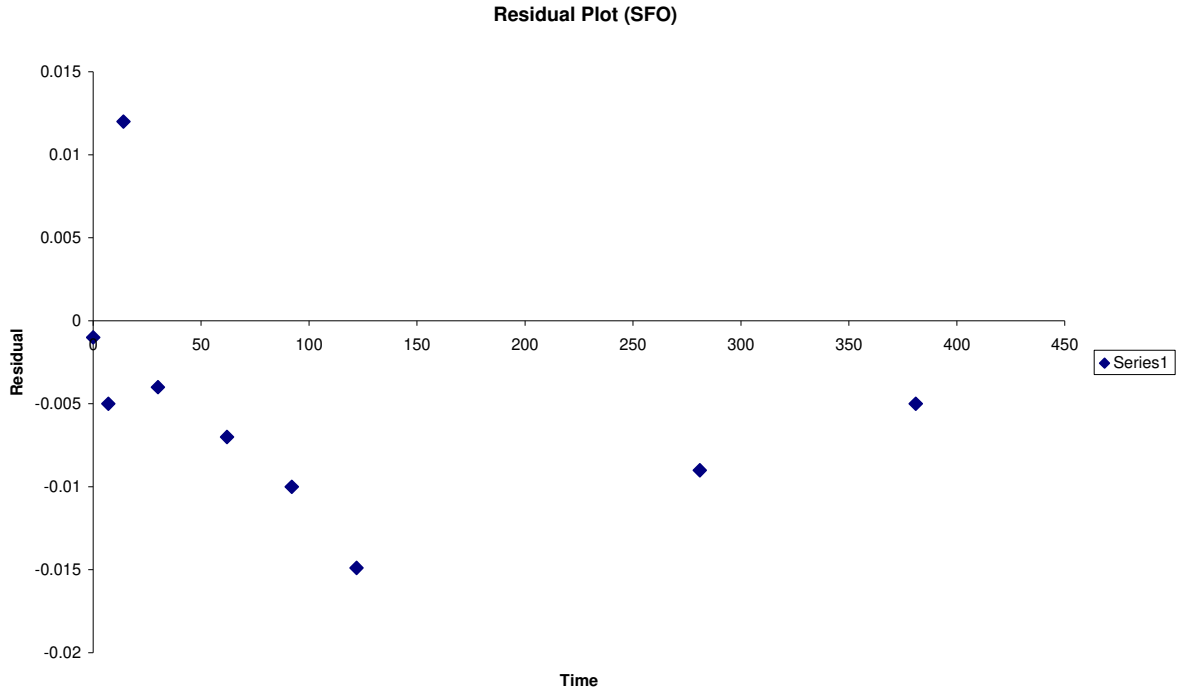
F2: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
SFO	13.8	45.9

F2: SFO: Graphs observed vs. fitted:



F2: SFO: Residual Graphs:



F2: SFO: Table χ^2-test results:

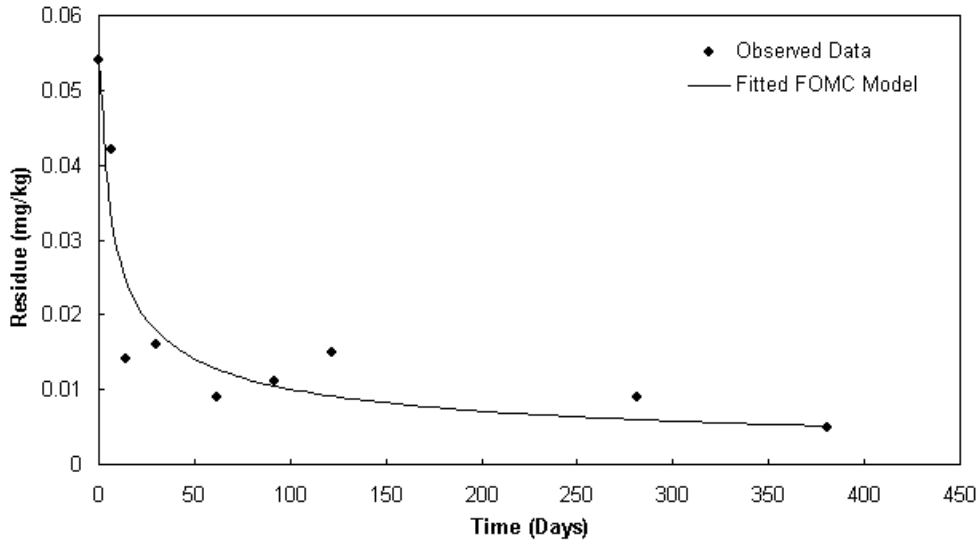
Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
SFO	2	36 %

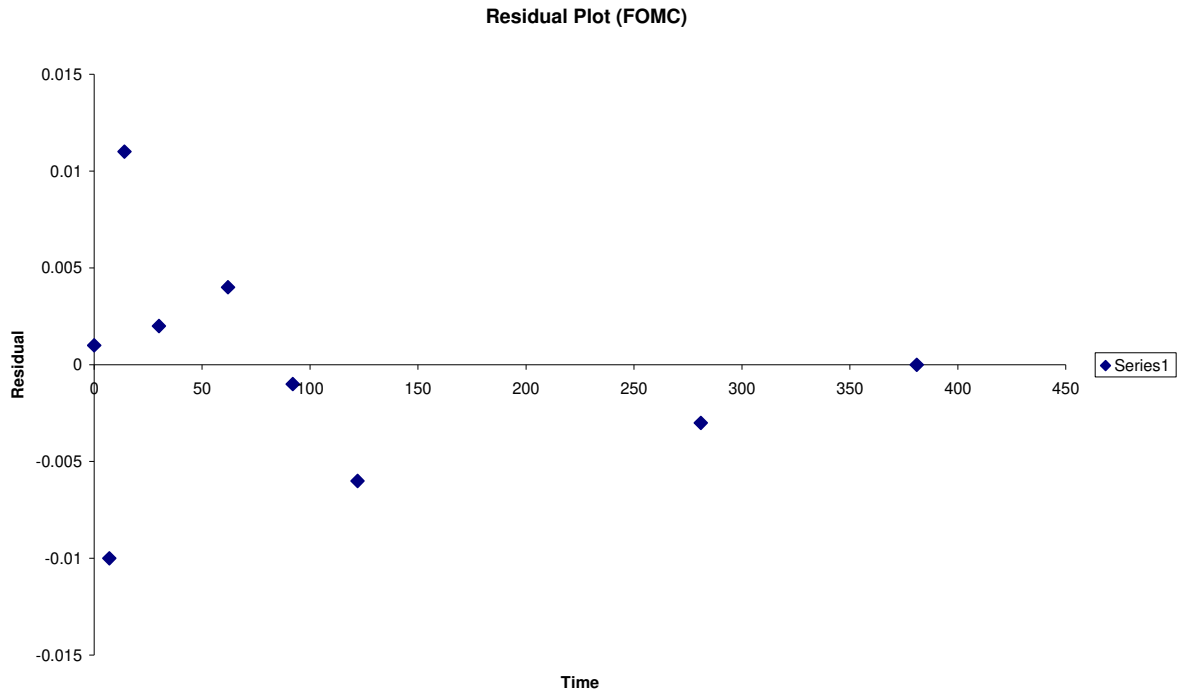
Conclusions:

- χ^2 error value for SFO at 36%.
- Residual graph of SFO indicates systematic deviation in sampling period up to the DT90.
- SFO considered not appropriate for modelling - > bi-phasic pattern (see 6.8)

- Improvement of χ^2 statistics for FOMC (25%).
- Improvement with regard to random nature of residuals, however absolute deviations remain high. Observed pattern is most likely due to scatter of measurements, degradation behaviour is well described by bi-phasic model.
- Use bi-phasic kinetics in modelling (e.g. FOMC: DT50= 10.8d; DT90= 333d)

F2: Additional Information: FOMC





Field Data: Example 3

F3: SFO: Table observed vs. fitted (mg/kg):

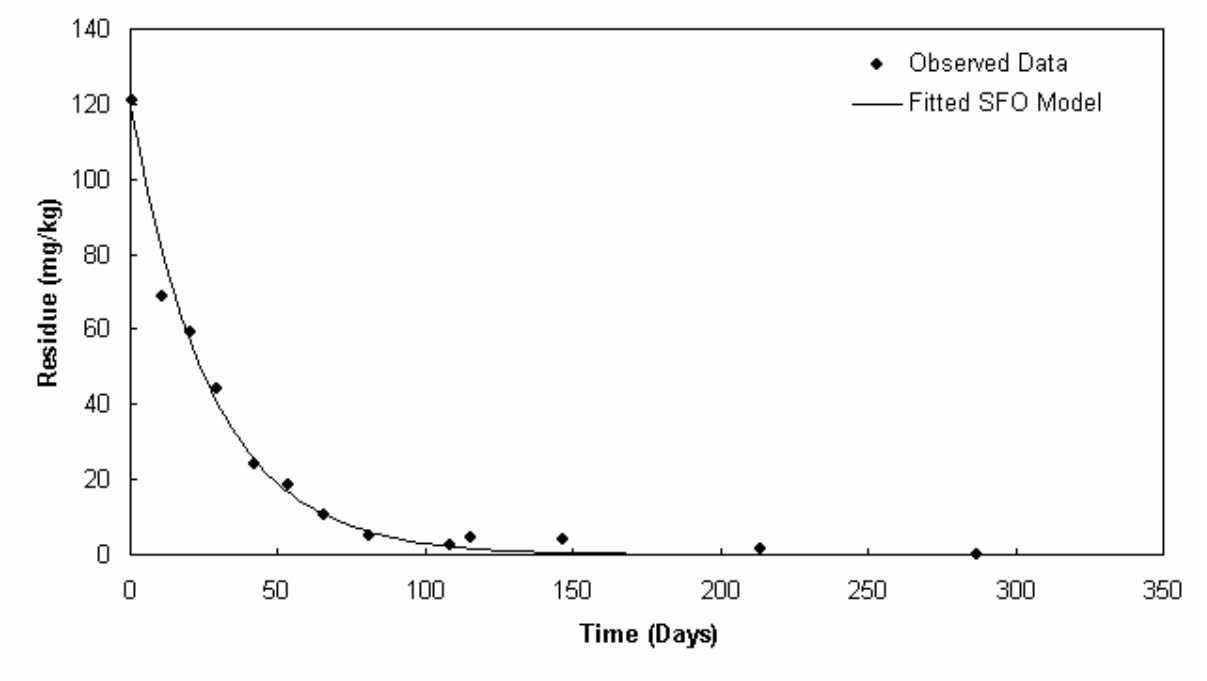
Time (d)	Parent Observed observed	Parent fitted SFO
0.9	121	115.64
10.7	68.6	80.45
20.2	59.1	56.60
29.6	44.2	39.96
42.3	24.3	24.97
53.8	18.7	16.31
65.8	10.3	10.46
80.9	5.2	5.98
108.5	2.6	2.15
115.5	4.3	1.66
146.9	3.8	0.52
213.7	1.7	0.04
287.1	0	0.00

Time (d)	Metabolite observed	Metabolite fitted SFO (parent SFO)
0.9	0.0	1.4
10.7	13.4	13.5
20.2	20.4	20.4
29.6	30.9	24.3
42.3	22.6	26.3
53.8	26.2	26.3
65.8	19.2	25.2
80.9	24.0	23.0
108.5	16.8	18.4
115.5	23.1	17.2
146.9	14.1	12.7
213.7	3.8	6.4
287.1	2.6	3.0

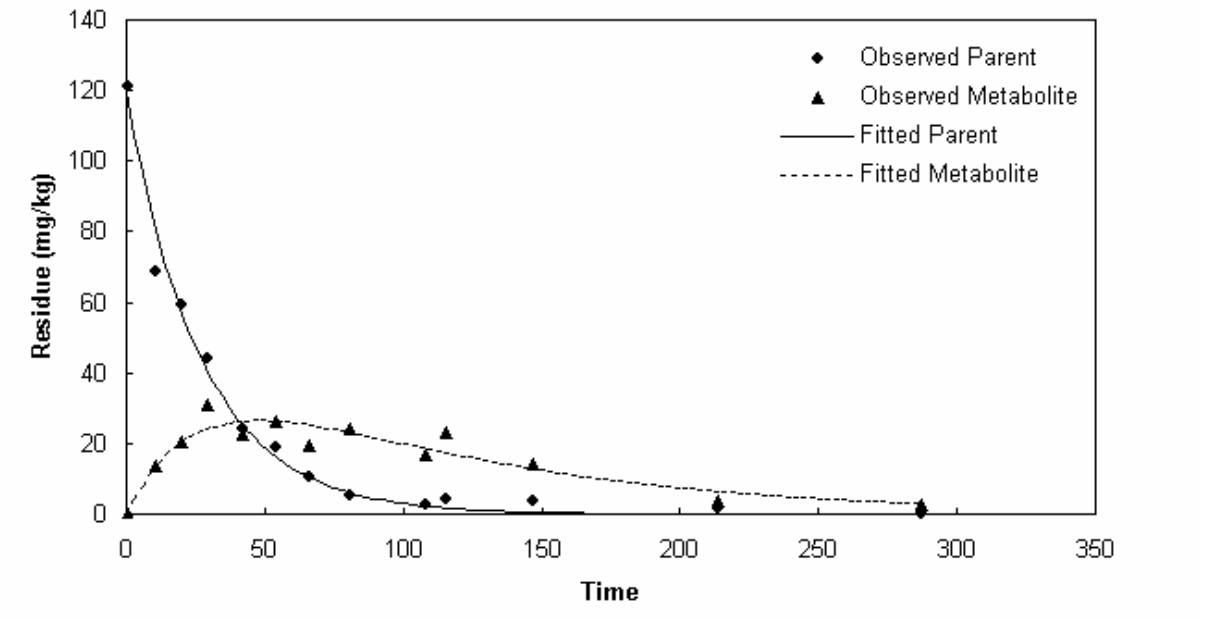
F3: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
Parent SFO	18.7	62.2
Met1 SFO (Parent SFO)	65.8	219

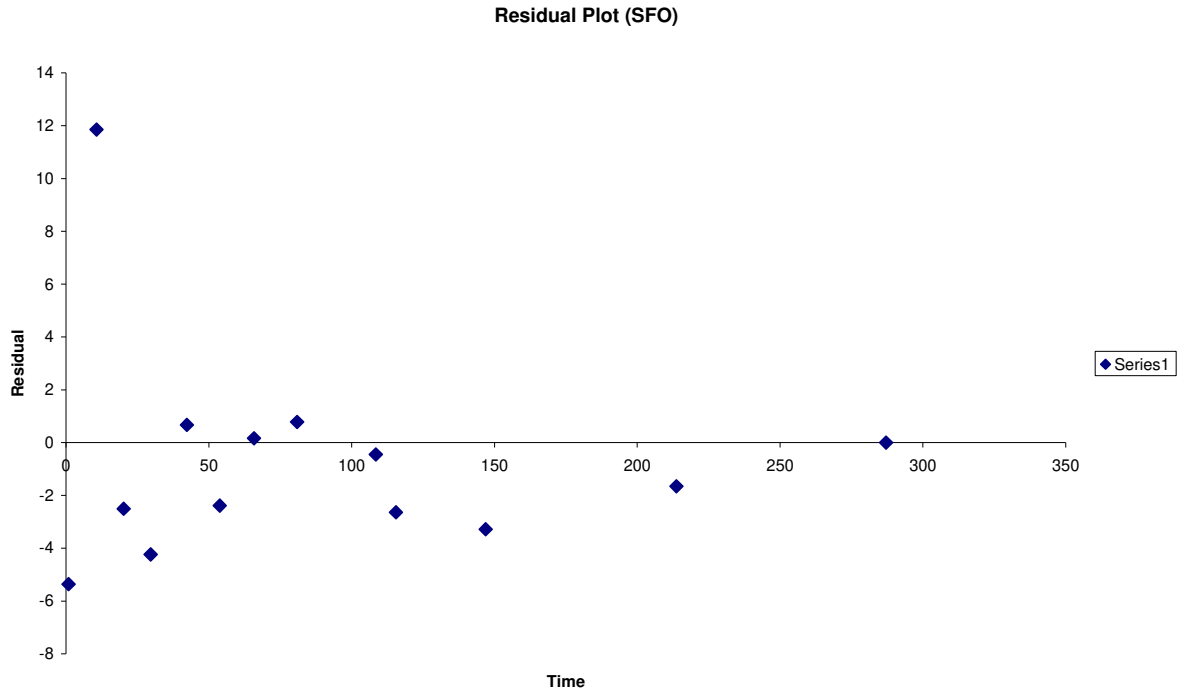
F3: Parent SFO: Graphs observed vs. fitted:



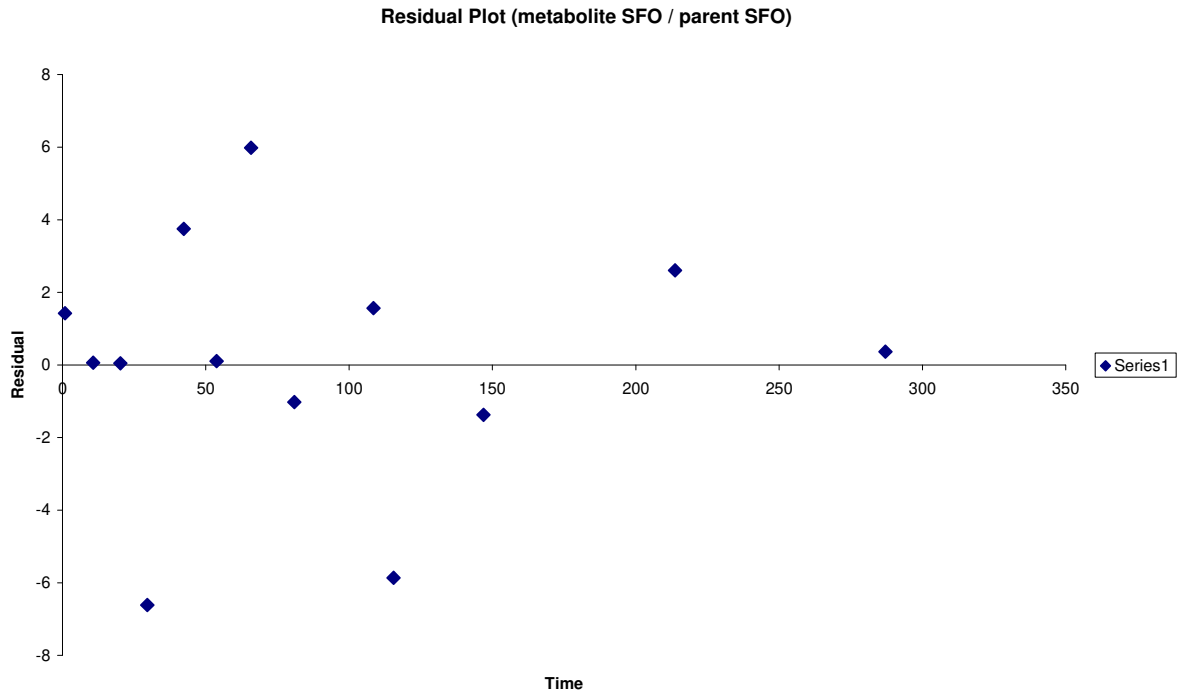
F3: Parent and Metabolite SFO: Graphs observed vs. fitted:



F3: Parent SFO: Residual Graphs:



F3: Metabolite SFO (Parent SFO): Residual Graphs:



F3: SFO: Table χ^2-test results:

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
Parent SFO	2	12%
Parent SFO Met1 SFO	2	17%

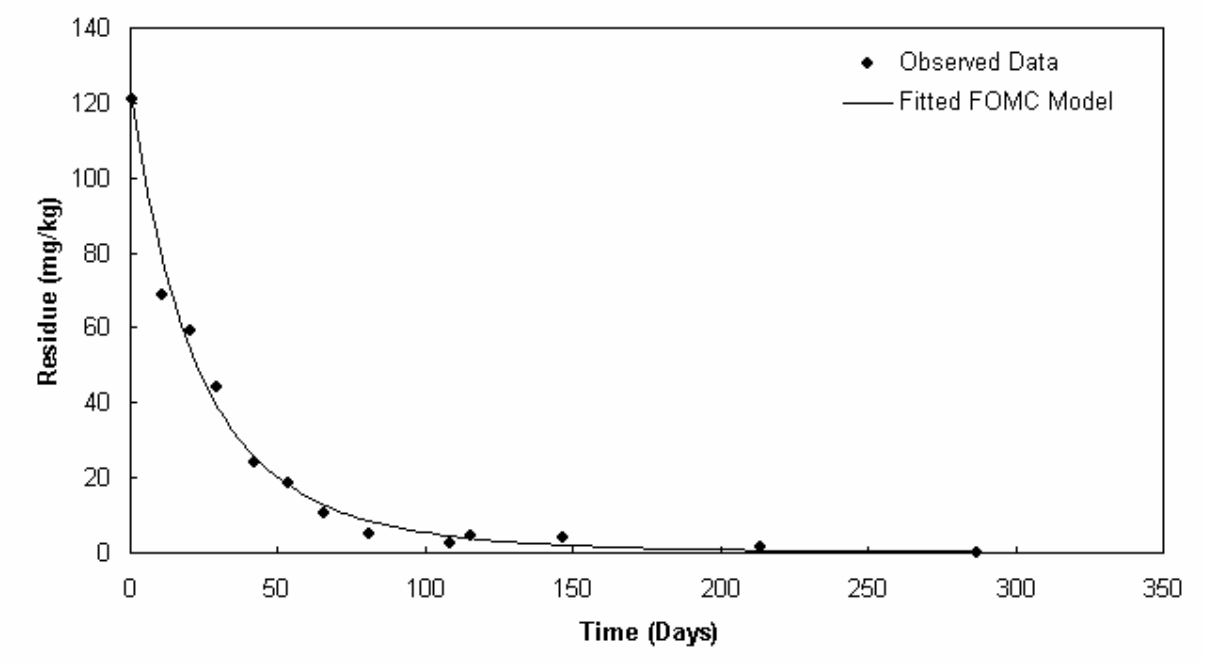
F3: Conclusions:

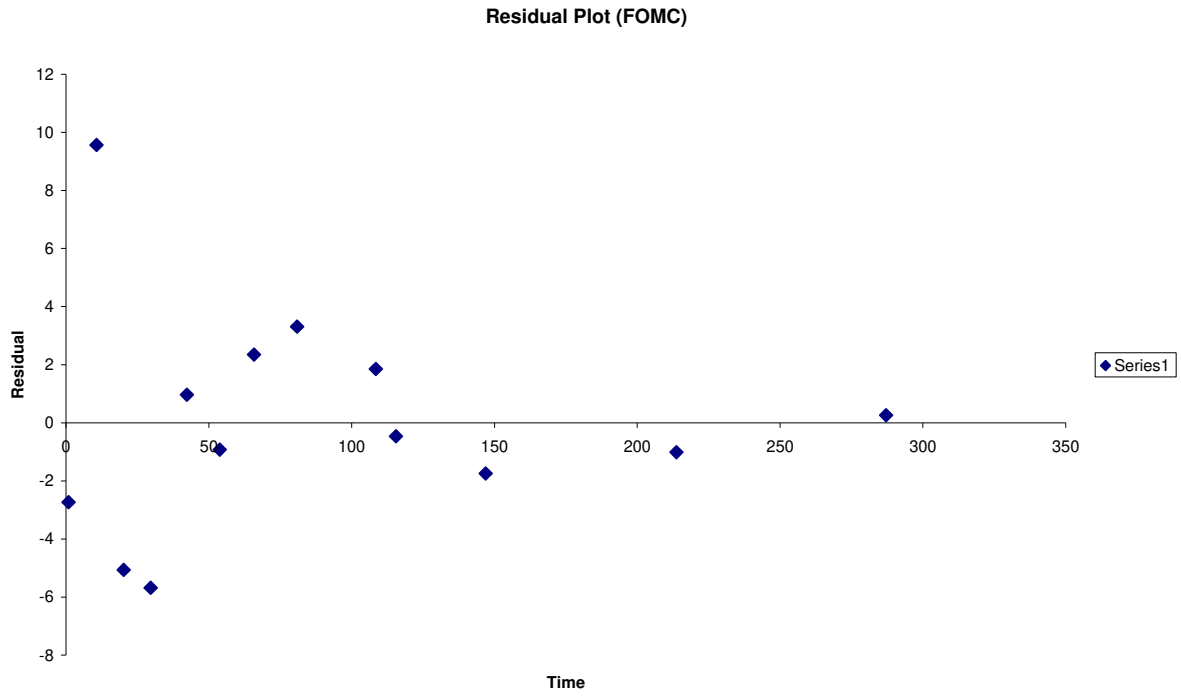
- χ^2 error value for parent SFO at 12%.
- Residuals plots indicate no systematic error of the SFO model.
- SFO considered appropriate for modelling of parent.
- χ^2 error value for metabolite SFO at 17%.
- Residuals plots indicate no systematic error of the SFO model.
- SFO considered appropriate for modelling of metabolite.

Additional information on potential bi-phasic behaviour of parent (see following section)

- No improvement of χ^2 statistics for FOMC (12%).
- No improvement with regard to random nature and absolute deviation of residuals.

F3: Additional information: FOMC





Field Data: Example 4

F4: SFO: Table observed vs. fitted (mg/kg):

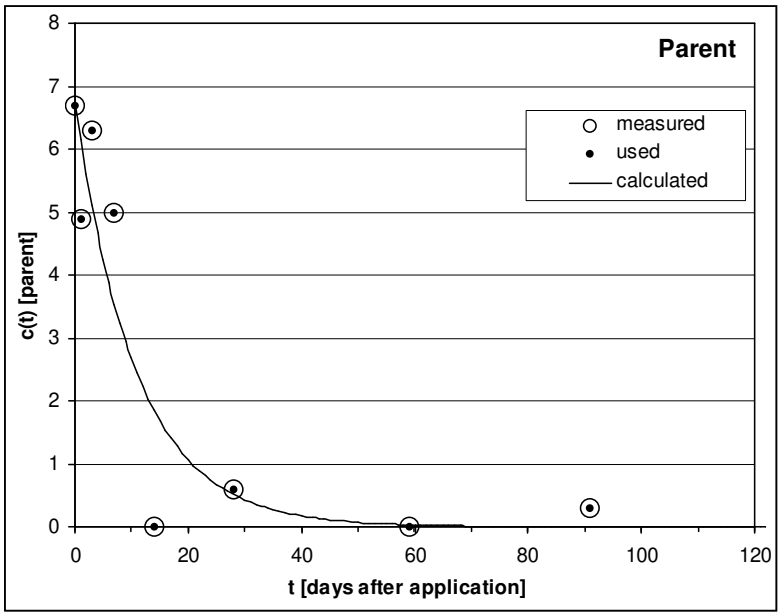
Time (d)	Parent observed	Parent fitted SFO
0	6.7	6.74
1	4.9	6.14
3	6.3	5.11
7	5	3.53
14	0	1.85
28	0.6	0.51
59	0	0.03
91	0.3	0.00

Time (d)	Met1 observed	Met1 fitted SFO (parent SFO)
0	0.694	0.000
1	1.110	0.504
3	0.972	1.313
7	2.360	2.314
14	3.054	2.847
28	1.943	2.203
59	0.416	0.625
91	1.249	0.135

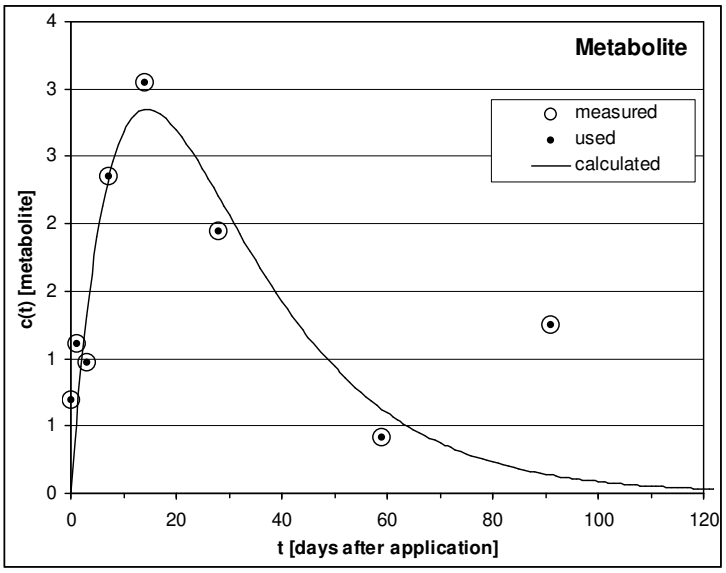
F4: SFO: Endpoints

Kinetic model	DT50 (d)	DT90 (d)
Parent SFO	7.5	25.0
Met1 SFO (Parent SFO)	13.9	46.3

F4: SFO: Graphs observed vs. fitted:

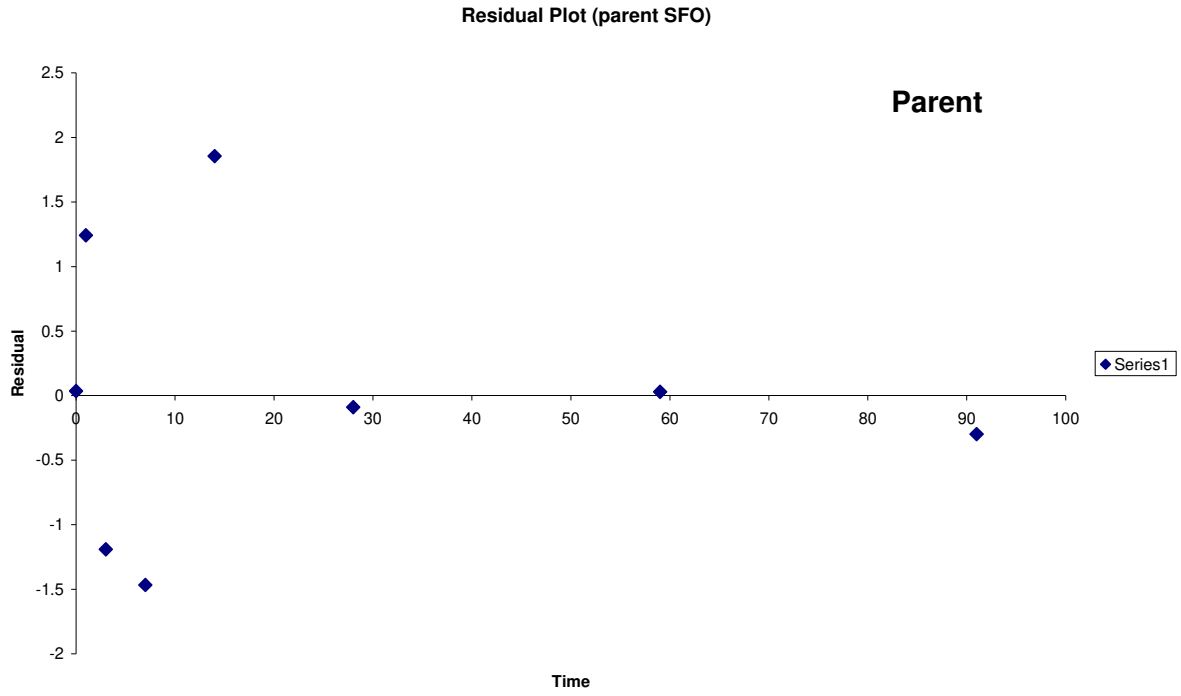


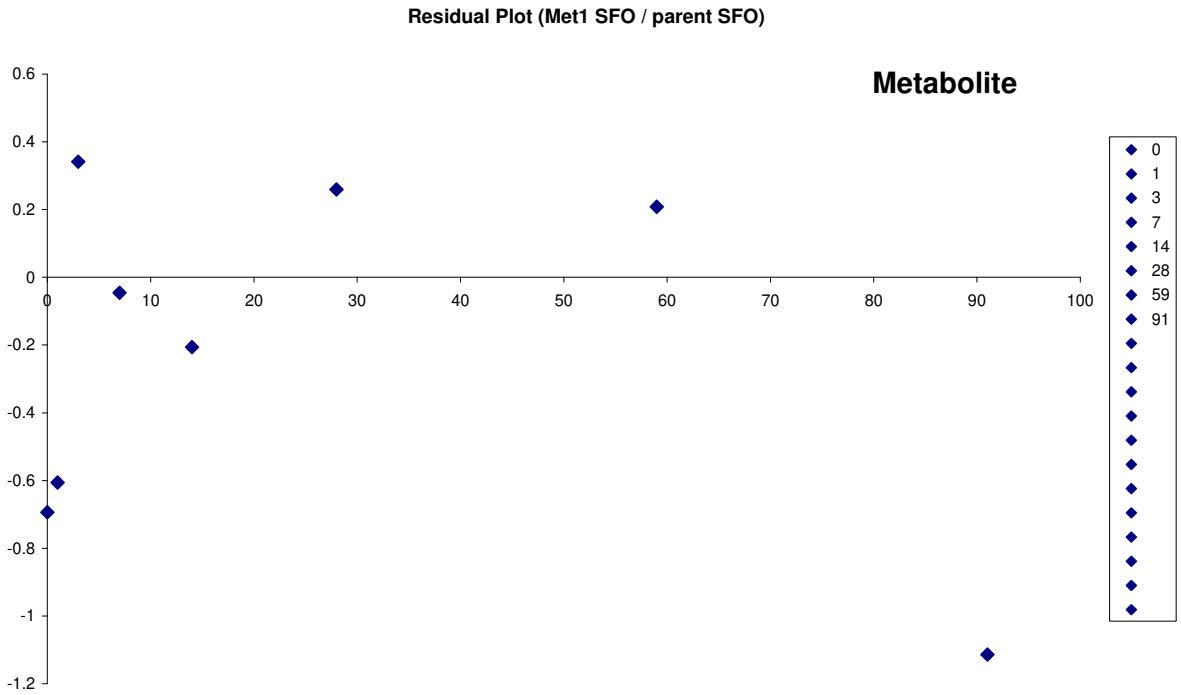
Parent SFO



Met. SFO (Parent SFO)

F4: SFO: Residual Graphs:





F4: SFO: Table χ^2-test results:

Kinetic model	Number of parameters	Error to pass χ^2-test at α=0.05
Parent SFO	2	28 %
(Parent SFO) Met1 SFO	2	30 %

F4: Conclusions:

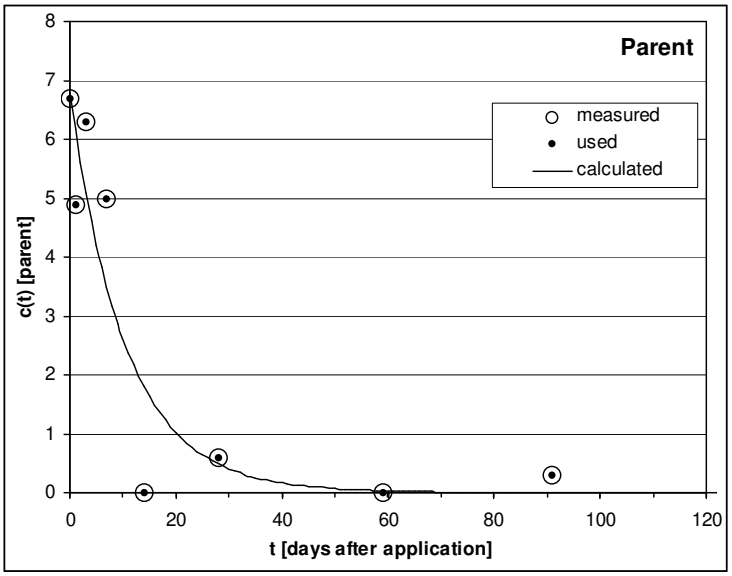
- χ^2 error value for parent and SFO at 28%.
- Residuals plots indicate no systematic error of the kinetic model, rather that the observed pattern is most likely due to scatter of measurements.
- Overall degradation behaviour of parent well described by SFO.
- Select SFO for modelling of parent.

- χ^2 error value for metabolite SFO at 30%.
- Residuals plots indicate no systematic error of the kinetic model, rather that the observed pattern is most likely due to scatter of measurements.
- SFO misses last measurement of metabolite. This result is probably an outlier. Additional information, e.g. from similar field studies, should be considered to decide if SFO for the metabolite is an appropriate description of overall degradation behaviour.

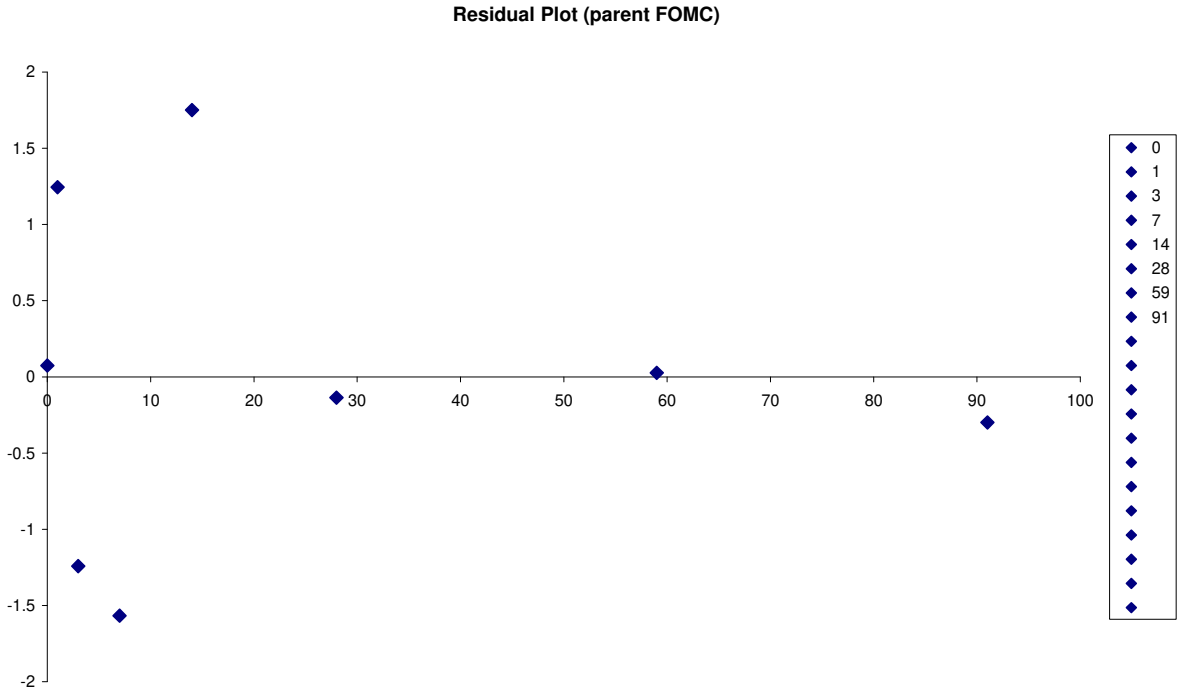
Additional information on potential bi-phasic behaviour of parent (see following section)

- Slight improvement of χ^2 statistics for FOMC (24%).
- No improvement with regard to random nature and absolute deviation of residuals, indicating that scatter of data is the root cause for deviations, not the kinetic description SFO vs. bi-phasic.

F4: Additional information: FOMC



Parent FOMC



APPENDIX 4: ESTIMATING DEGRADATION AND SORPTION PARAMETERS FROM LABORATORY DEGRADATION STUDIES FOR HIGHER-TIER CALCULATIONS WITH PEARL

A4.1. Introduction

In Section 7.1 the procedure is described to derive degradation parameters for pesticide fate modelling. Tier 1 approaches (if results differ from SFO) are based on derivation of conservative estimates of DegT50 values from either Gustafson-Holden or from the slow phase of a hockey-stick fit. This appendix describes a possible Tier-2 approach for such a case for the PEARL model.

The principles of the procedure are as follows:

1. PEARL assumes a Freundlich two-site sorption submodel: one site for equilibrium sorption and the second site for long-term sorption kinetics. PEARL also assumes SFO for the molecules present in liquid phase and sorbed to the equilibrium site; however, molecules sorbed on the kinetic site are not degraded.
2. The standard procedure for FOCUS scenarios is to ignore the second sorption site. Then PEARL reduces to a SFO system with a Freundlich isotherm.
3. As will be shown below, the PEARL sorption and degradation submodels (if applied to closed incubation systems) result in an approximately bi-exponential decline.
4. In this appendix the relationship between the parameters of the bi-exponential fit and the PEARL input parameters is described together with criteria for the acceptability of these input parameters.
5. The procedure implies that a bi-exponential decline is accepted as sufficient evidence for long-term sorption kinetics (also if no measurements on long-term sorption kinetics are available) provided that the resulting parameters for long-term sorption kinetics are more or less within the range of available measurements for these parameters. This approach is based on the ample evidence available in literature on long-term sorption kinetics (see for instance the review by Wauchope *et al*., 2002).

A4.2. Description of submodel for sorption and degradation kinetics used in PEARL

The submodel for sorption and degradation kinetics used in PEARL can be described as follows (see Leistra *et al*., 2001):

$$c^* = \theta c_L + \rho(X_{EQ} + X_{NE}) \qquad \text{(A4-1)}$$

$$X_{EQ} = K_{F,EQ}\, c_{L,R} \left(\frac{c_L}{c_{L,R}} \right)^N \tag{A4-2}$$

$$\frac{dX_{NE}}{dt} = k_d (K_{F,NE}\, c_{L,R} \left(\frac{c_L}{c_{L,R}} \right)^N - X_{NE}) \tag{A4-3}$$

$$K_{F,NE} = f_{NE}\, K_{F,EQ} \tag{A4-4}$$

$$\frac{dc^*}{dt} = -k_t (\theta c_L + \rho X_{EQ}) \tag{A4-5}$$

where

c^* = total concentration (mg/L)

c_L = concentration in the liquid phase (mg/L)

$c_{L,R}$ = reference concentration in the liquid phase (mg/L)

θ = volume fraction of water (-)

ρ = dry bulk density (kg/L)

X_{EQ} = content sorbed at equilibrium sites (mg/kg)

X_{NE} = content sorbed at non-equilibrium sites (mg/kg)

$K_{F,EQ}$ = equilibrium Freundlich sorption coefficient (L/kg)

$K_{F,NE}$ = non-equilibrium Freundlich sorption coefficient (L/kg)

N = Freundlich exponent (-)

k_d = desorption rate coefficient (d^{-1})

f_{NE} = factor for describing the ratio between the equilibrium and non-equilibrium Freundlich coefficients (-)

k_t = degradation rate coefficient (d^{-1})

A4.3. Analytical solution for incubation systems

An analytical solution for the system described by Equations A4-1 to A4-5 is only available for a linear sorption isotherm (so *N* = 1). Thus the sorption isotherm is assumed to be linear and the linearised sorption coefficients are further called $K_{L,EQ}$ and $K_{L,NE}$.

The parameters Φ and Q are defined as

$$\Phi = \frac{\rho\, f_{NE} K_{L,EQ}}{\theta + \rho K_{L,EQ}} \tag{A4-6}$$

$$Q = (1+\Phi)k_d + k_t \tag{A4-7}$$

The system then consists of two first-order linear differential equations in c* and X_{NE}. These equations can be rewritten as one second-order differential equation using the conventional mathematical solution procedure for such system. This second-order equation is (in terms of c*):

$$\frac{d^2c^*}{dt^2} + Q\frac{dc^*}{dt} + k_d k_t = 0 \tag{A4-8}$$

The second-order equation in terms of X_{NE} is identical to Equation A4-8.

The solution of the system is then given by

$$c^* = c_0^* \left[g\, \exp(-\lambda_1 t) + (1-g)\exp(-\lambda_2 t)\right] \tag{A4-9}$$

in which the constants are defined as follows:

$$\lambda_1 = 0.5Q - 0.5\sqrt{Q^2 - 4k_d k_t} \tag{A4-10a}$$

$$\lambda_2 = 0.5Q + 0.5\sqrt{Q^2 - 4k_d k_t} \tag{A4-10b}$$

$$g = \frac{\lambda_2(\Phi k_d + k_t - \lambda_2)}{k_d(\lambda_1 - \lambda_2)} \tag{A4-11}$$

Note that the solution is not a function of absolute values of $K_{L,EQ}$ or $K_{L,NE}$ but only of the quotient Φ.

From Equations A4-10a and A4-10b, the following equations for the product and sum of λ_1 and λ_2 can be derived:

$$\lambda_1 \lambda_2 = k_d k_t \tag{A4-12}$$

$$\lambda_1 + \lambda_2 = (1+\Phi)k_d + k_t \tag{A4-13}$$

The system parameters k_t , k_d and Φ were derived as follows. The parameter k_t was eliminated from equation A4-11 using Equation A4-13. This gives the following expression for k_d :

$$k_d = \frac{\lambda_1\lambda_2}{g\lambda_1 + (1-g)\lambda_2} \tag{A4-14}$$

Because λ_1 λ_2 equals k_t k_d , this implies that

$$k_t = g\lambda_1 + (1-g)\lambda_2 \tag{A4-15}$$

Substitution of Equations A4-14 and A4-15 in Equation A4-13, gives the following expression for Φ:

$$\Phi = \frac{g(1-g)(\lambda_1 - \lambda_2)^2}{\lambda_1\lambda_2} \tag{A4-16}$$

So if g, λ_1 and λ_2 are available, the three system parameters k_t , k_d and Φ can be calculated.

For the above system, also the course of the concentration in liquid phase can be calculated from the analytical solution:

$$c_L = c_{L,0}\left[h\exp(-\lambda_1 t) + (1-h)\exp(-\lambda_2 t)\right] \tag{A4-18}$$

with the following expression for *h*

$$h = \frac{\Phi k_d + k_t - \lambda_2}{\lambda_1 - \lambda_2} \tag{A4-19}$$

Note that the parameters h, g, λ_1 and λ_2 are not independent. Their dependency is described by

$$h = \frac{g\lambda_1}{g\lambda_1 + (1-g)\lambda_2} \qquad \text{(A4-20)}$$

The analytical solution described above was tested against a numerical solution (using simple Euler integration). The result in Figure A4-1 shows that there was good correspondence between the analytical and numerical solution for *c** (copy of computer programme available upon request).

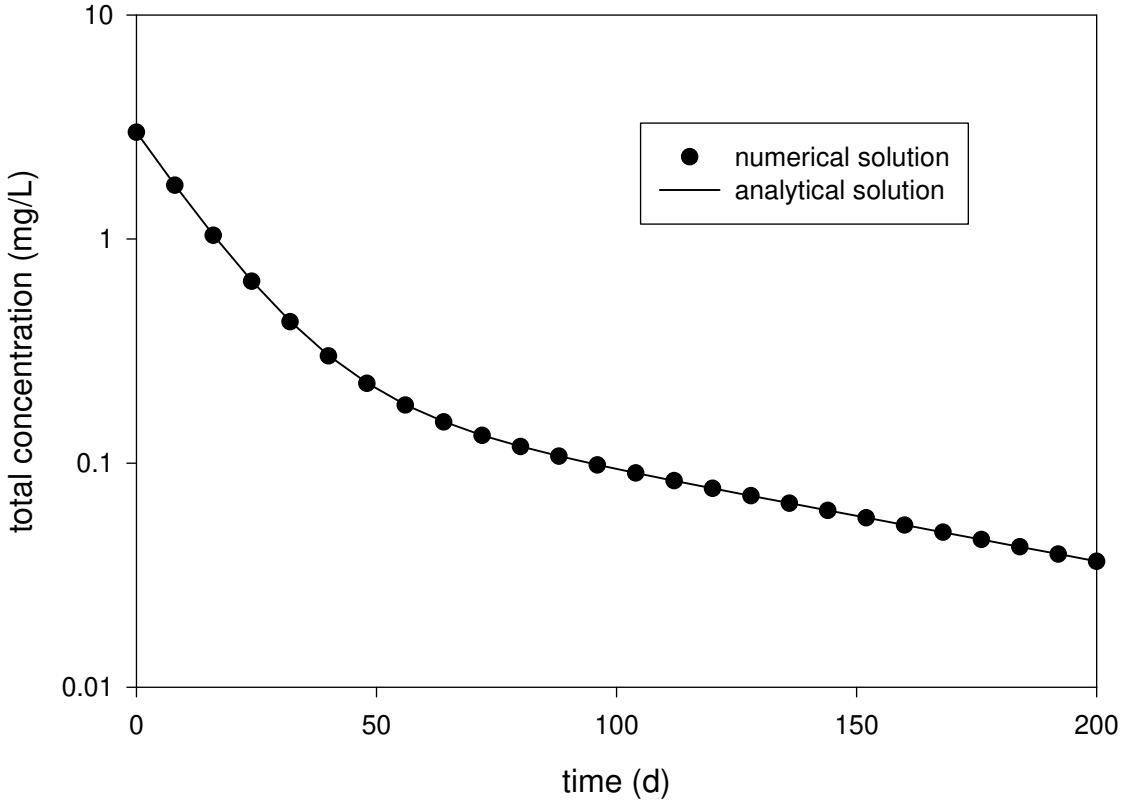


Figure A4-1. Comparison of numerical and analytical solution for a system with θ = 0.3, ρ = 1.5 kg/L, k_t = 0.0693 d^{-1} (i.e. DegT50 = 10 d), $K_{L,EQ}$ = 1.5 L/kg, f_{NE} = 0.5, k_d =0.01 d^{-1}, c* = 3 mg/L at start. The parameters of the analytical solution were λ_1 = 0.00932 d^{-1}, λ_2 = 0.07441 d^{-1}, g = 0.0783. Note that in this case λ_1 is close to k_d (0.01 d^{-1}) and λ_2 is close to k_t (0.0693 d^{-1}).

A4.4. Effect of non-linearity of sorption

PEARL 1.1.1 and 2.2.2 assume Freundlich sorption whereas linear sorption was assumed in the above approach. The aim is to derive sorption and degradation parameters that can be used for PEARL leaching calculations. Thus the acceptability of the assumption of linear sorption in the above approach needs to be checked.

Figure A4-2 shows the effect of non-linearity for a typical laboratory study. Assuming *N* = 0.7 (which is in practice more or less the strongest non-linearity), results in almost exactly the same decline curve compared to linear sorption. Table A4-1 gives the numerical results for the same system and a range of Freundlich exponents. The effect of non-linearity of sorption on the simulated decline in this case was very small. Consider, for instance, the total concentrations after 56 d when only 10% of the dose is left: the total concentration for N = 1 is then 0.094 mg/L whereas it is 0.099 mg/L for N = 0.7. Such small differences have probably only a small effect on the estimated degradation rate coefficient.

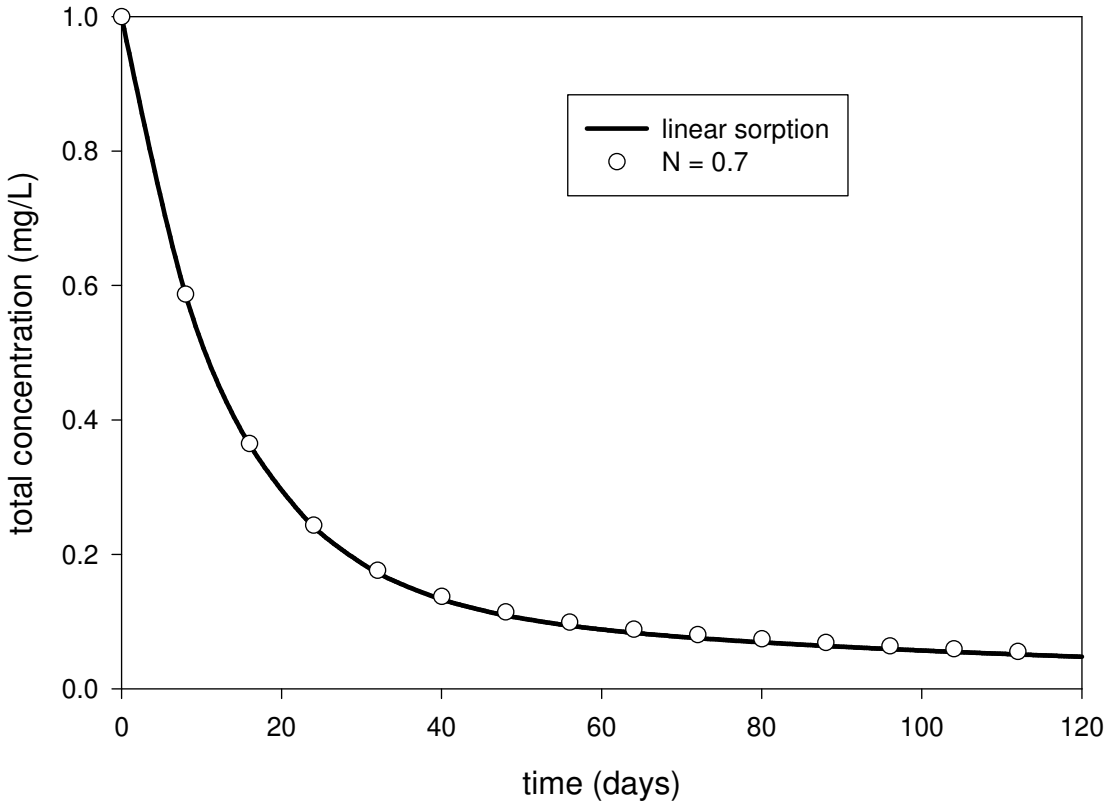


Figure A4-2. Total concentration as a function of time assuming linear sorption and Freundlich sorption with N = 0.7 for a laboratory incubation system with θ = 0.3, ρ = 1.5 kg/L, k_t = 0.0693 d^{-1} (i.e. DegT50 = 10 d), $K_{F,EQ}$ = 1.0 L/kg, f_{NE} = 1.0, k_d = 0.01 d^{-1} and c* = 1 mg/L at start.

Table A4-1. Total concentration (mg/L) as a function of time and the Freundlich exponent N for a system with θ = 0.3, ρ = 1.5 kg/L, k_t = 0.0693 d^{-1} (i.e. DegT50 = 10 d), $K_{F,EQ}$ = 1.0 L/kg, f_{NE} = 1.0, k_d = 0.01 d^{-1} and c* = 1 mg/L at start.

Time (d)	N = 1.0	N = 0.9	N = 0.8	N = 0.7	N = 0.6
0	1.000	1.000	1.000	1.000	1.000
8	0.587	0.587	0.587	0.587	0.587
16	0.363	0.363	0.364	0.365	0.365
24	0.240	0.241	0.242	0.243	0.245
32	0.172	0.173	0.175	0.176	0.178
40	0.133	0.134	0.136	0.137	0.139
48	0.109	0.111	0.112	0.114	0.116
56	0.094	0.095	0.097	0.099	0.101
64	0.083	0.085	0.087	0.089	0.090
72	0.075	0.077	0.079	0.081	0.082
80	0.069	0.071	0.072	0.074	0.076
88	0.064	0.065	0.067	0.069	0.070
96	0.059	0.061	0.062	0.064	0.065
104	0.055	0.056	0.058	0.059	0.061
112	0.051	0.053	0.054	0.055	0.057
120	0.048	0.049	0.050	0.052	0.053

To check whether the results shown in Figure A4-2 and Table A4-1 can be generalised, some 100 calculations were made in which k_t , k_t, f_{NE} and $K_{F,EQ}$ were systematically varied. For each run, the remaining concentrations calculated with N = 1 and N = 0.7 were compared when approximately 10% of the dose was left. If k_t ranged from 0.01-1 d^{-1}, k_d ranged from 0.005-0.05 d^{-1}, $K_{F,EQ}$ ranged from 0.1 to 10 L/kg and f_{NE} ranged from 0.1-1, the two concentrations differed usually not more than 1% from each other and never more than 10% (i.e. the result of in total 72 runs). Runs with f_{NE} = 10 in combination with $K_{F,EQ}$ = 1 L/kg or $K_{F,EQ}$ = 10 L/kg showed differences that did not exceed 10%. A 10% difference when 10% is left, implies a difference of only 1% of the dose which seems acceptable (scatter in measurements will often be much larger). In contrast, runs with f_{NE} = 10 in combination with $K_{F,EQ}$ = 0.1 L/kg showed differences up to 60%. However, this is an exceptional case: low equilibrium sorption in combination with high non-equilibrium sorption. These calculations indicate that the approximation of linear sorption is acceptable for f_{NE} values that do not appreciably exceed 1.0. For systems with higher f_{NE} values, the PEARLNEQ fitting tool

(Tiktak *et al*., 2000, p. 52), which uses equations A4-1 to A4-5 (thus including Freundlich sorption), should be used.

A4.5. Guidance for PEARL

The recommended procedure based on the previous analysis is as follows:

1. Fit the decline in total concentration to a bi-exponential equation (Equation A4-9) which results in values of g, λ_1 and λ_2. Note that λ_2 is per definition larger than λ_1 as follows from Equations A4-10a and A4-10b.

2. Calculate k_t , k_d and Φ from g, λ_1 and λ_2 using Equations A4-14 to A4-16 and calculate the corresponding PEARL input parameters.

From k_t the half-life to be used in PEARL can be derived via ln 2 / k_t (using the agreed procedures for standardisation to 20°C and field capacity if necessary). The parameter k_d can be directly used in PEARL. In contrast, the parameter Φ is not a PEARL input parameter: the corresponding input parameter is f_{NE} , which can be calculated from Equation A4-6:

$$f_{NE} = \Phi \frac{\theta + \rho K_{L,EQ}}{\rho K_{L,EQ}} \qquad \text{(A4-21)}$$

The quotient θ/ρ is equivalent to the mass of water divided by the mass of solid phase in the system (in $dm^3\ kg^{-1}$ or $cm^3\ g^{-1}$), which is available in laboratory degradation rate studies. Using the symbol *w* for this quotient, the above equation can be simplified to:

$$f_{NE} = \Phi \frac{w + K_{L,EQ}}{K_{L,EQ}} \qquad \text{(A4-22)}$$

$K_{L,EQ}$ for the soil in the laboratory degradation study can be estimated using the average K_{OC} and the average Freundlich exponent from the dossier. In the derivation of the parameters k_t, k_d, and Φ the sorption isotherm is assumed to be linear. Thus the value used for $K_{L,EQ}$ should be as close as possible to the Freundlich equilibrium sorption during the incubation. This can be achieved by calculating $K_{L,EQ}$ from the Freundlich isotherm using the

concentration level when 50% of the pesticide is degraded in the incubation system. The distribution over the solid/liquid phase in Freundlich systems has to be calculated via iteration using Equations A4-1 and A4-2 (while assuming zero X_{NE}). Figure A4-3 shows an example of a suitable FORTRAN function for this iteration procedure.

```
REAL FUNCTION FREUND(MPE,MSOL,VLIQ,KF,CREF,N)
C     this function calculates the equilibrium concentration in a system
C     according to the Freundlich isotherm:  X = KF * CREF * (C/CREF)**N
C     X    = mass of pesticide sorbed divided by mass of solid phase
C            (ug/g = mg/kg)
C     MPE  = mass of pesticide in the system (ug)
C     MSOL = mass of solid phase in the system (g)
C     VLIQ = volume of liquid in the system (mL)
C     KF   = Freundlich coefficient (mL/g = L/kg)
C     N    = Freundlich exponent (1)
C     C    = equilibrium concentration (ug/mL = mg/L)
C     CREF = reference value of C (ug/mL = mg/L)
C     OLDC = old value of C
C     RER  = acceptable relative error in C
C
      IMPLICIT REAL (A-Z)
      PARAMETER (RER=0.001E-2)
      C=CREF
1     CONTINUE
      OLDC=C
      SCO = KF * CREF**(1.-N) * ( AMAX1(C,1.E-30) )**(N-1.)
      C=MPE/(VLIQ+MSOL*SCO)
      IF (ABS(C-OLDC) .GT. RER*ABS(C)) GO TO 1
      FREUND=C
      END
```

Figure A4-3. Example of FORTRAN function for calculation of concentration in liquid phase in a Freundlich system.

This is illustrated with the following example. Consider a study where 0.1 mg/kg pesticide is incubated at a water content of 0.2 mL/g with the following Freundlich sorption parameters: $K_{F,EQ}$ = 1.0 L/kg, $c_{L,R}$ = 1 mg/L and N = 0.7. The content at which 50% is degraded is 0.05 mg/kg. The result of the iteration procedure in this case is c_L = 0.0128 mg/L and X_{EQ} = 0.0474 mg/kg (it can be easily verified that this is correct by checking that these values fit to the isotherm and also correspond with a total content of 0.05 mg/kg). The c_L and X_{EQ} values correspond with $K_{L,EQ}$ = 3.69 L/kg which is the value to be used in Equation 4A-22 when

calculating f_{NE} for this incubation. Note that in this example, the value to be used differs significantly from the $K_{F,EQ}$ value of 1.0 L/kg.

Note that the linearised sorption coefficient $K_{L,EQ}$ is only relevant for estimating f_{NE} from the laboratory degradation study with Eq. 22. In leaching calculations with PEARL, the Freundlich sorption coefficient $K_{F,EQ}$ and the Freundlich exponent N should be used that were derived from the batch adsorption studies.

3. The next step is to check whether the values obtained for k_d and f_{NE} are defensible. This is necessary because they are derived from a decline of the total amount without considering sorption studies on long-term kinetics. Boesten et al. (1989) found f_{NE} values of 0.3 to 0.4 and k_d values of 0.01-0.02 d^{-1} for cyanazine and metribuzin in a sandy soil. Boesten & Gottesbüren (2000) found f_{NE} =0.55 and k_d =0.015 d^{-1} for bentazone in a sandy soil. Using the same bentazone data, Tiktak et al. (2000) found f_{NE} =0.73 and k_d =0.019 d^{-1}. Boesten (personal communication) found f_{NE} =0.75 and k_d =0.005 d^{-1} for metamitron and hydroxychlorothalonil in a sandy soil. Based on this limited information, setting strict limits is not justifiable. Thus values for k_d are considered defensible if they are in the range between 0.002 and 0.1 d^{-1} and for f_{NE} the defensible range is from 0.1-1.0. If f_{NE} values exceed 1.0, the assumption of linear sorption may be not defensible as well as described in Section A4.4. If values are outside this range, additional studies are necessary (e.g. aged sorption studies) and more complex fitting tools need to be used.

4. If values obtained for k_d and f_{NE} are not defensible, stop and do not use PEARL as a higher-tier option in the context of the flow chart shown in Figure 7-2. If the values are defensible, use the average of all k_d and f_{NE} values for PEARL calculations.

5. If more data are available than only the decline of the total amount with time (e.g. also concentration in liquid phase as a function of time), then consider using the PEARLNEQ tool described by Tiktak *et al*. (2000; see p. 52).

A4.6. Case study

A data set was available on degradation studies with four soils. It was selected because the results showed a strong bi-phasic pattern. The decline was fitted to Equation A4-9 and results are shown in Table A4-2. In the fitting procedure the measurements from time zero were ignored because the decline in the first 0.1-0.3 d was extremely fast. This is interpreted

as an artefact. For the calculation of f_{NE} the moisture content of the soils during the incubation was necessary and was estimated to be 0.2 mL/g (no data available).

Table A4-2. Values of g, λ_1 and λ_2 (defined by Equation A4-9) obtained by fitting the decline in four soils and the resulting k_t , k_d , Φ and f_{NE} parameters calculated with Equations A4-14 to A4-16 and A4-22.

Soil number	g (-)	λ_1 (d^{-1})	λ_2 (d^{-1})	k_t (d^{-1})	k_d (d^{-1})	Φ (-)	f_{NE} (-)
1	0.137	0.00033	0.285	0.246	0.00038	102	142
2	0.563	0.0267	4.47	1.97	0.061	41	50
3	0.224	0.0112	0.337	0.264	0.014	4.9	8.3
4	0.557	0.0515	0.647	0.315	0.106	2.6	2.7

The results in Table A4-2 show that k_d values of two out of the four soils are in the acceptable range (0.002-0.1 d^{-1}) and that f_{NE} are all outside the acceptable range of 0.1-1. Thus none of the soils produces an acceptable set of parameters. Note that the data set was selected to be a case with strong bi-phasic behaviour so that the f_{NE} values being outside the normal range is not surprising. According to the guidance described above, the next step should be to check whether the linear-sorption approach is defensible and to analyse available aged adsorption studies. These studies were provided but did not contain sufficient detail (data deficiencies were: (1) the decline of the total amount with time during the studies, (2) water content during incubation, (3) solid-liquid ratio and equilibration time of the desorption measurements).

To illustrate the possible effect of the above analysis, the sorption parameters of Soil 3 were assumed to be correct (which implies a very strong effect of non-equilibrium sorption). Firstly, calculations were made for the Tier-1 approach shown in Figure 7-2. Because more than 10% was left at the end of the study, the DegT50 has to be estimated from the slow phase of a hockey-stick fit. The resulting value for Soil 3 was DegT50 = 57 d. The K_{OC} of this soil was 22 L/kg which gives a K_{OM} of 12.8 L/kg. No Freundlich exponents were available so N was set to 0.9 (the default value recommended by FOCUS, 2000). So a Tier-1 run was made using these parameters and ignoring long-term sorption kinetics. The Hamburg scenario was used with winter wheat and the standard FOCUS application. This resulted in a FOCUS leaching concentration of 86 µg/L. For the Tier-2 run, the DegT50 was derived from the k_t value for Soil 3, so ln2/0.264 which equals 2.6 d. Values of k_d and f_{NE} were set at 0.014 d^{-1} and 8.3 (see Table 4-2). The resulting leaching concentration for the Hamburg

scenario was as low as 0.001 μg/L so about five orders of magnitude lower than found in the Tier-1 approach.

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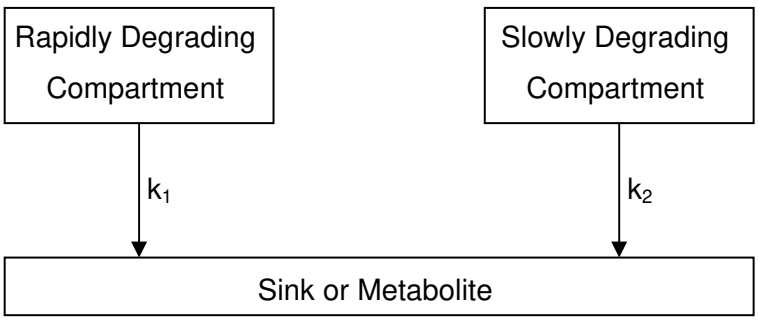
APPENDIX 5: IMPLEMENTING BI-PHASIC KINETICS IN LEACHING MODELS

Implementing some bi-phasic kinetic models in existing leaching models is not straightforward since parameters may depend on concentration and the time since the application. This appendix presents two bi-exponential approaches (DFOP—double first-order in parallel and FOTC—first-order two compartment) that can be used to simulate such kinetic behaviour. In both of these approaches there are two compartments, one with a rapid degradation rate and the other with a slow degradation rate. In both approaches the model is empirically fitted to the observed data from the degradation studies to obtain the parameters for the kinetic model. As discussed in Section 7.1.2.2.3, the DFOP and FOTC bi-phasic approaches should only be considered a pragmatic solution for representing bi-phasic kinetics, because the kinetic expressions are entirely empirical in nature.

Bi-Exponential Approach (DFOP)

The application of the DFOP bi-exponential approach is relatively straightforward, especially since the DFOP bi-exponential model is one of the recommended equations in Chapter 5. In the DFOP approach, a fraction of the amount applied is placed in the rapidly degrading compartment and the rest is placed in the slowly degrading compartment, as illustrated in the following diagram:



The bi-exponential model has three variables, the degradation rate in each of two compartments and the fraction of material in the rapidly degrading compartment (the fraction in the slowly degrading compartment is one minus the fraction in the rapidly degrading compartment).

The procedure for implementing the bi-exponential approach is to conduct two separate simulations. As an example, if the degradation rates corresponded to half-lives of 10 days and 100 days and 30 percent of the material went through the 10 day half-life, one simulation

would consist of applications made at 30 percent of the total application rate with the compound degrading with a half-life of 10 days and the other simulation would consist of applications made at 70 percent of the total application rate with the compound degrading with a half-life of 100 days. The concentrations would then be summed to get the total concentration.

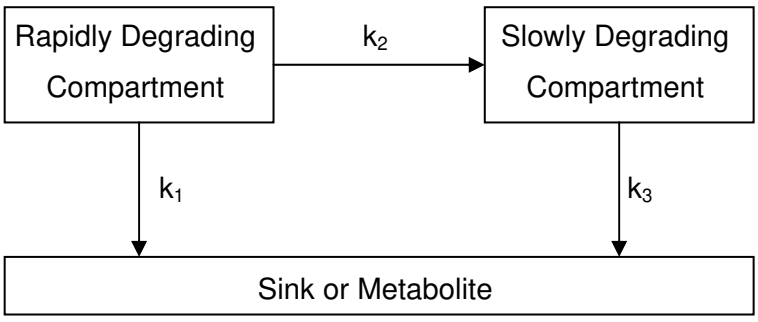
The simulation is strictly correct only when the Freundlich exponent is one (linear isotherm). However, a conservative estimate can be made when the Freundlich exponent is not one by doubling the application rate and then dividing the final answer by two.

This approach can also be used to simulate bi-phasic kinetics of a metabolite if the degradation of the parent is not bi-phasic. However, some minor re-coding would be required to be able to simulate the behaviour of a metabolite formed as a result of bi-phasic kinetics (either from parent or a predecessor compound). A way around the re-coding problem would be to have the parent break down rapidly (essentially in a day) into the two metabolites with the different degradation rates.

Because the partitioning occurs at the time of application, this approach can handle situations where the material in a first application has not degraded by the time of the second application.

Bi-Exponential Approach (FOTC)

This approach also considers parent as being present in two compartments. However, unlike the DFOP approach all of the material is initially placed in the rapidly degrading compartment. In this compartment, two processes occur: degradation to the first actual metabolite or sink and transformation to a slowly degrading compartment. These processes are illustrated in the following diagram.



Like the DFOP approach, three parameters are used to describe the kinetics using the FOTC approach, the degradation rate of the first parent compound to the first actual metabolite or sink (k_1), the degradation rate of the first parent compound to the second parent compound (k_2), and the degradation rate of the second parent compound to the first actual metabolite or sink (k_3). This approach is implemented in a leaching model by simulating the rapidly degrading compartment as a parent substance, the slowly degrading compartment as a metabolite, and the first actual metabolite as a second metabolite. The simulated concentrations of the rapidly and slowly degrading substances in leachate are then added to give the total concentration of the active substance. The three transformation rates are determined by fitting the kinetic model depicted above to the concentrations measured in the degradation study. Some leaching models require the input of a total rate of degradation from the rapidly degrading compartment and the fraction of this compartment which is transformed to the slowly degrading compartment. The total degradation rate in the rapidly degrading compartment is the sum of k_1 and k_2, the transformation fraction to the slowly degrading compartment is $k_2/(k_1 + k_2)$, and the transformation fraction of the rapidly degrading compartment to the first actual metabolite is $k_1/(k_1 + k_2)$.

As with the bi-exponential approach, the simulation is strictly correct only when the Freundlich exponent is one (linear isotherm). However, a conservative estimate can be made when the Freundlich exponent is not one by doubling the application rate and then dividing the final answer by two.

This approach can also be used to simulate first-order or bi-phasic kinetics of metabolites following either first-order or bi-phasic kinetics of parent or predecessor metabolites, subject to the constraints of the total number of compounds that can be handled by the specific model.

Because the transformation rate of the more rapidly degrading parent to the more slowly degrading is described as a first-order reaction rate, this approach can handle situations where the material in a first application has not degraded by the time of the second application.

Comparison of the Two Approaches

The DFOP and FOTC approaches are quite similar. In cases where the models provide good fits of the experimental data, the parameters for both models are related. For example, k_2 in the DFOP model is equal to k_3 in the FOTC model. Also, the sum of k_1 and k_2 in the FOTC model is equal to k_1 in the DFOP model.

The major advantage of both the DFOP and FOTC approaches over models such as the hockey-stick model is that they can be used in cases involving closely spaced multiple applications. There is also no problem on how to determine the appropriate break point when normalising field data.

The ability to express the fraction remaining in each compartment as a function of time makes the DFOP approach more appropriate for calculation of soil PEC values.

Example

These two techniques are illustrated in the following example using the Hamburg scenario for applications of 1 kg/ha to winter cereals one day before emergence. Degradation rates for the two models are as follows (these are presented to a large number of significant figures to facilitate comparison with the models):

> *DFOP.* Half-lives of 6.3021 and 29.003 days with 63.8682 percent being placed in the compartment with the shortest half-life.
>
> *FOTC.* Half-lives of 6.3138 and 29.0049 for the fast and slow degrading parent compounds with a formation fraction for the slow degrading parent of 0.28174.

For simplicity the Freundlich exponent was assumed to be one. All of the remaining pesticide properties are the same as dummy pesticide D in the FOCUS Groundwater Scenarios Workgroup final report. PRZM has been used as an example but the approach is similar for the other models.

The parameterisation of the bi-exponential DFOP model using two runs is shown in Figures A5-1 and A5-2. When the results of the two runs are combined by year to give an overall concentration the resulting 80th percentile value is 2.70 µg/L.

Figure A5-3 shows the parameterisation of the FOTC approach. When the results of the two compounds are combined by year the resulting 80th percentile value is essentially the same, 2.69 µg/L.

Figure A5-4 shows the parameterisation of the bi-exponential model using the approach of a parent compound rapidly breaking down into the two compounds with different degradation rates. This approach gives essentially the same result as with the individual runs with the bi-exponential model. The advantage is that this approach is that it allows the concentration of the metabolite to be predicted in a single run, which is essential if this metabolite degrades to another metabolite.

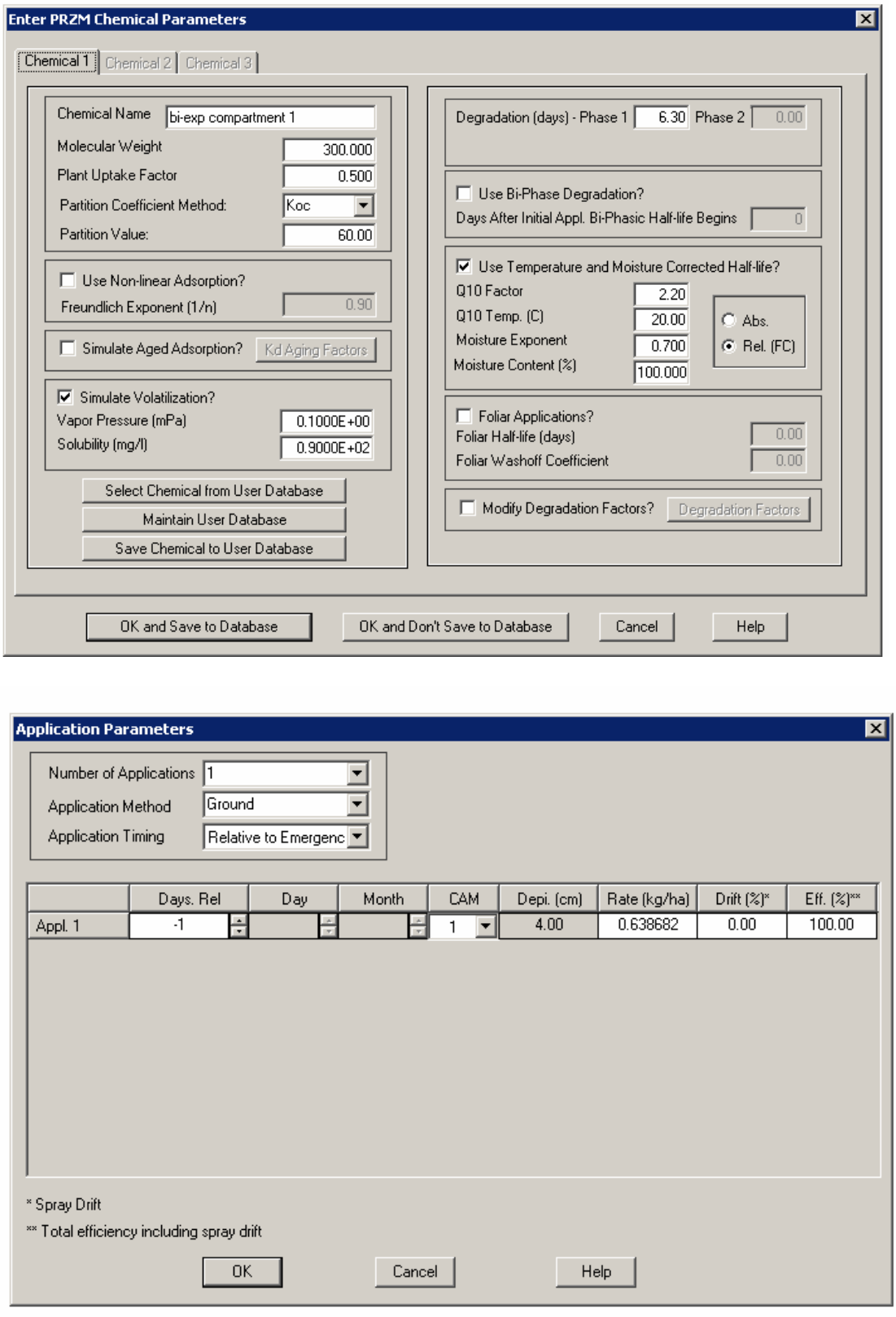


Figure A5-1. Parameterisation of the rapidly degrading compartment of the bi-exponential model in PRZM.

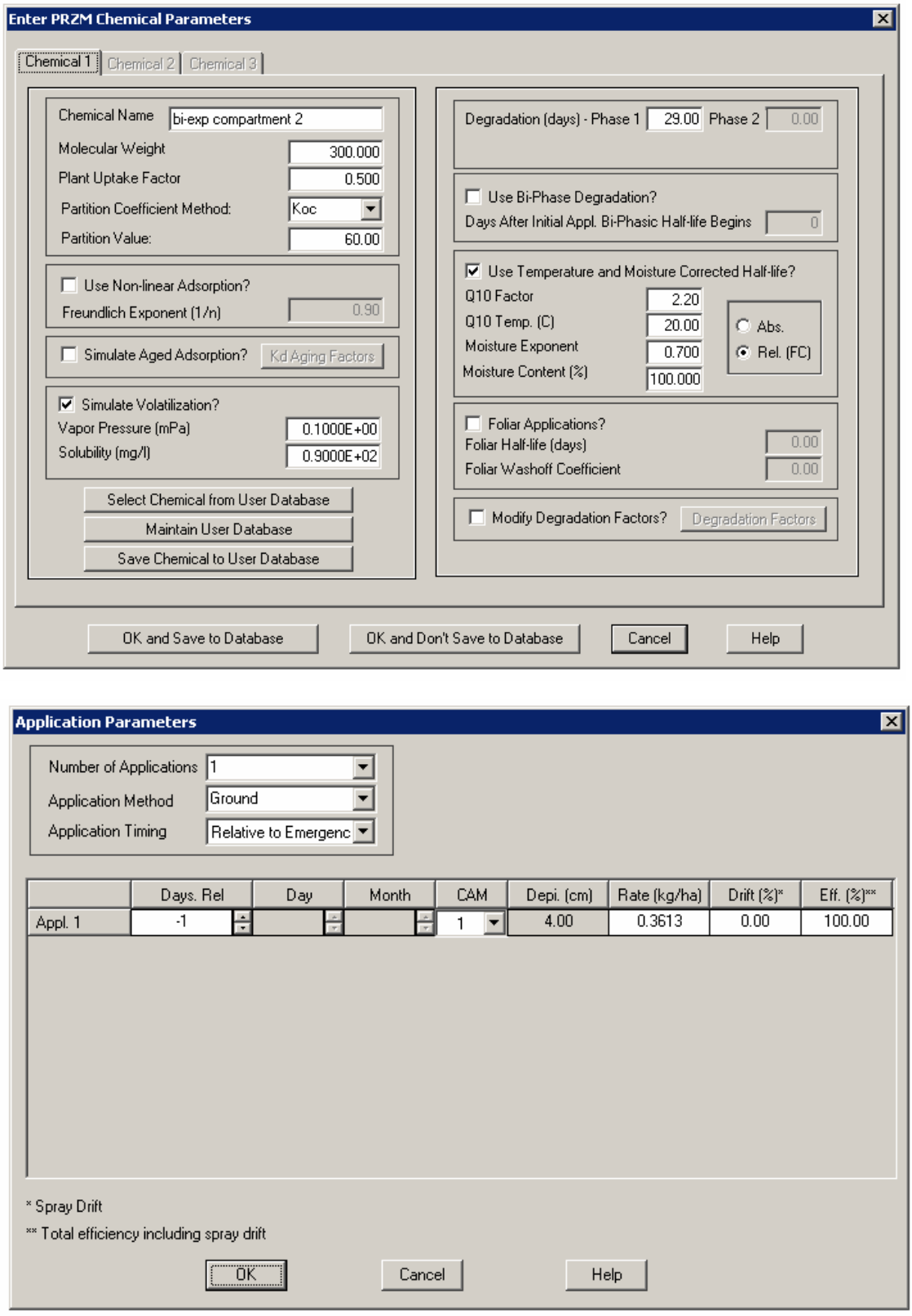


Figure A5-2. Parameterisation of the slowly degrading compartment of the bi-exponential model in PRZM.

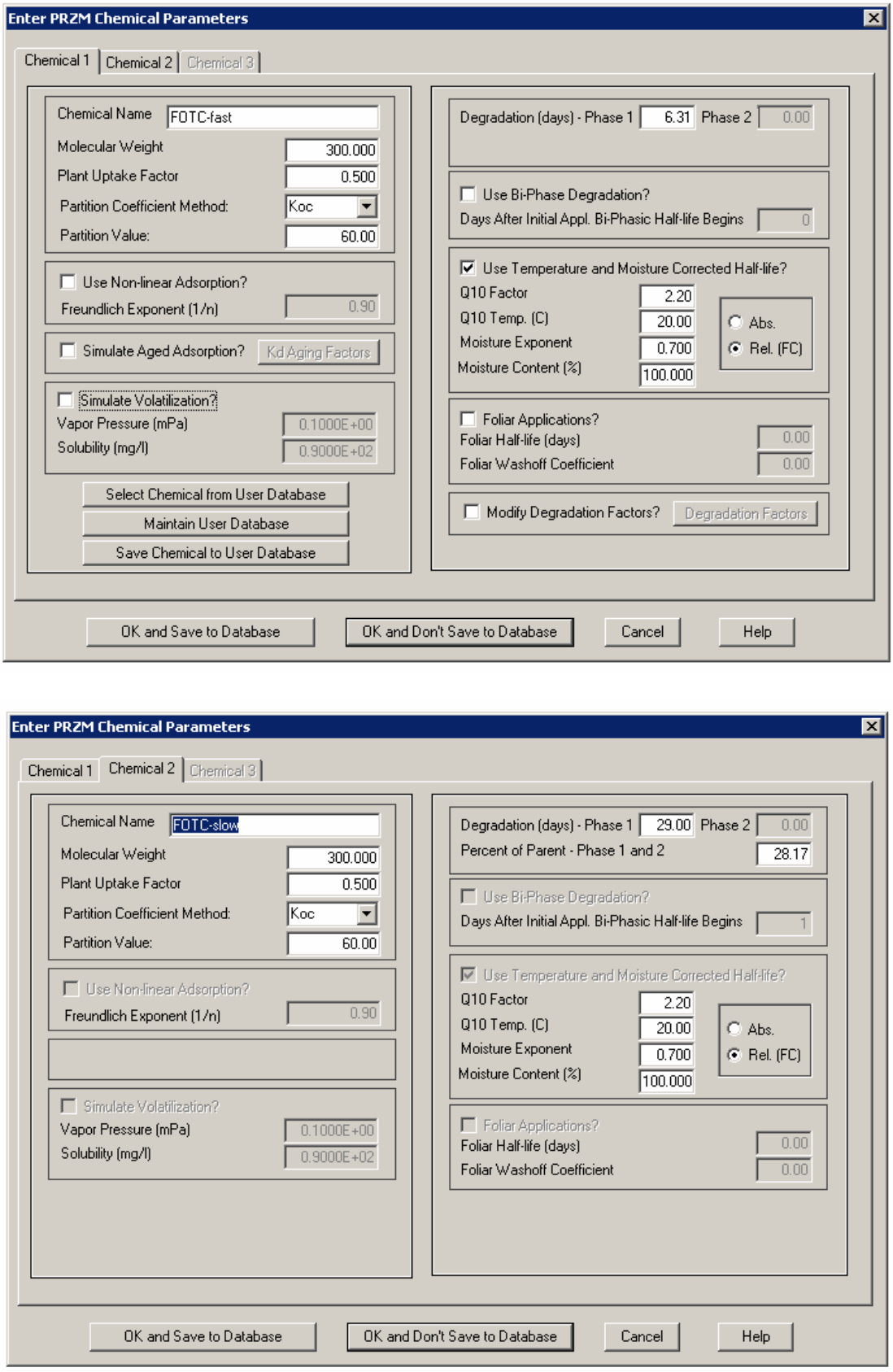


Figure A5-3. Parameterisation of the FOTC approach in PRZM.

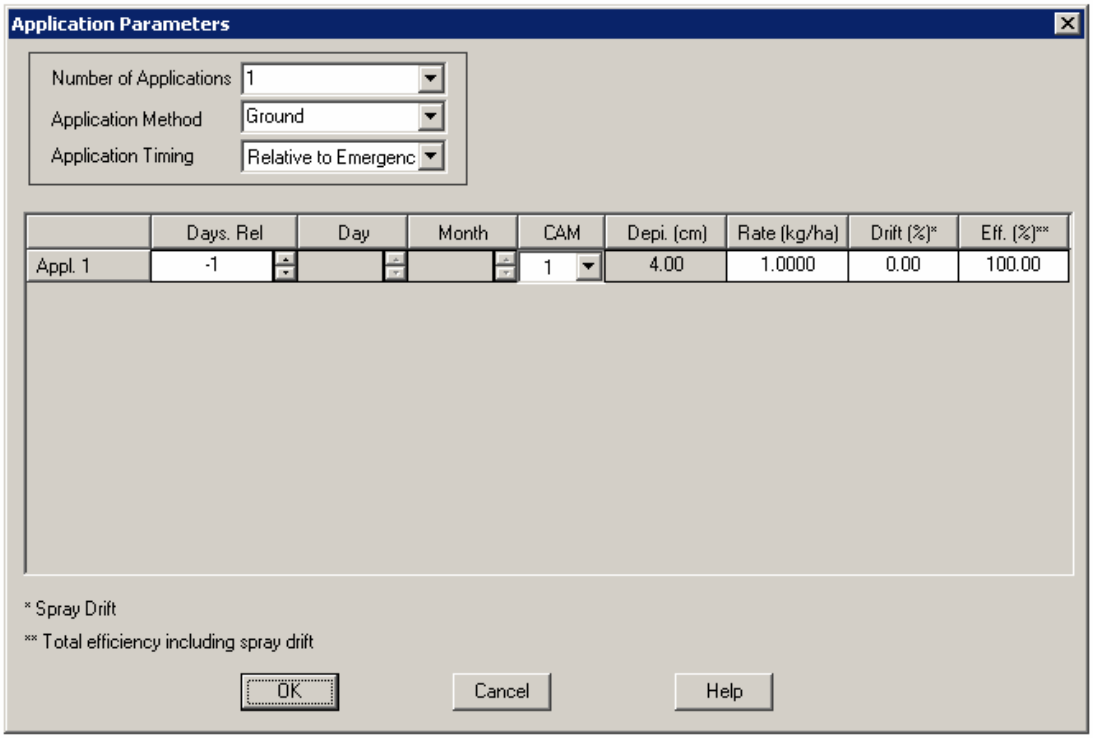


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Figure A5-3 (continued). Parameterisation of the FOTC approach in PRZM

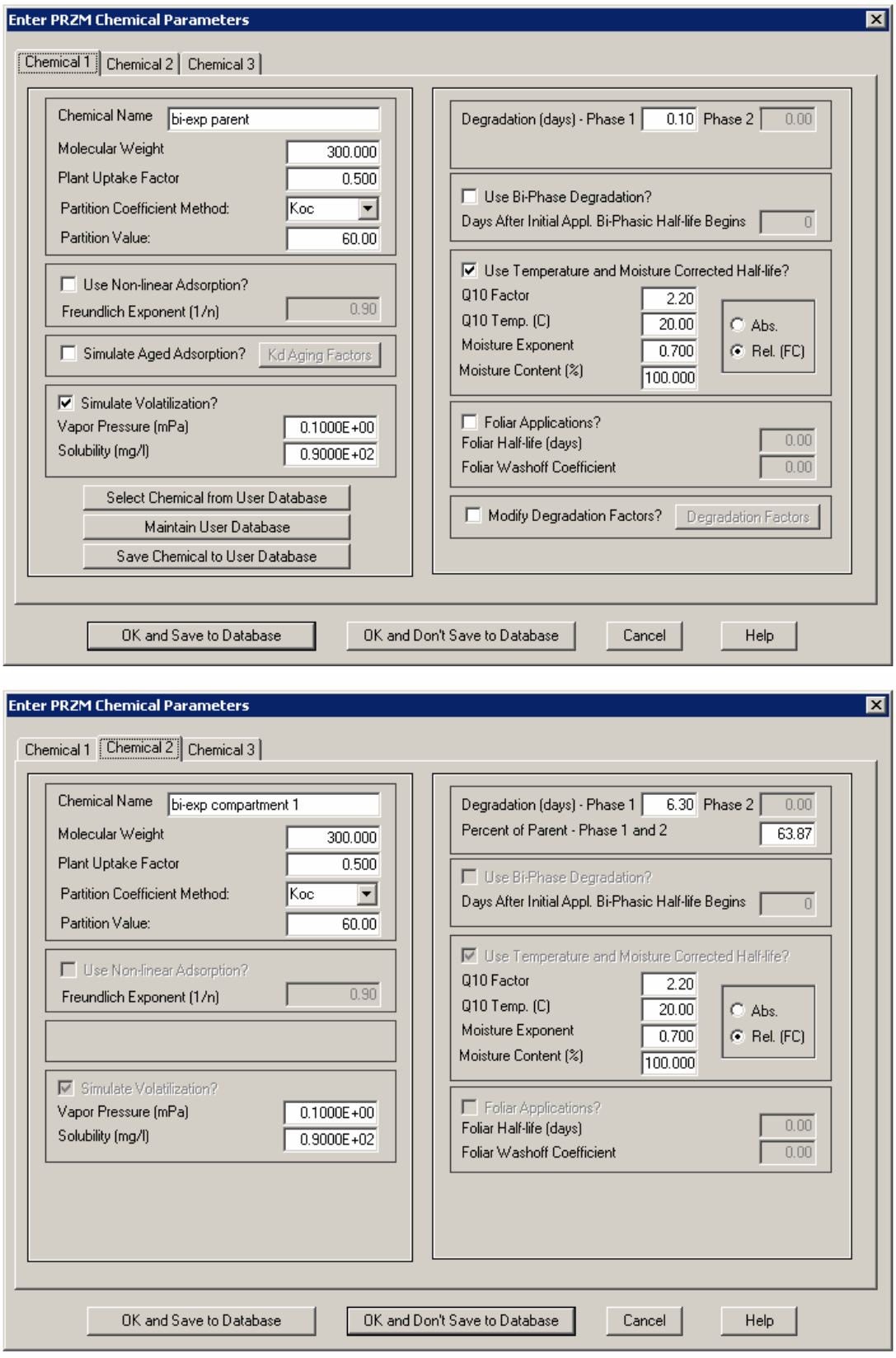
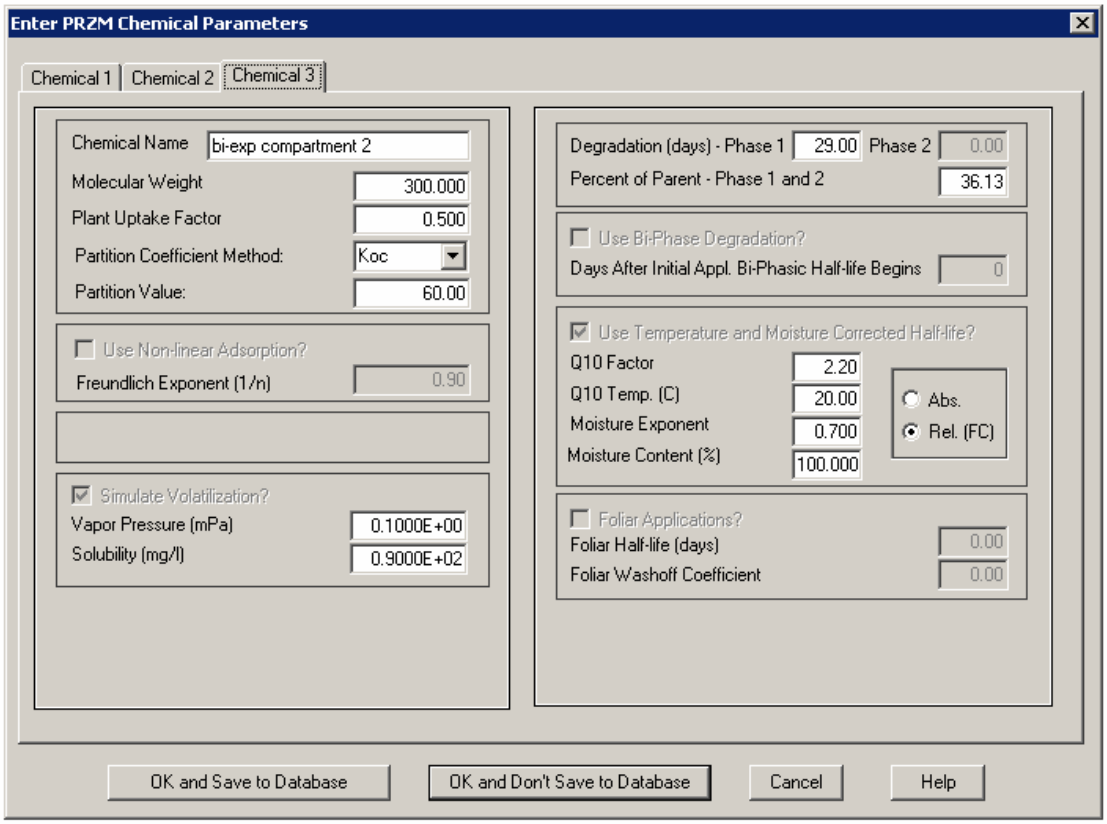


Figure A5-4. Parameterisation of bi-exponential model using a rapidly degrading parent.



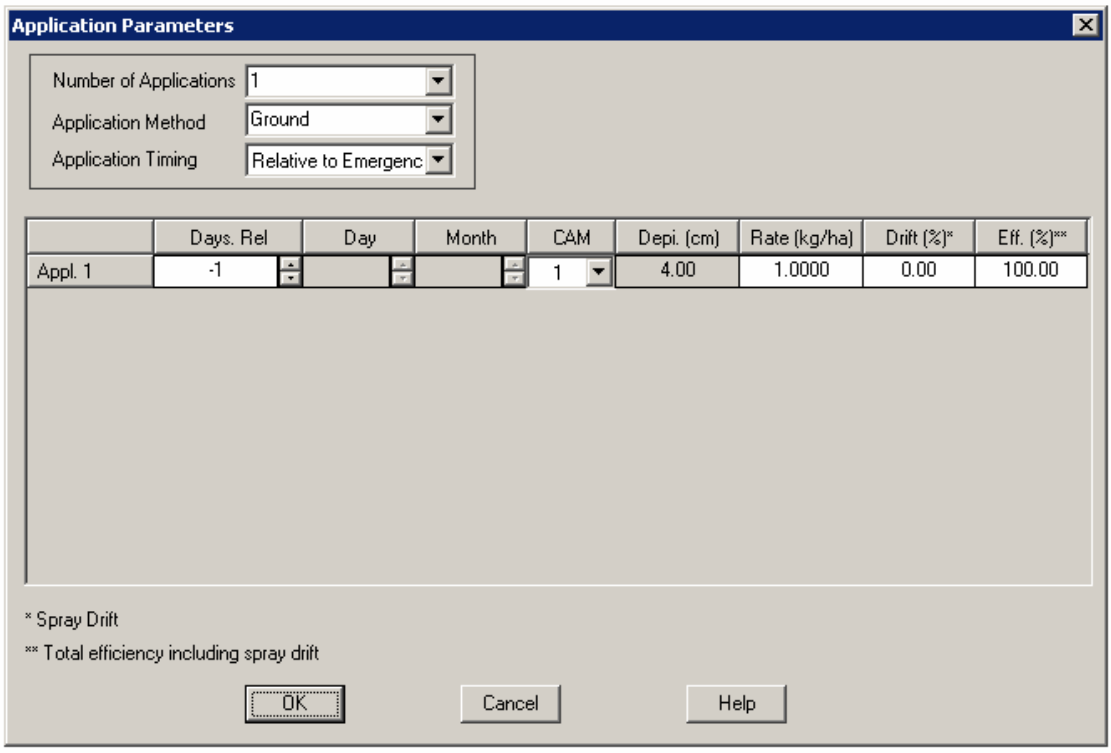


Figure A5-4 (continued). Parameterisation of bi-exponential model using a rapidly degrading parent.

APPENDIX 6: ILLUSTRATION OF THE INFLUENCE OF DATA QUALITY ON THE ESTIMATION OF METABOLITE PARAMETERS

The influence of the number and distribution of data points on the quality of the parameter estimation was tested with a number of generated data sets with all-SFO kinetics.

A simple model (Model I) of parent forming one major metabolite and bound residues and/or minor metabolites (sink compartment) was used to generate four different data sets depending on the values assigned to the various rate constants (Examples 1 to 4). A more complex model (Model II), considering two metabolites, was used (Example 5) to generate data. In this model the parent formed metabolite 1 and metabolite 1 is transformed to metabolite 2. In addition, there were flows from all substances (parent, metabolite 1 and metabolite 2) to the sink compartment. Graphical representations of the compartments of Model I and II are given in Box A6-1 and Box A6-2, respectively.

An error of 10 % was introduced in the data points using the random function in ModelMaker with two different seed values for duplicates. The parameter values used for the generation of Example A6-1 to A6-4 are given in Table A6-1 and for Example A6-5 in Table A6-2. The data sets were generated for a 100-day degradation period. Data subsets were then generated by picking a data points every 10 days (i.e. sampling times at 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 days) or in a pattern representative of a typical laboratory study (i.e. sampling times at 0, 1, 3, 7, 14, 21, 35, 50, 75 and 100 days). To show the influence of the study duration (and number of sampling points) on the estimation of the parameter (uncertainty of the parameter value) the study duration was truncated in some of the example cases to either 75 or 50 days.

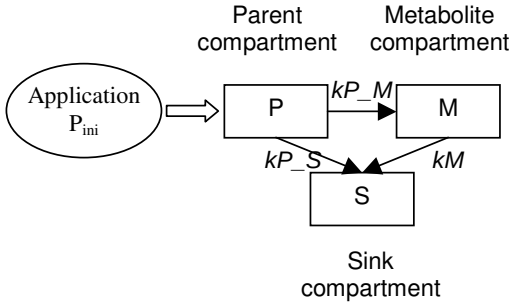
For each example, the full data set (all points, only for Examples A6-1 to A6-3) and each data subset (with 10-day interval for Examples A6-1 to A6-3 or laboratory sampling pattern, and truncated sets), all including 10% error in the data set, were then fitted with the same model as used to generate the data to estimate the initial amount of parent and the various degradation rate constants.

The compartment models used for the generation and estimation of parameters are described as follows:

Model definitions:

The model definitions were formulated by using separate rate constants. The model equations are given in Box A6-1 for model I and in Box A6-2 for model II. Additionally, for example A6-4 and example A6-5 calculations were conducted using formation fractions for the description of the degradation of the Parent and the Parent and Metabolite 1, respectively (see Box A6-1 and Box A6-2).

Model I (formulated with rate constants)



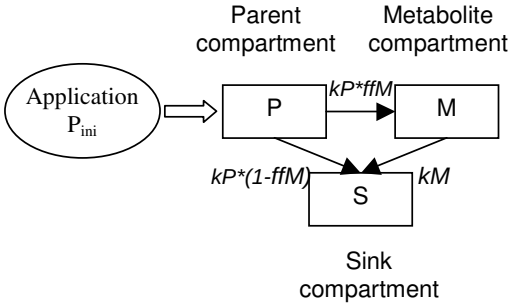
Rate equations (differential form):

$$\frac{dP}{dt} = -(kP\_M + kP\_S) \cdot P$$

$$\frac{dM}{dt} = kP\_M \cdot P - kM \cdot M$$

$$\frac{dS}{dt} = kP\_S \cdot P + kM \cdot M$$

Model I (formulated with formation fraction)



Rate equations (differential form):

$$\frac{dP}{dt} = -kP \cdot P$$

$$\frac{dM}{dt} = kP \cdot ffM \cdot P - kM \cdot M$$

$$\frac{dS}{dt} = kP \cdot (1 - ffM) \cdot P + kM \cdot M$$

where

P	=	Total amount of the parent at time *t*
P_{ini}	=	Total amount of the parent at time *t=0*
M	=	Total amount of the metabolite at time *t*
M_{ini}	=	Total amount of the metabolite at time *t=0* (fixed to 0)
S	=	Total amount of the sink at time *t* (S at time *t*=0 is fixed to 0)
kP, kP_M, kP_S, kM	=	First-order rate constants
ffM	=	Formation fraction of metabolite [0...1]

Parameters to be determined:

P_{ini}, *kP_M*, *kP_S*, *kM*

Parameters to be determined:

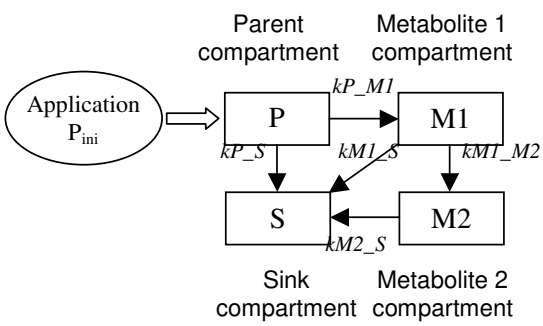
P_{ini}, *kP*, *kM*, *ffM*

Endpoints:

Parent-DT_{50}	= ln (2)/(*kP_M* + *kP_S*)	Parent-DT_{50}	= ln (2)/*kP*
Metabolite-DT_{50}	= ln (2)/*kM*	Metabolite-DT_{50}	= ln (2)/*kM*

Box A6-1. Compartment model I (formulated with rate constants and with formation fraction)

Model II (formulated with rate constants)



Rate equations (differential form):

$$\frac{dP}{dt} = -(kP\_M1 + kP\_S) \cdot P$$

$$\frac{dM1}{dt} = kP\_M1 \cdot P - (kM1\_M2 + kM1\_S) \cdot M1$$

$$\frac{dM2}{dt} = kM1\_M2 \cdot M1 - kM2 \cdot M2$$

$$\frac{dS}{dt} = kP\_S \cdot P + kM1\_S \cdot M1 + kM2 \cdot M2$$

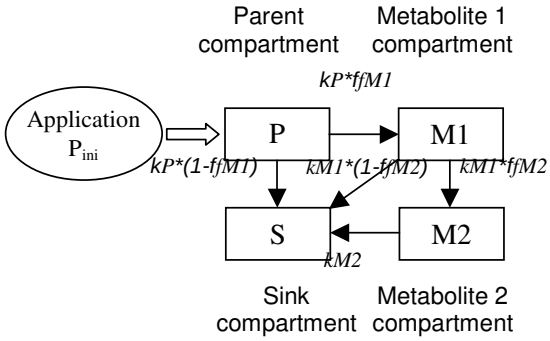
Parameters to be determined:

P_{ini}, *kP_M1*, *kP_S*, *kM1_M2*, *kM1_S*, *kM2*

Endpoints:

Parent-DT_{50} = ln (2)/(*kP_M1* + *kP_S*)
Metabolite 1-DT_{50} = ln (2)/(*kM1_M2* + *kM1_S*)
Metabolite 2-DT_{50} = ln (2)/*kM2*

Model II (formulated with formation fraction)



Rate equations (differential form):

$$\frac{dP}{dt} = -kP \cdot P$$

$$\frac{dM1}{dt} = kP \cdot ffM1 \cdot P - kM1 \cdot M1$$

$$\frac{dM2}{dt} = kM1 \cdot ffM2 \cdot M1 - kM2 \cdot M2$$

$$\frac{dS}{dt} = kP \cdot (1 - ffM1) \cdot P + kM1 \cdot (1 - ffM2) \cdot M1 + kM2 \cdot M2$$

Parameters to be determined:

P_{ini}, *kP*, *kM1*, *kM2*, *ffM1*, *ffM2*

Endpoints:

Parent-DT_{50} = ln (2)/*kP*
Metabolite 1-DT_{50} = ln (2)/*kM1*
Metabolite 2-DT_{50} = ln (2)/*kM2*

where

P	=	Total amount of the parent at time *t*
P_{ini}	=	Total amount of the parent at time *t=0*
M1, M2	=	Total amount of the metabolite 1 and 2 at time *t*
$M1_{ini}$, $M2_{ini}$	=	Total amount of the metabolite 1 and 2 at time *t=0* (fixed to 0)
S	=	Total amount of the sink at time *t* (S at time *t*=0 is fixed to 0)
kP - kM	=	First-order rate constants
ffM_1, ffM_2	=	Formation fraction of metabolite 1 and 2 [0...1]

Box A6-2. Compartment model II
(formulated with rate constants and with formation fraction)

Table A6-1. Parameters used for the generation of data and initial values used for the estimation of parameters in model I, examples 1 to 4

	Model formulated with rate constants								
	parameter values:				initial values:				
	P_{ini} (*t=0*)	*kP_M* P→M	*kP_S* P→S	*kM* M→S	P_{ini}	M_{ini},S_{ini}	*kP_M* P→M	*kP_S* P→S	*kM* M→S
Example 1	100	0.03	0.05	0.005	100	Set to 0	0.01	0.01	0.01
Example 2	100	0.01	0.01	0.005	100	Set to 0	0.02	0.02	0.02
Example 3	100	0.05	0.05	0.090	100	Set to 0	0.02	0.02	0.02
Example 4	100	0.05	0.01	0.020	100	Set to 0	0.02	0.02	0.02
	Model formulated with formation fraction								
	parameter values:				initial values:				
	P_{ini} (*t=0*)	*kP* P→M+S	*ffM*	*kM* M→S	P_{ini}	M_{ini},S_{ini}	*kP* P→M+S	*ffM*	*kM* M→S
Example 4	100	0.06	0.833	0.020	100	Set to 0	0.02	0.5	0.02

Table A6-2. Parameters used for the generation of data and initial values used for the estimation of parameters in model II, example 5

Model formulated with rate constants							
Parameter values:							
P_{ini} (*t=0*)	*kP_M1* (P→M1)	*kP_S* (P→S)	*kM1_M2* (M1→M2)	*kM1_S* (M1→S)	*kM2* (M2→S)		
100	0.06	0.03	0.07	0.02	0.06		
Initial values:							
P_{ini}	$M1_{ini}$, $M2_{ini}$, S_{ini}	*kP_M1*	*kP_S*	*kM1_M2*	*kM1_S*	*kM2*	
100	set=0	0.02	0.02	0.02	0.02	0.02	
Model formulated with formation fractions							
Parameter values:							
P_{ini} (*t=0*)	*kP* (P→M1+S)	*ffM1*	*kM1* (M1→M2+S)	*ffM2*	*kM2* (M2→S)		
100	0.09	0.666	0.09	0.777	0.06		
Initial values:							
P_{ini}	$M1_{ini}$, $M2_{ini}$, S_{ini}	*kP* (P→M1+S)	*ffM1*	*kM1* (M1→M2+S)	*ffM2*	*kM2* (M2→S)	
100	set=0	0.02	0.5	0.02	0.5	0.06	

Results of the parameter estimations

The results of the parameter estimations are presented in Tables A6-3 to A6-7, for examples A6-1 to A6-5, respectively, each time using different sampling dates as input data. All estimates of the parent initial amount, Pini, were close to 100 with low associated error, and are not reported in the result tables. The model description of the data points in the case of a typical study design with two replicates (sampling on day 0, 1, 3, 7, 14, 21, 35, 50, 75 and 100) is shown in Figures A6-1 to A6-5, for examples A6-1 to A6-5, respectively.

EXAMPLE A6-1:

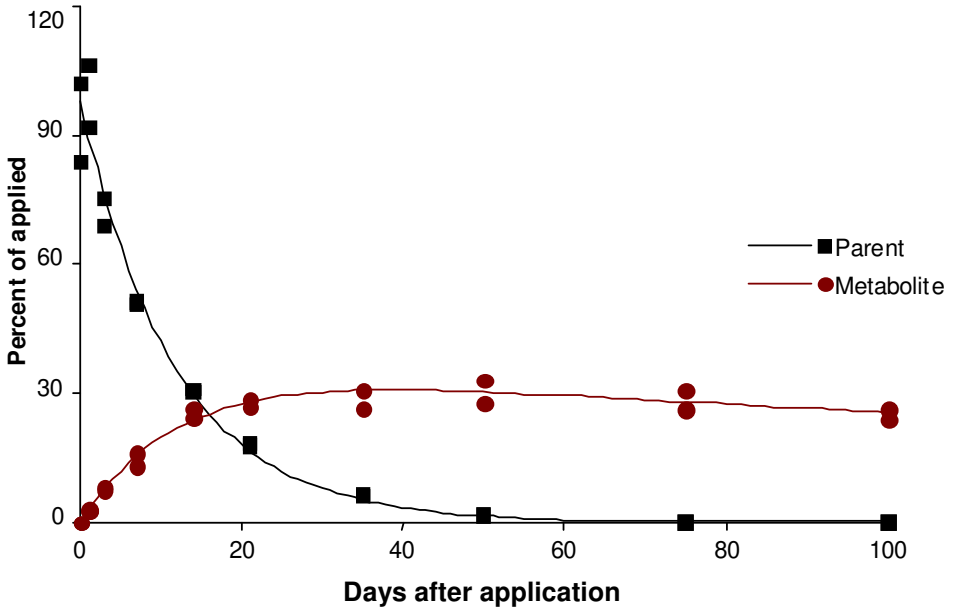


Figure A6-1. Result of fitting of "laboratory" sub-set generated for Example A6-1 data set, data used up to day 100

Table A6-3: Results of the parameter estimations for Example A6-1 using different sampling points as input data.

Parameters used for the generation of data						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* $_2$ (P→S)	P DT50	*kM* (M→S)	M DT50
		0.03	*0.05*	*8.7*	*0.005*	*138.6*
Parameter estimation using different sampling points						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* $_2$ (P→S)	P DT50	*kM* (M→S)	M DT50
	Data used up to day 100					
1	every day (101)	0.031±0.0004	0.050±0.0012	8.6	0.005±0.0002	147.5
2	every 10 days: 0,10,20...100	0.032±0.0015	0.043±0.0028	9.3	0.005±0.0009	133.3
3	0,1,3,7,14,21,35,50,75,100	0.030±0.0023	0.054±0.0047	8.2	0.004±0.0016	192.5
	Data used up to day 75					
4	every day (76)	0.031±0.0006	0.049±0.0013	8.6	0.005±0.0005	141.5
5	every 10 days: 0,10, 20...75	0.032±0.0021	0.043±0.0036	9.3	0.005±0.0018	128.4
6	0,1,3,7,14,21,35,50,75	0.030±0.0027	0.054±0.0052	8.2	**0.003±0.0025①**	**203.9***
	Data used up to day 50					
7	every day (51)	0.031±0.0008	0.049±0.0019	8.6	0.005±0.0013	128.4
8	every 10 days: 0, 10, 20...50	0.031±0.0030	0.044±0.0048	9.3	**0.004±0.0038②**	**165.0***
9	0,1,3,7,14,21,35,50	0.031±0.0035	0.054±0.0061	8.2	**0.004±0.0047③**	**161.2***

①, ② and ③ The probability corresponding to the calculated t-value for the highlighted parameter is above the significance level of 10% (0.120, 0.153 and 0.201, respectively), indicating that the parameter is not significantly different from zero.

*Because of lack of confidence in the rate constant parameter estimate for M from this fit, the DT50 value for M calculated from the parameter may not be reliable.

EXAMPLE A6-2:

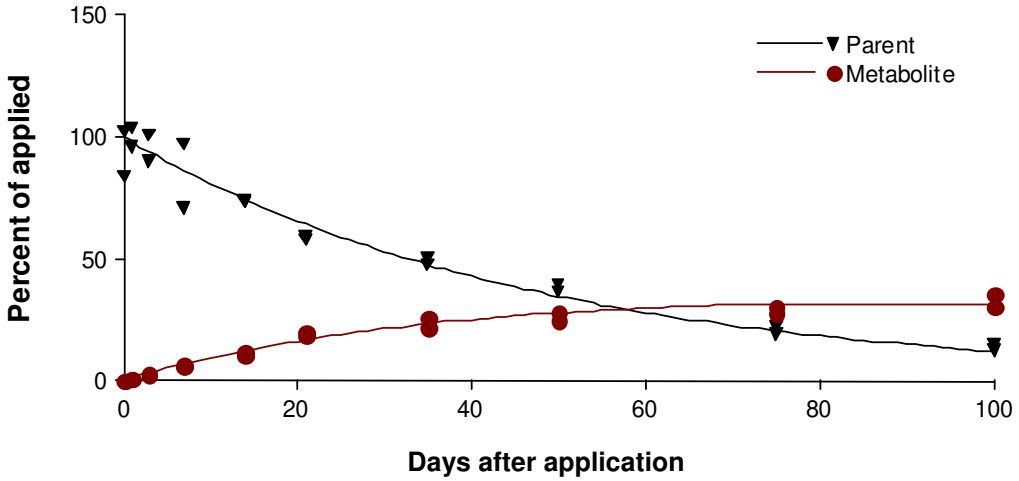


Figure A6-2. Result of fitting of "laboratory" subset generated for Example A6-2 data set, data used up to day 100

Table A6-4. Results of the parameter estimations for Example A6-2 using different sampling points as input data

Parameters used for the generation of data						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* (P→S)	P DT50	*kM* (M→S)	M DT50
		0.01	*0.01*	*34.7*	*0.005*	*138.6*
Parameter estimation using different sampling points						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* (P→S)	P DT50	*kM* (M→S)	M DT50
	Data used up to day 100					
1	every day (101)	0.010±0.0003	0.010±0.0004	35.0	0.005±0.0008	130.8
2	0,1,3,7,14,21,35,50,75,100	0.011±0.0013	0.010±0.0018	34.0	**0.005±0.0031①**	**133.3***
3	inclusion of data: 0,1,3,7, 14,21,35,50,**62**,75,**87**,100	0.011±0.0012	0.009±0.0015	34.5	0.006±0.0027	115.5
	Data used up to day 75					
4	every day (76)	0.010±0.0005	0.010±0.0006	34.5	0.006±0.0016	119.5
5	0,1,3,7,14,21,35,50,75	0.011±0.0018	0.009±0.0023	33.8	**0.008±0.0055②**	**84.5***
	Data used up to day 50					
6	every day (51)	0.010±0.0009	0.010±0.0011	34.8	**0.006±0.0042③**	**119.5***
7	0,1,3,7,14,21,35,50	0.011±0.0027	0.009±0.0032	34.1	**0.009±0.0116④**	**79.7****
8	inclusion of data: 0,1,3,7, 14,21,**28**,35,**42**, 50	0.012±0.0026	0.008±0.0029	35.2	**0.011±0.0110⑤**	**61.3****

①, ② and ③ The probability corresponding to the calculated t-value for the highlighted parameter is in the range of significance level of 5 to 10% (0.058, 0.078, and 0.077, respectively), indicating that the parameter may not be significantly different from zero. The parameter results and goodness of fit need to be further examined based on all available data for the substance to decide whether the estimate may be accepted or not.

④ and ⑤ The probability corresponding to the calculated t-value for the highlighted parameter is above the significance level of 10% (0.222 and 0.162, respectively), indicating that the parameter is not significantly different from zero.

*The parameter results and goodness of fit need to be further examined based on all available data for the substance to decide whether the DT50 may be considered or not.

**Because of lack of confidence in the rate constant parameter estimate for M from this fit, the DT50 value for M calculated from the parameter may not be considered reliable.

EXAMPLE A6-3:

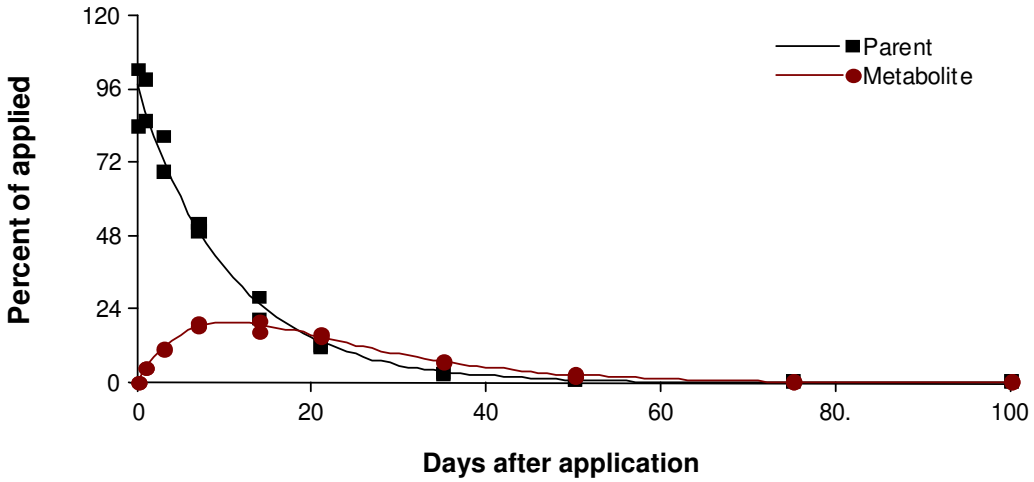


Figure A6-3. Result of fitting of "laboratory" subset generated for Example A6-3 data set, data used up to day 100

Table A6-5. Results of the parameter estimations for Example A6-3 using different sampling points as input data

Parameters used for the generation of data						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* $_2$ (P→S)	P DT50	*kM* (M→S)	M DT50
		0.05	*0.05*	*6.9*	*0.09*	*7.7*
Parameter estimation using different sampling points						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* (P→S)	P DT50	*kM* (M→S)	M DT50
	Data used up to day 100					
1	every day (101)	0.053±0.0014	0.045±0.0019	7.1	0.093±0.0030	7.4
2	0,1,3,7,14,21,35,50,75,100	0.051±0.0072	0.044±0.0087	7.3	0.090±0.0184	7.7
	Data used up to day 75					
3	every day (76)	0.053±0.0016	0.045±0.0022	7.1	0.094±0.0035	7.4
4	0,1,3,7,14,21,35,50,75	0.051±0.0076	0.044±0.0092	7.3	0.090±0.0197	7.7
	Data used up to day 50					
5	every day (51)	0.053±0.0021	0.045±0.0027	7.1	0.094±0.0044	7.4
6	0,1,3,7,14,21,35,50	0.051±0.0082	0.044±0.0096	7.3	0.090±0.0207	7.7
	Data used up to day 35					
7	every day (36)	0.054±0.0027	0.044±0.0034	7.1	0.095±0.0061	7.3
8	0,1,3,7,14,21,35	0.051±0.0090	0.044±0.0105	7.3	0.090±0.0232	7.7

EXAMPLE A6-4:

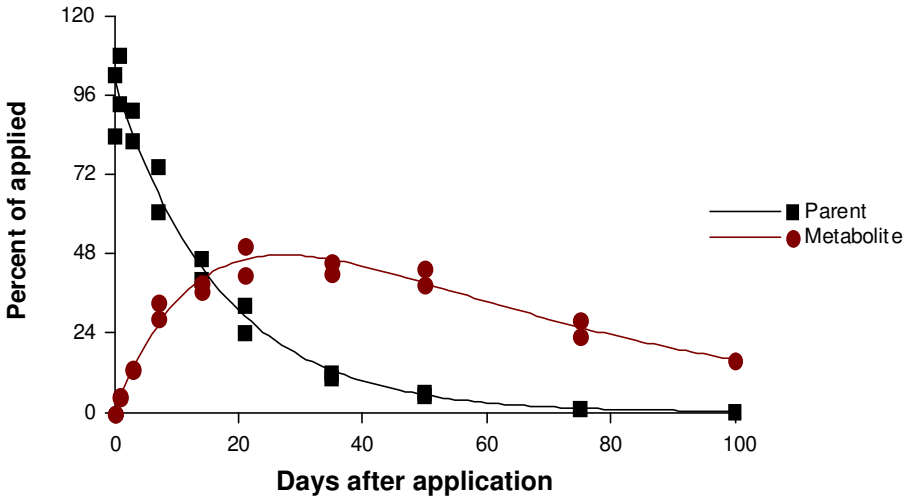


Figure A6-4. Result of fitting of "laboratory" subset generated for Example A6-4 data set, data used up to day 100

Table A6-6: Results of the parameter estimations for Example A6-4 using different sampling points as input data

I: Model formulated with rate constants						
Parameters used for the generation of data						
		kP_M (P→M)	*kP_S* (P→S)	P_DT50	*kM* (M→S)	M_DT50
		0.05	*0.01*	*11.6*	*0.02*	*34.7*
Parameter estimation using different sampling points						
Case	Sampling days (Number)	*kP_M* (P→M)	*kP_S* (P→S)	P_DT50	*kM* (M→S)	M_DT50
	Data used up to day 100					
1	0,1,3,7,14,21,35,50,75,100	0.049±0.0030	0.010±0.0045	11.7	0.021±0.0025	33.5
	Data used up to day 100, data of metabolite for day 21 and 35 deleted					
2	0,1,3,7,14,21,35, 50,75,100	0.051±0.0039	**0.008±0.0053**①	**11.8***	0.021±0.0027	33.2
	Data used up to day 100, data of metabolite for day 14, 21, 35 and 50 deleted					
3	0,1,3,7,14,21,35, 50,75,100	0.056±0.0063	**0.003±0.0074**②	**11.8***	0.023±0.0031	30.3
	Data used up to day 100, data of metabolite for day 50, 75 and 100 deleted					
4	0,1,3,7,14,21,35, 50,75,100	0.052±0.0052	**0.007±0.0066**③	**11.8***	0.027±0.0076	26.1
	Data used up to day 100, data of metabolite for day 35, 50, 75 and 100 deleted					
5	0,1,3,7,14,21,35, 50,75,100	0.054±0.0081	**0.005±0.0092**④	**11.8***	**0.032±0.0169**	**21.7***
II: Model formulated with formation fraction						
Parameters used for the generation of data						
		kP (P→M+S)	P_DT50	*ffM*	*kM* (M→S)	M_DT50
		0.06	*11.6*	*0.833*	*0.02*	*34.7*
Parameter estimation using different sampling points						
Case	Sampling days (Number)	*kP* (P→M+S)	P_DT50	*ffM*	*kM* (M→S)	M_DT50
	Data used up to day 100, data of metabolite for day 14, 21, 35 and 50 deleted					
3	0,1,3,7,14,21,35, 50,75,100	0.059±0.0037	11.8	0.952±0.1252	0.023±0.0031	30.2
	Data used up to day 100, data of metabolite for day 35, 50, 75 and 100 deleted					
6	0,1,3,7,14,21,35, 50,75,100	0.059±0.0039	11.8	0.924±0.1550	0.032±0.0170	21.7

① The probability corresponding to the calculated t-value for the highlighted parameter is in the range of significance level of 5 to 10% (0.071), indicating that the parameter may not be significantly different from zero. The parameter results and goodness of fit would need to be further examined to decide whether the estimate may be accepted or not.

②, ③ and ④ The probability corresponding to the calculated t-value for the highlighted parameter is far above the significance level of 10% (0.344, 0.149 and 0.296, respectively), indicating that the parameter is not significantly different from zero.

*Because of the low confidence or lack of confidence in the rate constant parameter estimate for *kP_S* from this fit, the DT50 value calculated from the parameter may not be considered reliable. However, in this case, the overall parent DT50 is calculated in the model formulated with rate constants from the sum of *kP_M* + *kP_S*. The fact that *kP_S* is not significantly different from 0 in cases 2, 3, 4 and 5 does not directly imply that the overall DT50 of the parent is not reliable, as the contribution of *kP_M* also needs to be considered. This problem can be circumvented by using the model formulation with formation fraction, as in this case the overall rate constant of the parent, *kP*, is estimated together with its standard error.

EXAMPLE A6-5:

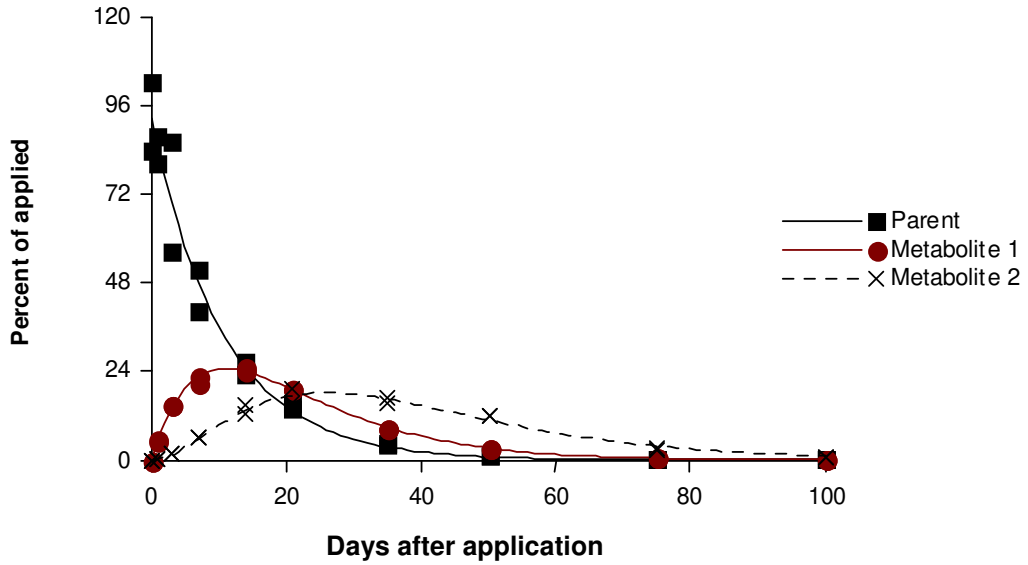


Figure A6-5. Result of fitting of "laboratory" subset generated for Example A6-5 data set, data used up to day 100

Table A6-7: Results of the parameter estimations for Example A6-5 using different sampling points as input data.

	I: Model formulated with rate constants					
	Parameters used for the generation of data					
		kP_M1 (P→M1)	*kP_S* (P→S)	*kM1_S* (M1→S)	*kM1_M2* (M1→M2)	*kM2* (M2→S)
		0.06	*0.03*	*0.02*	*0.07*	*0.06*
		P_DT50 = 7.7		M1_DT50 = 7.7		M2_DT50=11.6
	Parameter estimation using different sampling points of a data set:					
	Sampling days	*kP_M1* ± std.	*kP_S* ± std.	*kM1_S* ± std.	*kM1_M2* ± std.	*kM2* ± std.
	Data used up to day 100					
1	0,1,3,7,14,21,35, 50,75,100	0.0662 ± 0.0078	0.0268 ± 0.0096	**0.0218 ± 0.0228①**	0.0671 ± 0.0129	0.0555 ± 0.0151
		P_DT50 = 7.5		**M1_ DT50 = 7.8***		M2_DT50 = 12.5
	Data used up to day 100, data of metabolite 2 for day 21 and 35 deleted					
2	0,1,3,7,14,21,35, 50,75,100	0.0661 ± 0.0080	0.0270 ± 0.0099	**0.0187 ± 0.0263②**	0.0700 ± 0.0170	0.0557 ± 0.0166
		P_DT50 = 7.4		**M1_ DT50 = 7.8***		M2_DT50 = 12.4
	II: Model formulated with formation fraction					
	Parameters used for the generation of data					
		kP (P→M1+S)	*ffM1*	*kM1* (M1→M2+S)	*ffM2*	*kM2* (M2→S)
		0.09	*0.666*	*0.09*	*0.777*	*0.06*
		P_DT50 = 7.7		M1_DT50 = 7.7		M2_DT50=11.6
	Parameter estimation using different sampling points of a data set:					
	Sampling days	*kP* ± std.	*ffM1* ± std.	*kM1* ± std.	*ffM2* ± std.	*kM2* ± std.
	Data used up to day 100					
1	0,1,3,7,14,21,35, 50,75,100	0.0931 ± 0.0051	0.7099 ± 0.0954	0.0887 ± 0.0156	0.7590 ± 0.2278	0.0557 ± 0.0155
		P_DT50 = 7.5		M1_ DT50 = 7.8		M2_DT50 = 12.4
	Data used up to day 100, data of metabolite 2 for day 21 and 35 deleted					
2	0,1,3,7,14,21,35, 50,75,100	0.0931 ± 0.0051	0.7090 ± 0.0959	0.0884 ± 0.0156	0.8051 ± 0.2488	0.0567 ± 0.0158
		P_DT50 = 7.4		M1_ DT50 = 7.8		M2_DT50 = 12.2

① and ② The probability corresponding to the calculated t-value for the highlighted parameter is far above the significance level of 10% (0.172 and 0.240, respectively), indicating that the parameter is not significantly different from zero.

*Because of the lack of confidence in the rate constant parameter estimate for *kM1_S* from this fit, the DT50 value calculated from the parameter may not be considered reliable. However, in this case, the overall DT50 for M1 is calculated in the model formulated with rate constants from the sum of *kM1_M2* + *kM1_S*. The fact that *kM1_S* is not significantly different from 0 in cases 1 and 2 does not directly imply that the overall DT50 of the metabolite is not reliable, as the contribution of *kM1_M2* also needs to be considered. This problem can be circumvented by using the model formulation with formation fraction, as in this case the overall rate constant of M1, *kM1*, is estimated together with its standard error.

Goodness-of-fit

The χ^2-test was performed on the optimisation results for all data sets. The test provided an error of ~10 % for all the examples presented (actual values for each test not shown). Therefore, the χ^2-test confirms the error in the data, which was introduced during the generation process of the data.

The parameter estimates and their standard deviations are presented for the parent and the metabolite(s) for Examples 1 to 5. The probability corresponding to the calculated t-value for each individual estimated parameter was calculated in Excel as described in 6.3.1.3 (t-test). All parameters were significantly different from zero at a significance level of 5% except when highlighted in the tables. For those parameter values and resulting DT50 values that are highlighted, the high uncertainty indicates that the calculated half-life may not be considered reliable.

Conclusions from Examples A6-1 to A6-4:

The following conclusions can be drawn from the results of Examples A6-1 to A6-4:

- The number and distribution of the sampling times have a strong impact on the results of the estimated parameters in the examples of slow degrading metabolites, i.e. Example A6-1 for fast forming and slow degrading metabolite, and Example A6-2 for slow forming and slow degrading metabolite. Decreasing the number of sampling times and increasing the interval between sampling times at the later stages of the study, as in a typical laboratory experiment, resulted in the highest error associated to the parameter estimates for the metabolite and uncertainty in the calculated DT50 values (see different DT50 values obtained for the metabolite in Table A6-3 and Table A6-4, for the different cases of input data). DT50 values that differed widely from the "true" values were generally identified as non-reliable based on the result of the t-test on the rate constant parameter.
- The study duration (100, 75 and 50 days) has a strong impact on the value and the quality of the estimated parameters for the slow degrading metabolites. When truncating the data set from the typical study sampling schedule (Example A6-1: case 3, 6 and 9 in Table A6-4 and Example A6-2: case 2, 5 and 7 in Table A6-6), the DT50 values obtained from the fits varied from 161 to 193 days for the metabolite in Example A6- and from 78 to 133 days in Example A6-2. The uncertainty of the parameters describing the metabolite increased when the study duration decreased.

- The inclusion of additional sampling dates for the parent and metabolite as shown in Example A6-2 leads to a decrease of the uncertainty of the parameter describing the degradation of the metabolite, although the high error associated to the parameter estimates still indicates high uncertainty, reflected in the calculated DT50 values of the metabolite, which differ from the “true” DT50 values used for the generation of the data (see cases 3 and 8 in Table A6-4).
- Generally, the uncertainty of the parameters for the metabolites is low, when these show an observable increase and decrease during the study. For a fast forming and fast degrading metabolite showing a clear formation and decline pattern early-on in the study, as in Example A6-3, the number and distribution of sampled dates have less impact on the results of the estimated parameters. While the parameter values and DT50 values calculated from the parameters remained constant, the uncertainty of the parameters describing the parent and metabolite increased when the study duration decreased. All parameter estimates in this example were still considered significantly different from zero as concluded from the t-test. When typical study data is used (case 2, 4, 6 and 8 in Table A6-5) the calculated DT50 was always 7.7 days, which is the "true" value for this example.
- The uncertainty of the estimation of parameters for a metabolite formed in high amounts is low as long the sampling points include the decrease phase of the metabolite (see Example A6-4). The reduction of information (non-consideration of sampling points, especially toward the end of the study) for the metabolite increases the uncertainty of the parameter estimates for the metabolite (see Table A6-6).
- The parameter estimations with the model formulated with individual rate constants in Example A6-4 shows that the uncertainty of a parameter describing the degradation of the parent substance to the sink compartment, for which measured data is not available, can be high. This is especially the case if the rate constant to this compartment is much lower than the rate constants to the other compartments (i.e. the individual rate constant to this compartment is relatively low compared to the overall rate constant of the substance). In Example A6-4, the rate from parent to sink (*kP_S*) is much lower than the rate from parent to the metabolite (*kP_M*). The estimate of the rate constant *kP_S* shows an error between 50 and >100 % of the estimate, resulting in a failed t-test at a 5% significance level, while the error for the rate constant *kP_M* is lower than 20 % in the cases 2, 3, 5 and 6 (Table A6-6). This problem can be circumvented by using the model formulation with formation fraction, as in this case the overall rate constant of the parent, *kP*, is estimated together with its standard error. In the two cases tested with the formation fraction model formulation, case 3 and 6, the error associated to *kP* is less than

10% of the estimate and the t-test for the parameter is passed. The uncertainty associated to the flow to the sink is still reflected in the metabolite formation fraction parameter, and, since they are related, in the degradation rate of the metabolite, which both become more uncertain as data points for the metabolite are removed from the fit.

Conclusions from Example A6-5:

The following conclusion can be drawn from the results for this example with two metabolites formed sequentially in low amounts:

- The two metabolites showed a clear formation and decline pattern during the study, and as a result, the uncertainty of the estimated metabolite parameters was relatively low. However, the parameter estimations with the model formulated with individual rate constants show that the uncertainty of the parameter describing the degradation of metabolite 1 to the sink compartment, for which measured data is not available, can be high. In this example, the rate from metabolite 1 to sink (*kM1_S*) is much lower than the rate to the second metabolite (*kM1_M2*). The estimate of the rate constant *kM1_S* shows an error >100 % of the estimate, resulting in a failed t-test at a 5% significance level, while the error for the rate constant *kM1_M2* is lower than 20 % of the estimate in the two cases tested (Table A6-7). This problem can be circumvented by using the model formulation with formation fraction, as in this case the overall rate constant of metabolite 1, *kM1*, is estimated together with its standard error. Using the formation fraction model formulation, the error associated to *kM1* is less than 20% of the estimate and the t-test for the parameter is passed.
- Because the formation and degradation of metabolite 2 can be observed during the study and the degradation of this metabolite is reasonably fast, non-consideration of two sampling points during the peak phase have little impact on the results of the estimated parameters for the metabolite (case 2 in Table A6-7).

APPENDIX 7: ILLUSTRATION OF STEPWISE APPROACH WITH PARENT AND THREE METABOLITES

Introduction

In the following example, the stepwise approach is used to evaluate the degradation kinetics and determine the modelling endpoints of pesticide Z and its soil metabolites Z1, Z2 and Z3 from a study conducted with the parent substance. The proposed metabolic pathway in soil involves the successive formation of 3 metabolites Z1, Z2, Z3. The kinetic endpoints for modelling were generated according to the recommended procedure outlined in the decision flowchart in Section 8.4.2.1, with an all-SFO model. All the fits were performed using the software tool ModelMaker 4.0, unweighted (ordinary least-squares fitting procedure), with unmodified data and without constraint on the initial concentration of the parent substance. This summary includes a detailed account of the approach followed to arrive to the final, simultaneous fit of the parent substance with its 3 metabolites, and provides detailed results at each step. Such a level of details is not necessary in a kinetic evaluation report, as long as enough information is provided to be able to reproduce the kinetic evaluation results.

Data handling

The experimental data for pesticide Z and its metabolites and the corresponding model input data after data treatment is shown in Table A7-1. An LOD of 0.5% AR was selected for this exercise, and therefore the last sampling time before detectable amounts of the metabolites were observed and first sampling time after the decline at which the metabolite was not detected were set to ½ LOD = 0.25% AR. The data for the time 0 for the metabolites were set to 0.

The data from all sampling times up to and including 124 days were used. Considering that microbial activity is not sustainable after 4 months, data at further time points (not shown) were not included in the input data.

Implementation of the conceptual model

The proposed degradation pathway of pesticide Z in soil involves the successive formation of three metabolites, noted Met Z1, Met Z2 and Met Z3. The kinetic model for the degradation of pesticide Z and formation and degradation of the metabolites was built step-by-step based

on this proposed pathway. Compartment models of increasing complexity were implemented in ModelMaker, as illustrated in Figure A7-1. At each step, flows to the sink were initially included, using models formulated with formation fraction parameters. These flows were then deleted if the formation fraction of the metabolite converged to 1 (100% formation).

Table A7-1. Experimental data and model input data for the kinetic evaluation of Pesticide Z and metabolites Z1, Z2 and Z3 (in % AR)

Time (d)	Experimental data				Model input data			
	Pesticide Z	Met Z1	Met Z2	Met Z3	Pesticide Z	Met Z1	Met Z2	Met Z3
0	100	<LOD	<LOD	<LOD	100	Set to 0	Set to 0	Set to 0
0.04	81.7	18.3	<LOD	<LOD	81.7	18.3	-	-
0.125	70.4	29.6	<LOD	<LOD	70.4	29.6	0.25	-
0.29	51.1	46.3	2.6	<LOD	51.1	46.3	2.6	-
0.54	41.2	55.1	3.8	<LOD	41.2	55.1	3.8	-
1	6.6	65.7	15.3	<LOD	6.6	65.7	15.3	0.25
2	4.6	39.1	37.2	9.2	4.6	39.1	37.2	9.2
3	3.9	36	31.7	13.1	3.9	36	31.7	13.1
4	4.6	15.3	35.6	22.3	4.6	15.3	35.6	22.3
7	4.3	5.6	14.5	28.4	4.3	5.6	14.5	28.4
10	6.8	1.1	0.8	32.5	6.8	1.1	0.8	32.5
14	2.9	1.6	2.1	25.2	2.9	1.6	2.1	25.2
21	3.5	0.6	1.9	17.2	3.5	0.6	1.9	17.2
42	5.3	<LOD	<LOD	4.8	5.3	0.25	0.25	4.8
61	4.4	<LOD	<LOD	4.5	4.4	-	-	4.5
96	1.2	<LOD	<LOD	2.8	1.2	-	-	2.8
124	0.7	<LOD	<LOD	4.4	0.7	-	-	4.4

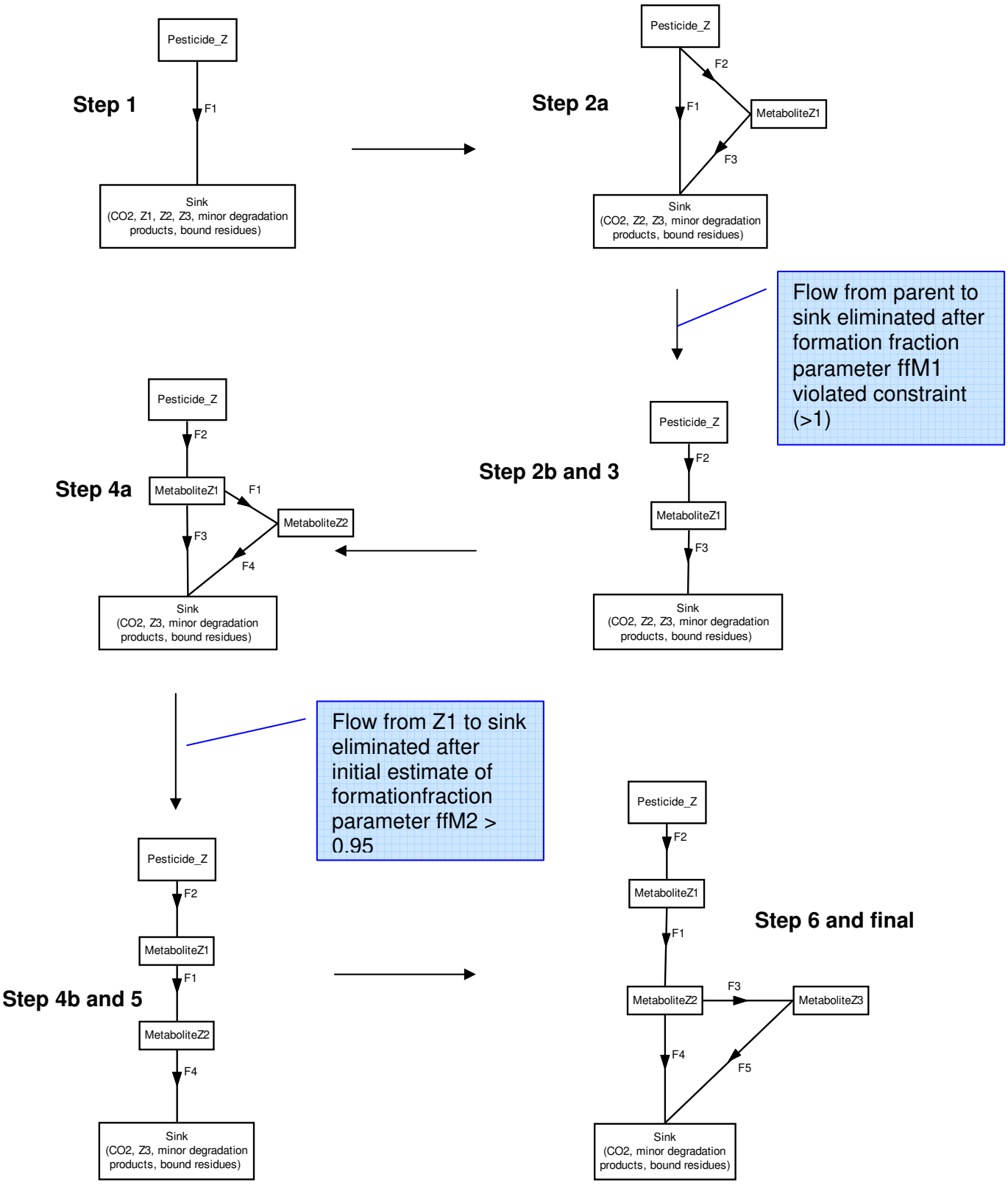


Figure A7-1. Building the kinetic model step-by-step.

Results

***Step 1**: Parent only*

The results of the fitting of the parent data with the SFO model are shown in Table A7-2 and Figure A7-2. Considering the whole incubation period, the fit passes the χ^2 test at an error level of 17%, and the plot of residuals shows a systematic error after 1 day. However, the degradation of the parent substance was extremely rapid, and the DT90 was reached within one day (see close-up graph). Therefore, it is necessary to examine the goodness of the fit over the first day of incubation. Considering the first day of incubation, which included six time points, the fit passes the χ^2 test at an error level of 8%, and is visually acceptable, with a random distribution of the residuals. The standard error of the parameter estimates is sufficiently low to assure that these are reliable. The SFO fit of pesticide Z is deemed acceptable and the parameter estimates for the parent substance can be used in step 2 with the first metabolite Z1 added.

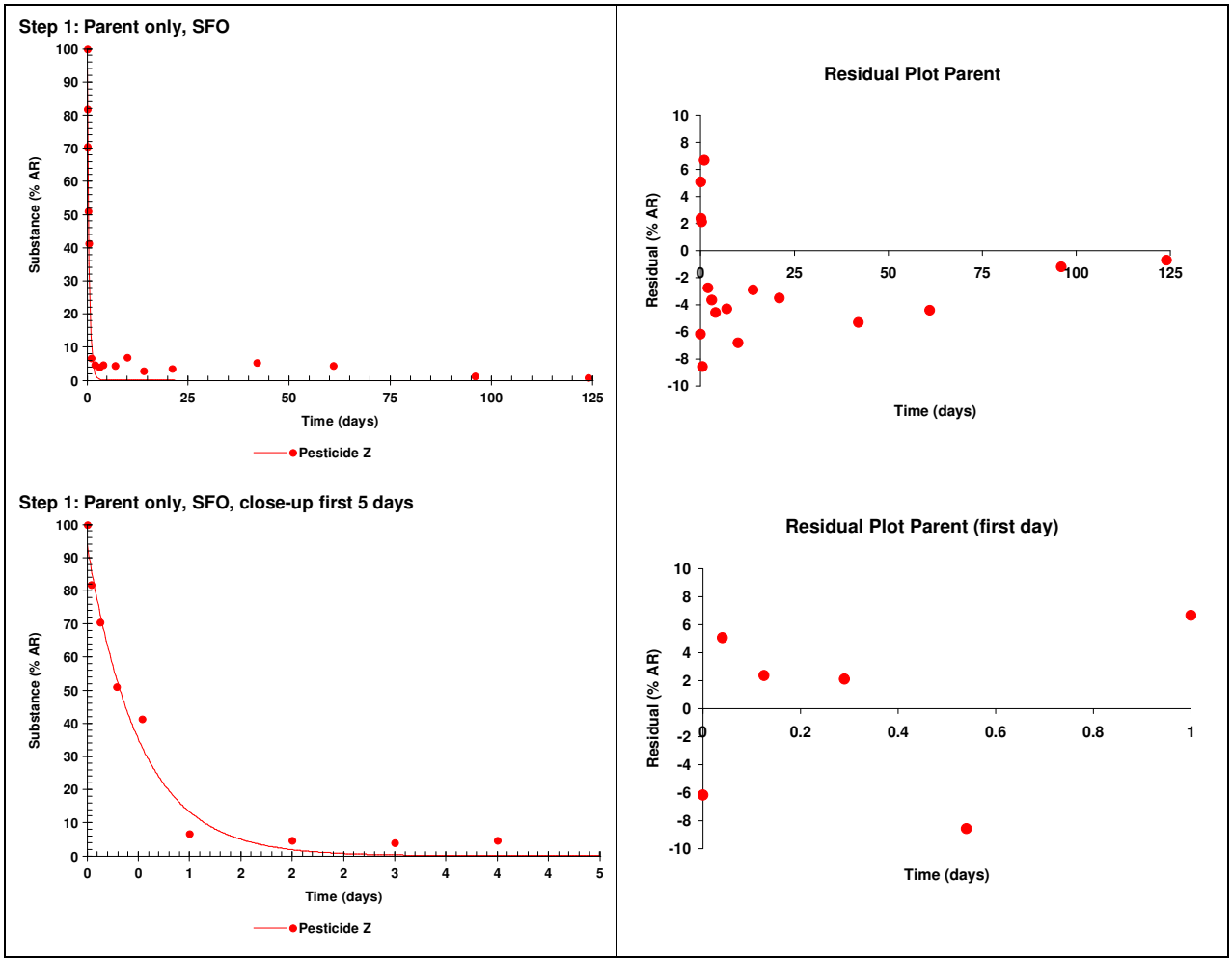


Figure A7-2. Description of the observed data for pesticide Z (parent only) with SFO kinetics.

Table A7-2. Results of the SFO fit with parent only in step 1.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	100	93.85 ± 3.49
k parent (d^{-1})	1	1.955 ± 0.207
Goodness of fit (χ^2 error)		
parent (121 days)	17	
parent (1 day)	8	

***Step 2a**: Parent and Met Z1, parent parameters fixed*

The first metabolite, Met Z1 is added to the model. At this step, parent was assumed to degrade to Met Z1 and to other unidentified metabolites or bound residues. However, the formation fraction parameter for Met Z1, *ffM1*, which was constrained between 0 and 1 as recommended, violated its constraint range (upper constraint) in the parameter optimisation, and no results were obtained at this step. Consequently, the formation fraction was set to 1 in a modified model in Step 2b.

Table A7-3. Starting values for Step 2a fit with parent and Metabolite Z1.

Model parameters	
	Starting value
Pini (% AR)	93.85 (fixed)
kP (d^{-1})	1.955 (fixed)
ffM1	0.5
kM1 (d^{-1})	0.1

***Step 2b**: Parent and Met Z1 (100% formation), parent parameters fixed*

The model in step 2a was simplified by removing the flow from parent to sink. The results of the fitting of the Met Z1 data with the SFO model are shown in Table A7-4 and Figure A7-3. The fit of Met Z1 passes the χ^2 test at an error level of 19%, and the plot of residuals shows an acceptable fit. The standard error of the parameter estimate is sufficiently low to assure that it is reliable. The overall pattern of formation and decline of the metabolite is described well by the model (see close-up graph), and the SFO fit of the metabolite is deemed acceptable. The estimated rate constant can be used as starting value in step 3.

Table A7-4. Results of the SFO fit of Met Z1 in step 2b.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	93.85 (fixed)	-
kP (d^{-1})	1.955 (fixed)	-
kM1 (d^{-1})	0.1	0.4614 ± 0.0413
Goodness of fit (χ^2 error)		
χ^2 error Met Z1	19	

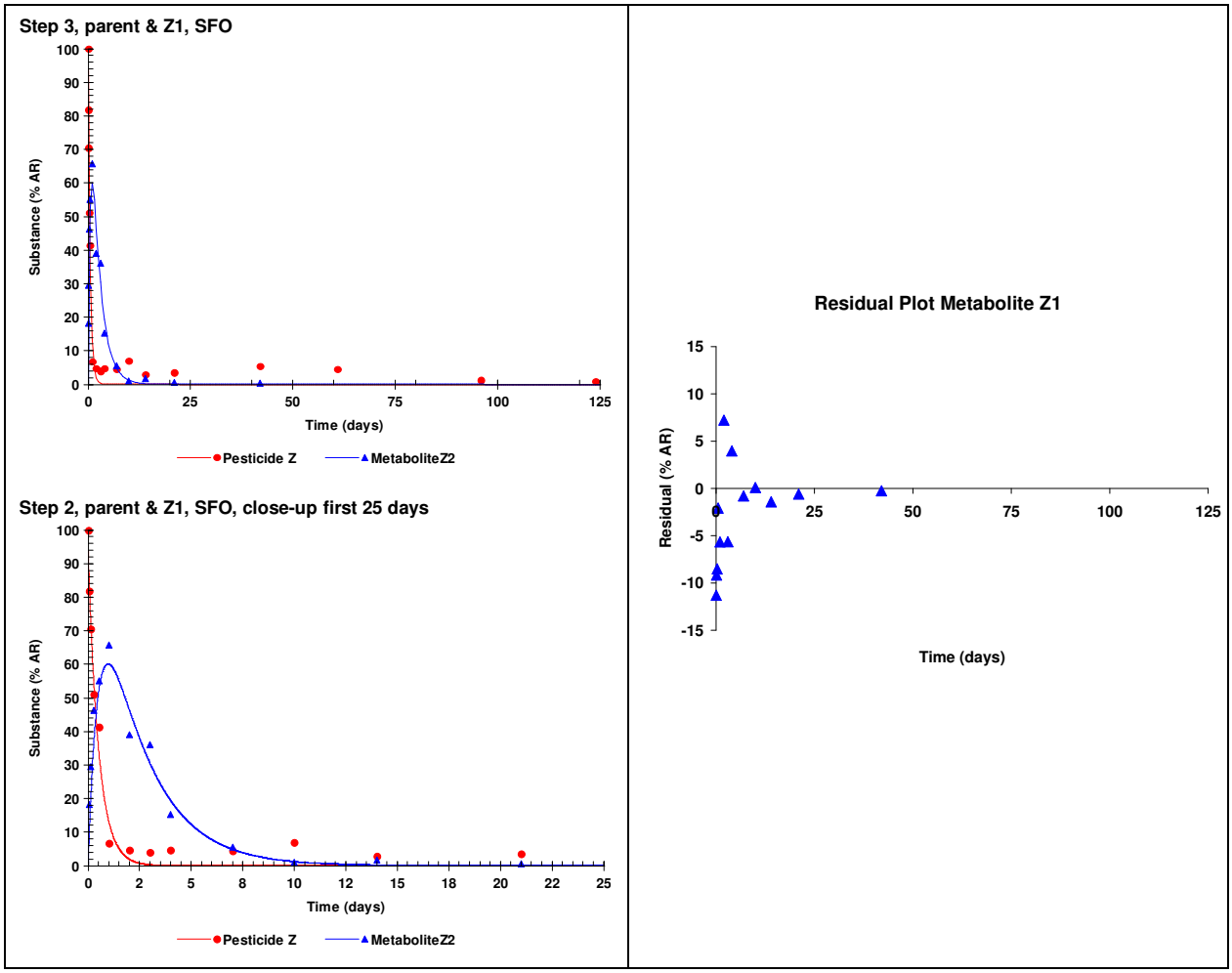


Figure A7-3. Description of the observed data for metabolite Z1 with SFO kinetics, with parent parameters fixed.

***Step 3**: Parent and Met Z1 (100% formation), all parameters optimised*

All parameters for Pesticide Z and Metabolite Z1 are optimised in this step, using the estimates from step 1 and step 2b as starting values for the parent and metabolite parameters, respectively. The results of the fitting of the data with the model are shown in Table A7-5 and Figure A7-4. The fits of pesticide Z and Met Z1 pass the χ^2 test with acceptable error levels, as previously discussed, and the plots of residuals show acceptable fits. The standard error of the parameter estimates is sufficiently low to assure that these are reliable. The estimated rate constant can be used as starting value in step 4 with the second metabolite Z2 added.

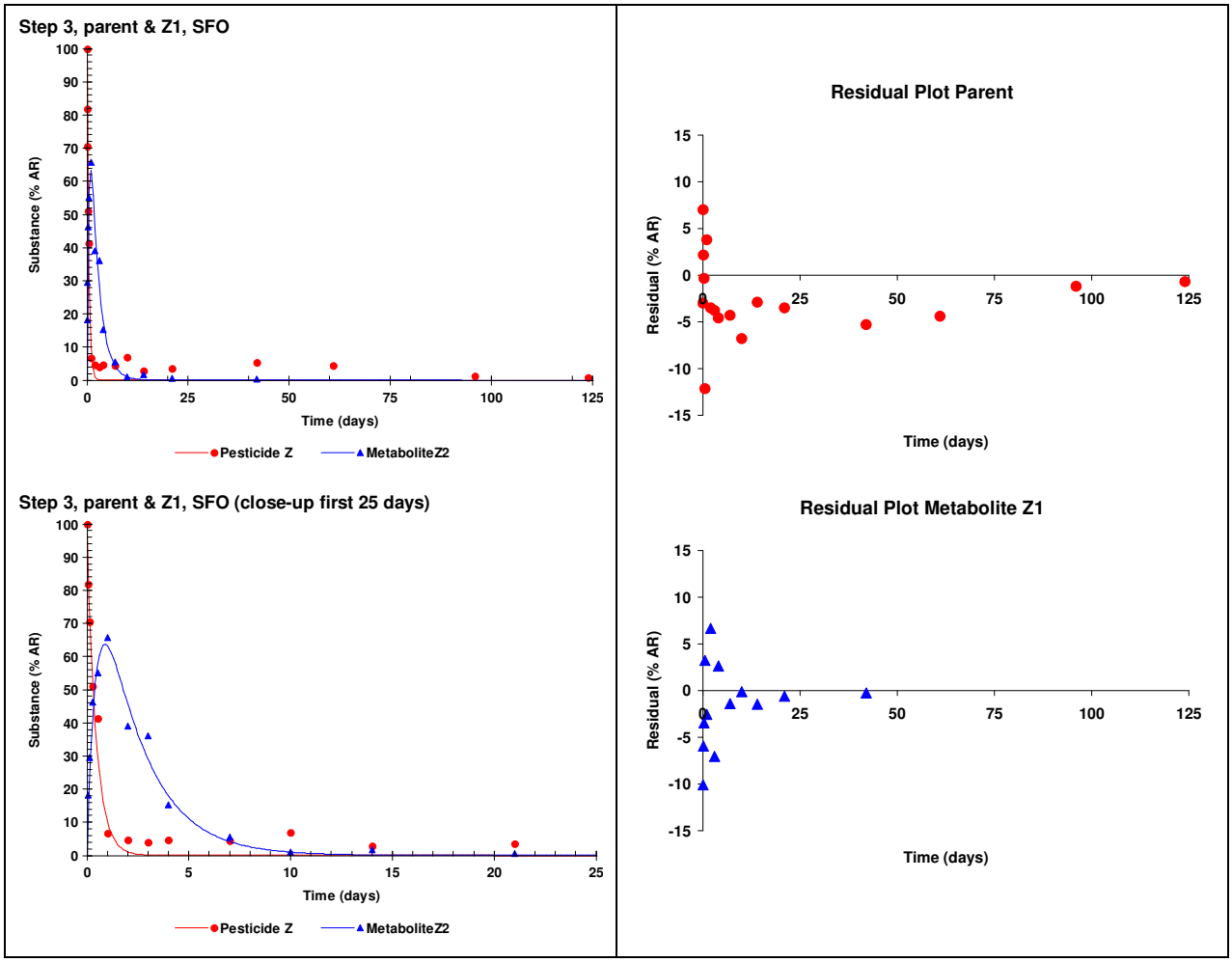


Figure A7-4. Description of the observed data for pesticide Z and metabolite Z1 with SFO kinetics, all parameters optimised.

Table A7-5. Results of the simultaneous fit of pesticide Z and Met Z1 in step 3.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	93.85	96.99 ± 2.73
kP (d^{-1})	1.955	2.232 ± 0.150
kM1 (d^{-1})	0.4614	0.4816 ± 0.0437
Goodness of fit (χ^2 error)		
parent (121 days)	18	
Met Z1	15	

***Step 4a**: Parent, Met Z1 (100% formation) and Met Z2, parent and Met Z1 parameters fixed*

The second metabolite, Met Z2 is added to the model. At this step, Met Z1 is assumed to degrade to Met Z2 and to other unidentified metabolites or bound residues. The results of the fitting of the data with the model are shown in Table A7-6. The formation fraction parameter, *ffM2*, constrained between 0 to 1, converged to 0.959 with a standard error of 0.135. Because the estimated value is so close to 1 and with the associated error would include 1, a reasonable assumption is that the deviation from 1 results from the natural error associated to the data. Such an assumption needs to be supported by additional information on the substance degradation pathway, to provide weight of evidence. In this example, Metabolite Z1 was assumed to have been shown to quickly and exclusively hydrolyse to Metabolite Z2 in a number of aquatic and soil laboratory studies. Consequently, the formation fraction of Metabolite Z2 was set to 1 in a modified model in Step 4b.

Table A7-6. Results of the fit of Met Z2 in step 4a.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	96.99 (fixed)	-
kP (d^{-1})	2.231 (fixed)	-
kM1 (d^{-1})	0.4816 (fixed)	-
ffM2	0.5	0.9591 ± 0.1348
kM2 (d^{-1})	0.1	0.4279 ± 0.0902

***Step 4b**: Parent, Met Z1 (100% formation), and Met Z2 (100% formation), parent and Met Z1 parameters fixed*

The model in step 4a was simplified by removing the flow from Met Z1 to sink. The results of the fitting of the Met Z2 data with the SFO model are shown in Table A7-7 and Figure A7-5. The fit of Met Z2 passes the χ^2 test at an error level of 20%, and the plot of residuals with its random distribution of residuals shows an acceptable fit. The standard error of the parameter estimate is sufficiently low to assure that it is reliable. The overall pattern of formation and decline of metabolite Z2 is described well by the model (see close-up graph), and the SFO fit of the metabolite is deemed acceptable. The estimated rate constant can be used as starting value in step 5.

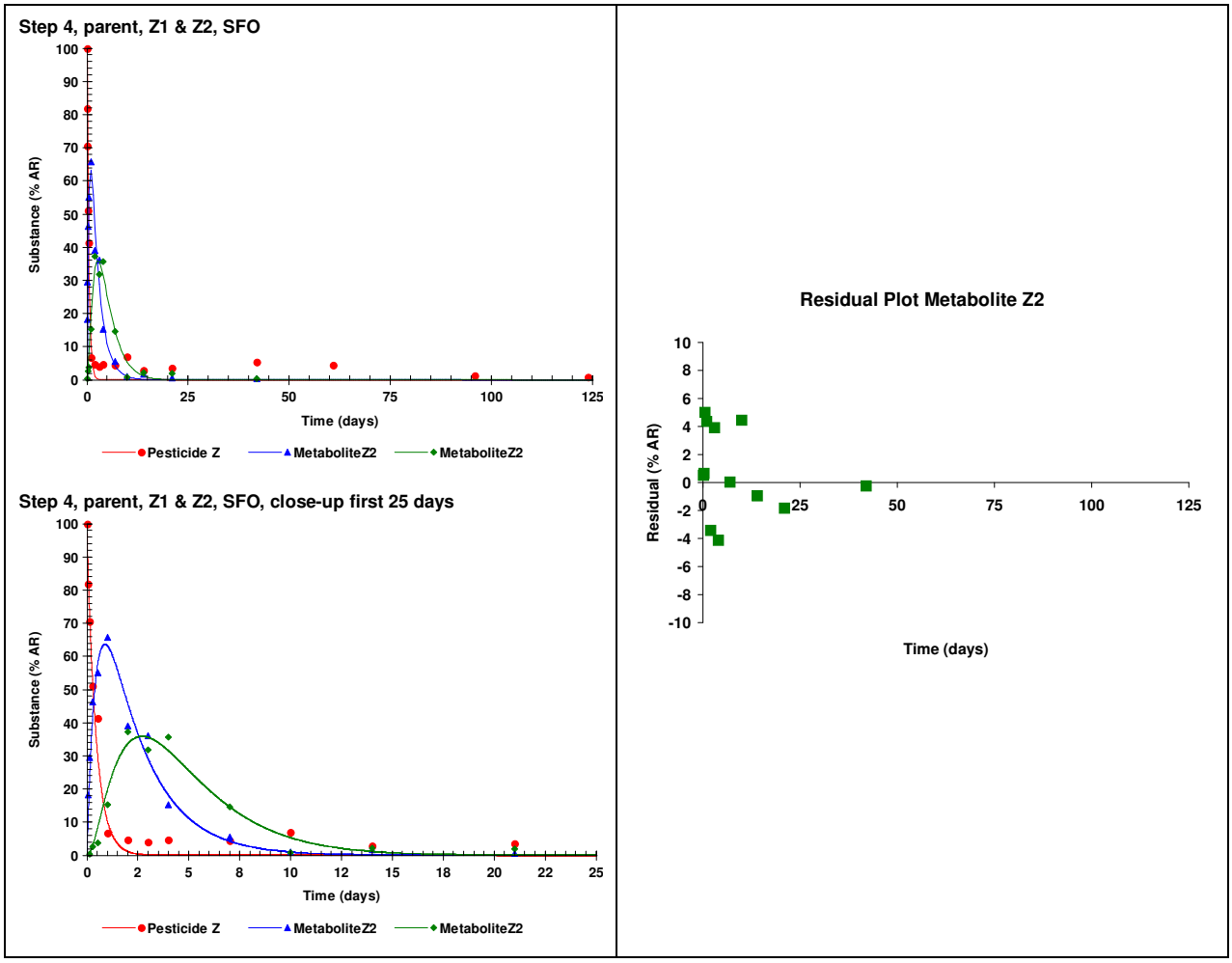


Figure A7-5. Description of the observed data for metabolite Z2 with SFO kinetics, with parent and metabolite Z1 parameters fixed.

Table A7-7. Results of the fit of Met Z2 in step 4b.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	96.99 (fixed)	-
kP (d^{-1})	2.231 (fixed)	-
kM1 (d^{-1})	0.4816 (fixed)	-
kM2 (d^{-1})	0.1	0.4505 ± 0.0462
Goodness of fit (χ^2 error)		
χ^2 error Met Z2	20	

***Step 5**: Parent, Met Z1 (100% formation), and Met Z2 (100% formation), all parameters optimised*

All parameters for Pesticide Z, Metabolite Z1 and Metabolite Z2 are optimised in this step, using the estimates from step 3 as starting values for the parent and metabolite Z1 parameters, and from step 4b for Metabolite Z2. The results of the fitting of the data with the model are shown in Table A7-8 and Figure A7-6. The fits of pesticide Z, Met Z1 and Met Z2 all pass the χ^2 test with error levels ≤ 20%, and the plots of residuals show acceptable fits. The standard error of the parameter estimates is sufficiently low to assure that these are reliable. The estimated rate constant can be used as starting value in step 6 with the last metabolite Z3 added.

Table A7-8. Results of the fit of pesticide Z, Met Z1 and Met Z2 in step 5.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	96.99	96.74 ± 2.33
kP (d^{-1})	2.231	2.207 ± 0.133
kM1 (d^{-1})	0.4816	0.4759 ± 0.0335
kM2 (d^{-1})	0.4505	0.4478 ± 0.0513
Goodness of fit (χ^2 error)		
parent (121 days)	18	
Met Z1	16	
Met Z2	20	

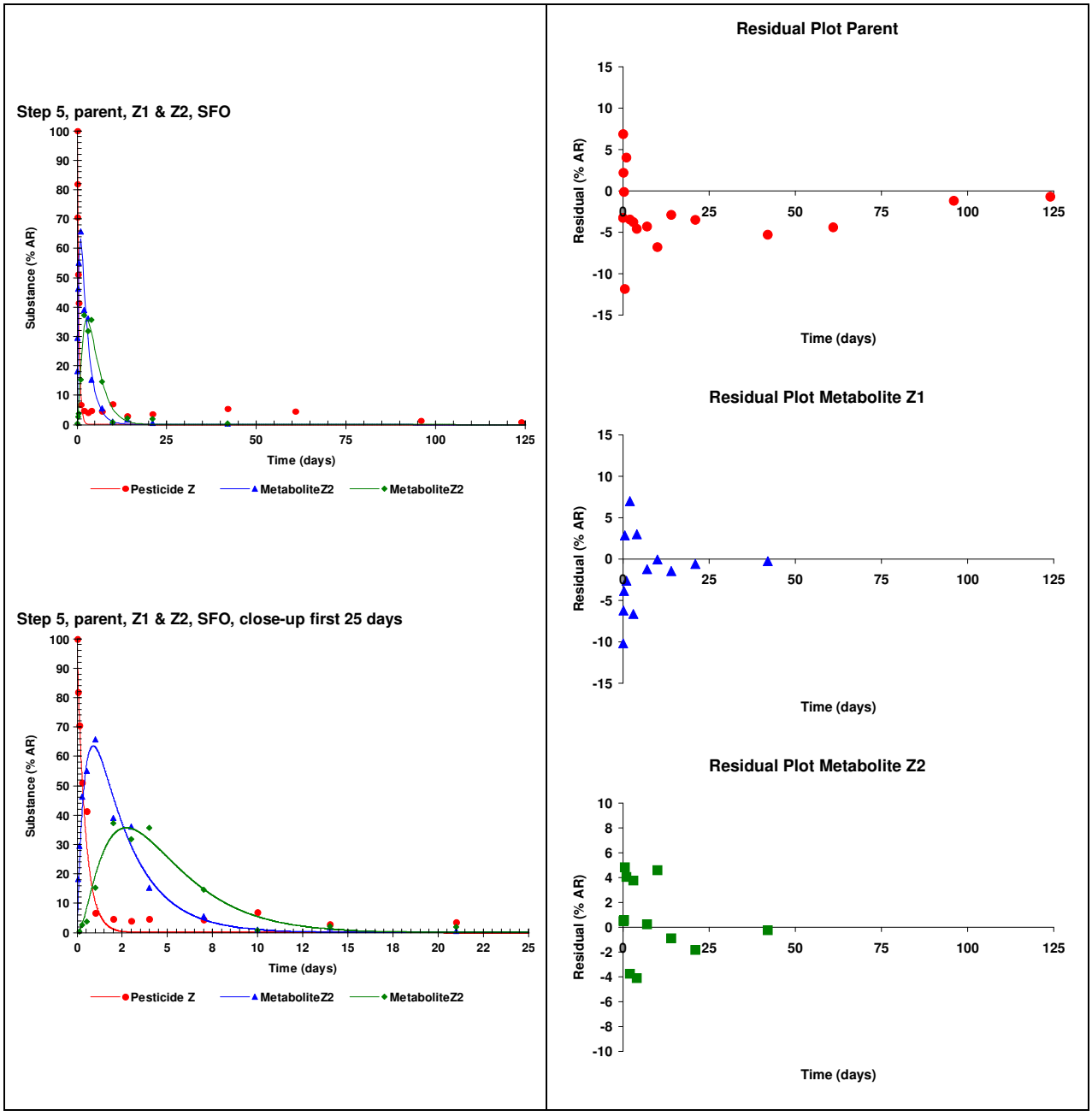


Figure A7-6. Description of the observed data for pesticide Z, metabolite Z1 and metabolite Z2 with SFO kinetics, all parameters optimized.

***Step 6**: Parent, Met Z1 (100% formation), Met Z2 (100% formation), and Met Z3, with parent, Met Z1 and Met Z2 parameters fixed*

The third and last metabolite in the estimation procedure, Met Z3 is added to the model. At this step, Met Z2 is assumed to degrade to Met Z3 and to other unidentified metabolites or bound residues. The results of the fitting of the data with the model are shown in Table A7-9 and Figure A7-7. The fit of Met Z3 passes the χ^2 test at a low error level of 13%, indicating a

good fit of the data, but the plot of residuals shows a systematic error at the later time points. The tailing is assumed to be attributed to experimental artefacts, and that no such tailing of Met Z3 has been observed in other soils. The standard error of the parameter estimates for metabolite Z3 is sufficiently low to assure that these are reliable. Apart from the apparent tailing in the last three sampling points, the overall pattern of formation and decline of metabolite Z3 is described very well by the model, and the SFO fit of the metabolite is deemed acceptable. The estimated rate constant can be used as starting value in the final step.

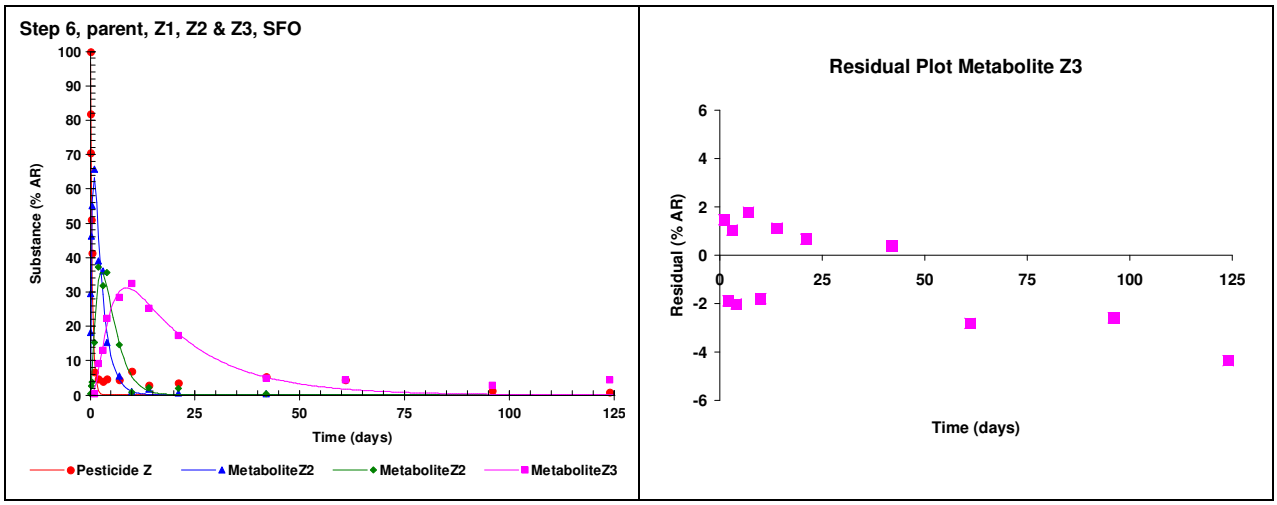


Figure A7-7. Description of the observed data for metabolite Z3 with SFO kinetics, with parent, metabolite Z1 and metabolite Z2 parameters fixed.

Table A7-9. Results of the fit of Met Z3 in step 6.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	96.74 (fixed)	-
kP (d^{-1})	2.207 (fixed)	-
kM1 (d^{-1})	0.4759 (fixed)	-
kM2 (d^{-1})	0.4478 (fixed)	-
ffM3	0.5	0.4724 ± 0.0501
kM3 (d^{-1})	0.1	0.0591 ± 0.0136
Goodness of fit (χ^2 error)		
χ^2 error Met Z3	13	

***Final step**: Parent, Met Z1 (100% formation), Met Z2 (100% formation), and Met Z3, with all parameters optimised*

In the final step of the parameter estimation procedure, all the model parameters for Pesticide Z, Metabolite Z1, Metabolite Z2 and Metabolite Z3 are optimised, using the estimates from step 5 as starting values for the parent, metabolite Z1 and metabolite Z2 parameters, and from step 6 for Metabolite Z3. The results of the fitting of the data with the model are shown in Table A7-10 and Figures A7-8 (fit of the experimental data) and A7-9 (plots of residuals). The fits of pesticide Z, Met Z1, Met Z2 and Met Z3 all pass the χ^2 test with error levels ≤ 20%, and the plots of residuals show acceptable fits. The standard errors of the parameter estimates are sufficiently low to assure that all the parameters are reliable and can be used as modelling endpoints.

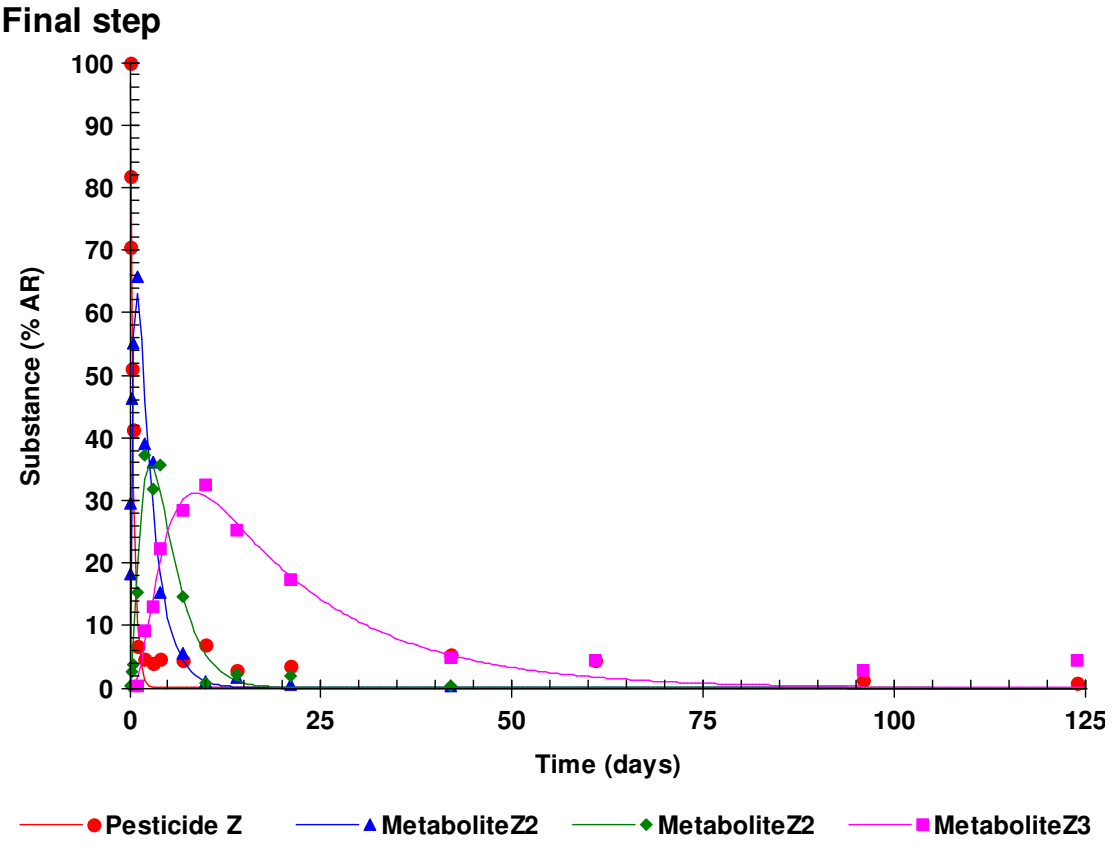


Figure A7-8. Description of the observed data for pesticide Z, metabolite Z1, metabolite Z2 and metabolite Z3 with SFO kinetics in the final fit with all parameters optimised.

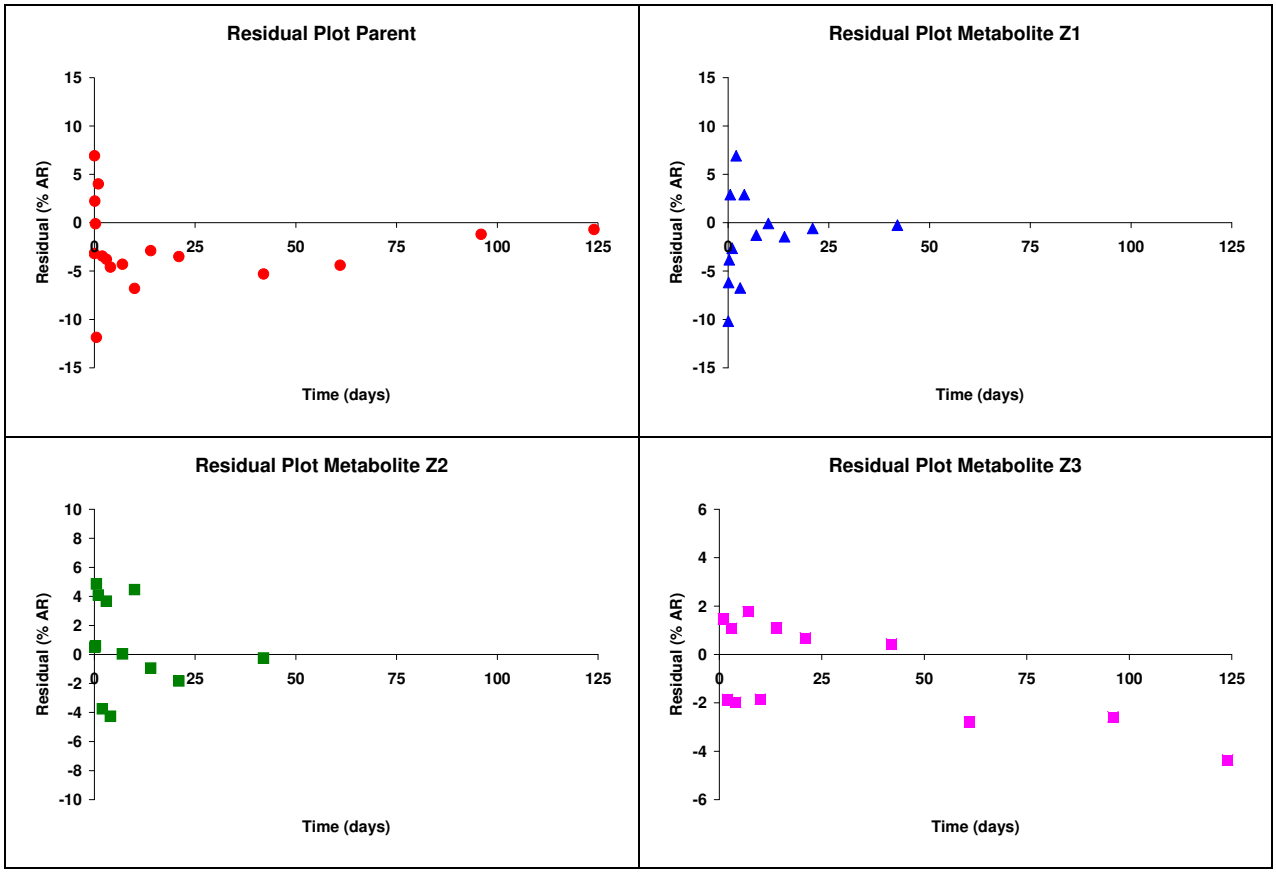


Figure A7-9. Plots of residuals for pesticide Z, metabolite Z1, metabolite Z2 and metabolite Z3 described with SFO kinetics in the final fit with all parameters optimized.

Table A7-10. Results of the final, simultaneous fit of pesticide Z, Met Z1, Met Z2 and Met Z3.

Model parameters		
	Starting value	Estimate ± standard error
Pini (% AR)	96.74	96.81 ± 2.12
kP (d^{-1})	2.207	2.209 ± 0.122
kM1 (d^{-1})	0.4759	0.4776 ± 0.0303
kM2 (d^{-1})	0.4479	0.4516 ± 0.0459
ffM3	0.4724	0.4716 ± 0.0588
kM3 (d^{-1})	0.0591	0.0587 ± 0.0148
Goodness of fit (χ^2)		
parent (121 days)	18	
Met Z1	16	
Met Z2	20	
Met Z3	13	

Conclusion

Reliable endpoints for modelling, formation fraction of the metabolites, rate constant parameters and corresponding calculated first-order DT50 values for pesticide Z, metabolite Z1, metabolite Z2, and metabolite Z3 are listed in Table A7-11.

Table A7-11. Modelling endpoints for pesticide Z, Met Z1, Met Z2 and Met Z3 for the example soil.

	Pesticide Z	Metabolite Z1	Metabolite Z2	Metabolite Z3
1^{st}-order rate constant (d^{-1})	2.207	0.4776	0.4516	0.0587
1^{st}-order DT50 (d)	0.314	1.45	1.53	11.8
Formation fraction (-)	-	1	1	0.4716

The stepwise approach is very helpful in determining which flows to the sink are relevant in the conceptual model. In this example, the flows from the parent and metabolite Z1 to the sink were eliminated and a simplified model could be built. In any case, the decision to remove flows or modify the conceptual model should be discussed based on the available information from laboratory or field studies on the behaviour of the substances of interest. All dissipation or degradation flows in the conceptual model must be realistic regarding the processes involved and should be justified accordingly. The fitting procedure for this complex model with four substances could be carried on successfully with the stepwise approach, using starting values for the parameters that were calculated in the previous steps.

APPENDIX 8: NORMALISATION OF FIELD DISSIPATION HALF-LIVES TO REFERENCE CONDITIONS

Method 1: Time-Step Normalisation

The normalisation procedure is carried out by reducing or increasing day lengths depending on soil temperature and moisture by means of correction factors identical to those used in most regulatory leaching models.

$$D_{Norm} = D \cdot f_{Temp} \qquad \text{(A8-1)}$$

$$f_{Temp} = Q_{10}^{(T_{act} - T_{ref})/10} \qquad \text{for } T_{act} > 0°C \qquad \text{(A8-2)}$$

$$f_{Temp} = 0 \qquad \text{for } T_{act} \leq 0°C$$

Where: D_{Norm} = Normalised day length

D = 1 d

f_{temp} = Correction factor for soil temperature

Q_{10} = 2.2 (FOCUS, 2000)

T_{act} = Actual soil temperature

T_{ref} = Reference soil temperature (e.g. 20 °C)

$$D_{Norm} = D \cdot f_{Moisture} \qquad \text{(A8-3)}$$

$$f_{Moisture} = \left(\frac{theta_{act}}{theta_{ref}} \right)^{0.7} \qquad \text{(A8-4)}$$

$$f_{Moisture} = 1 \qquad \text{for } theta_{act} \geq theta_{ref}$$

Where: D_{Norm} = Normalised day length

D = 1 d

$f_{moisture}$ = Correction factor for soil moisture

$theta_{act}$ = Actual soil moisture (v/v)

$theta_{ref}$ = Reference soil moisture (water content at pF2 = 100 % field capacity)

Combining the two:

$$D_{Norm} = D \cdot f_{Moisture} \cdot f_{Temp} \qquad \text{(A8-5)}$$

Cumulative corrected day lengths are calculated between each sampling interval to result in 'normalised' days after applications. The practical impact of the normalisation procedure is that days with an average soil temperature > 20 °C are longer whereas days with temperatures < 20 °C are shorter than reported days after application. Days with soil moisture contents less than the reference soil moisture will become shorter. The normalised day scale and residue data for parent compounds and metabolites may then be re-analysed to obtain kinetic parameters used in leaching modelling on the basis of field data. Note that the Q_{10} response function is only applied for temperatures above 0 °C. As a consequence it is assumed that no degradation occurs below 0 °C, i .e. D_{Norm} is set to 0.

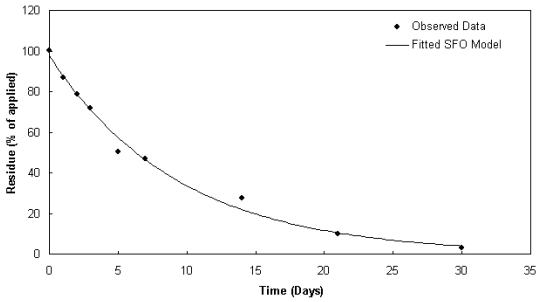
In cases where soil temperature data is not available, average daily soil temperatures may be estimated with suitable methods. Unlike soil temperature, soil moisture data are not readily available for many field soil dissipation experiments. A constant soil moisture of 100% FC during the study period may be used in a very conservative approach. For a more realistic assessment, average daily soil moisture contents may be estimated with predictive models.

Validity check

The first example illustrates the validity of the concept of normalised day lengths. A laboratory study conducted at 25°C resulted in the decline curve of the parent compound over a period of 30 days as shown in Figure A8-1.

The degradation clearly follows single first-order kinetics, the corresponding DT50 value is 6.5 days.

Days after appl.	Residues (% AR)
0	100.0
1	87.0
2	78.9
3	72.0
5	50.3
7	47.0
14	27.2
21	10.0
30	2.9



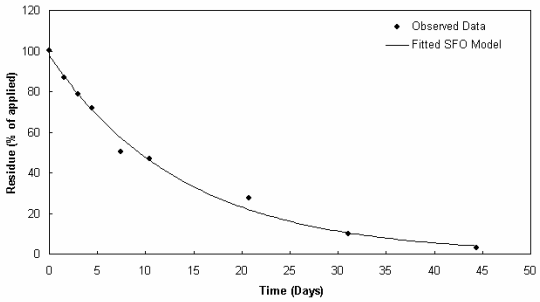
Study at 25℃

DT50 = 6.5 days

Figure A8-1. Laboratory degradation at 25 ℃.

A conventional normalisation to 20℃ results in a h alf-life of 9.6 days [6.5*(2.2^((25-20/10))]. When applying the conceptual approach which was described above, the day length for each day of the study is (2.2^((25-20/10))= 1.48 days. As a consequence the approach gives rise to normalised cumulative days after application as shown in Figure A8-2.

Days after appl.	Residues (% AR)
0	100.0
1.5	87.0
3.0	78.9
4.4	72.0
7.4	50.3
10.4	47.0
20.7	27.2
31.1	10.0
44.4	2.9



Study normalised to 20℃

DT50 = 9.6 days

Figure A8-2: Normalised laboratory degradation at 20 ℃.

Again, the degradation follows single first-order kinetics, the corresponding DT50 value is 9.6 days, identical to the value of the conventional approach. Therefore the normalisation of

day-lengths leads to the same result as the standardised normalisation of laboratory half-lives.

Method 2: Rate Constant Normalisation

A direct normalisation of degradation rates can be performed by incorporating the Q_{10} approach (see Equation A8-2) in a ModelMaker model or similar software tool. During the kinetic fitting procedure, ModelMaker accounts for daily temperature variations and thus provides a first-order field dissipation half-life at 20 °C.

Daily degradation rates are corrected by means of a correction factor $f_{temp,}$ which is derived according to Equation A8-6. Multiplying the fitted degradation rate at reference temperature (k_{Tref}) with the respective correction factor (Equation A8-6) eventually yields the degradation rate (k_{Tact}) at actual temperatures. Again degradation is assumed to occur only at temperatures > 0 °C.

$$f_{temp} = 0 \qquad \text{for } T_{act} \leq 0\ ^\circ C$$

$$f_{temp} = Q_{10}^{\frac{Tact - Tref}{10}} \qquad \text{for } T_{act} > 0\ ^\circ C \qquad \text{(A8- 6)}$$

$$k_{Tact} = k_{Tref} \cdot f_{temp}$$

Where f_{temp}	= Temperature correction factor		[-]
k_{Tact}	= Degradation rate constant at actual temperature T		[1/d]
k_{Tref}	= Degradation rate constant at a reference temperature T_{ref}		[1/d]
T_{act}	= Actual temperature		[°C]
T_{ref}	= Reference temperature (20 °C)		[°C]
Q_{10}	= Factor of increase of degradation rate with an increase in temperature of 10°C (Q_{10} = 2.2, FOCUS recommendation)		[-]

As with method 1, all temperatures refer to soil temperatures. In cases where the respective soil temperatures are not available, these may be estimated with a suitable tool.

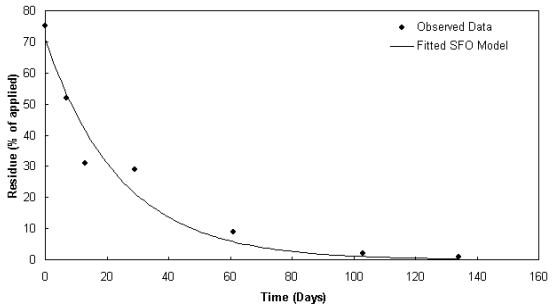
Soil moisture content may also be used during the optimisation procedure using equation A8-4 to derive a moisture correction factor, which can be combined as follows:

$$k_{Tact} = k_{Tref} \cdot f_{temp} \cdot f_{moisture} \qquad \text{(A8-7)}$$

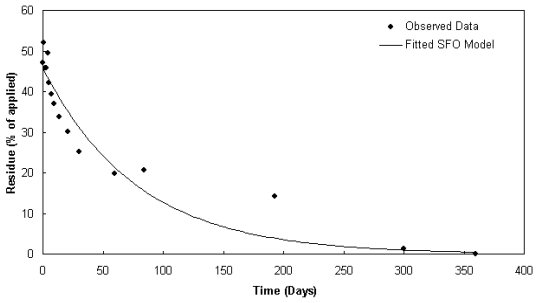
Unlike standard first-order fits, the inclusion of daily temperature fluctuations does not result in smooth curves. The slope of the dissipation curves usually flattens during cooler periods whereas higher temperatures lead to a more pronounced slope. In this way the curve reflects realistically the effect of the temperature fluctuations during the study with higher degradation rates in warmer periods and lower degradation rates in cooler periods.

Comparison of method 1 and 2

In principle, methods 1 and 2 should lead to identical results since both methods are based on the same conceptual approach, i.e. the Q10 relationship. To illustrate the inherent similarity of both methods two field dissipation studies are normalised to 20 °C using method 1 and 2. The first study represents a spring application whilst the second trial was initiated in autumn. In both cases soil moisture was assumed to be constant at 100 % FC, so that effectively no soil moisture correction was made. The uncorrected field dissipation half-life for the spring trial is 17 days, for the autumn trial the uncorrected half-life is 54 days.



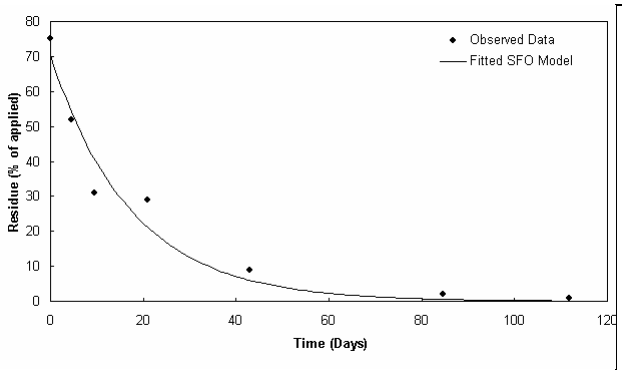
Spring application
(uncorrected half-life 17 d)



Autumn application
(uncorrected half-life 54 d)

Figure A8-3: Example data sets.

Days after appl.		Residues (% AR)
Reported	at 20 °C	
0	0.0	75
7	4.5	52
13	9.4	31
29	21.0	29
61	42.9	9
103	84.5	2
134	111.9	1

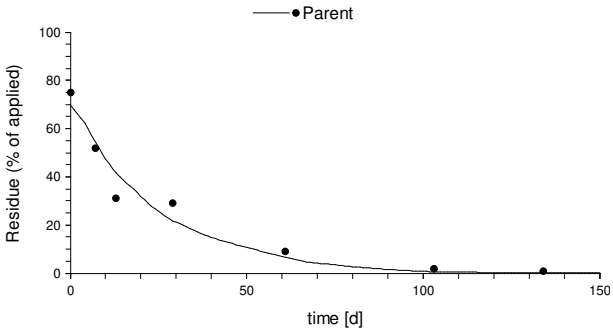


Day-length normalisation

Study normalised to 20°C

DT50 = 12.1 days

Days after appl.		Residues (% AR)
Reported	at 20 °C	
0	-	75
7	-	52
13	-	31
29	-	29
61	-	9
103	-	2
134	-	1



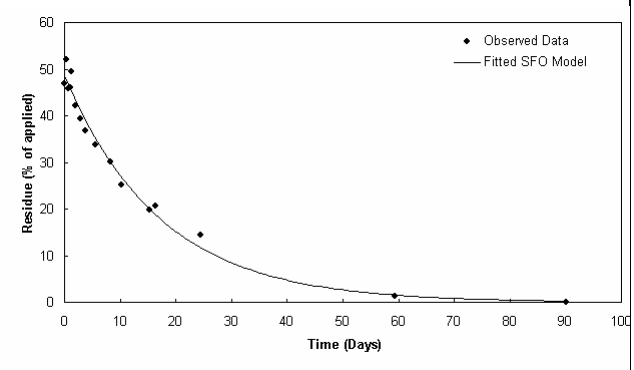
Rate normalisation

Study normalised to 20°C

DT50 = 12.5 days

Figure A8-4: Normalised field dissipation half-life at 20 °C following spring application.

Days after appl.		Residues (% AR)
Reported	at 20 °C	
0	0.0	47.0
1	0.3	52.1
2	0.7	45.8
3	1.0	45.9
4	1.3	49.4
5	1.9	42.2
7	2.9	39.3
9	3.7	36.9
14	5.5	33.7
21	8.3	30.1
30	10.3	25.1
60	15.2	19.8
84	16.4	20.6
193	24.4	14.3
300	59.3	1.3
360	90.1	0.0

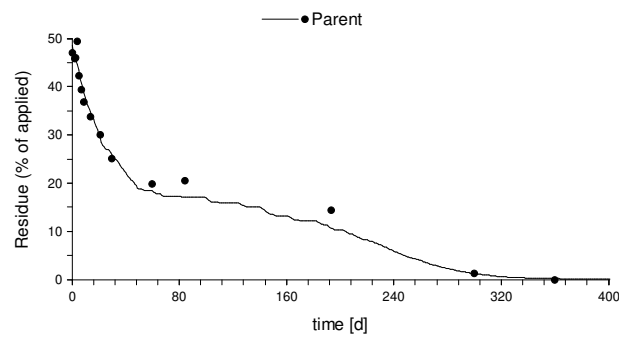


Day-length normalisation

<u>Study normalised to 20°C</u>

DT50 = 11.9 days

Days after appl.		Residues (% AR)
Reported	at 20 °C	
0	-	47.0
1	-	52.1
2	-	45.8
3	-	45.9
4	-	49.4
5	-	42.2
7	-	39.3
9	-	36.9
14	-	33.7
21	-	30.1
30	-	25.1
60	-	19.8
84	-	20.6
193	-	14.3
300	-	1.3
360	-	0.0



Rate normalisation

<u>Study normalised to 20°C</u>

DT50 = 11.3 days

Figure A8-5: Normalised field dissipation half-life at 20 °C following autumn application.

Conclusion

The normalisation of field dissipation half-lives to reference conditions was carried out for two field dissipation trials, which represent autumn and spring applications. Both the daily correction of degradation rates as well as the correction of day-lengths yield similar half-lives, though small differences remain. These differences are however very small and seem to be within the numerical accuracy of kinetic fitting programs. Therefore, both methods result in equally valid results.

The example in this chapter demonstrates the usefulness of the normalisation process. The unnormalised autumn half-lives were more than a factor of three higher that the half-lives from spring applications. However, there was little difference between the normalised half-lives from the two application periods, suggesting that the effect of climatic conditions on the degradation rate is well described by the normalisation process.

APPENDIX 9: REPRESENTATION AND FITTING OF TRANSFER BETWEEN THE WATER COLUMN AND SEDIMENT BY REVERSIBLE FIRST-ORDER KINETICS

The purpose of this appendix was to provide:

- An example illustrating that transfer between the water column and sediment can be represented approximately by reversible first-order kinetics
- Methods for calculating the fraction of a substance in sediment when transfer is at equilibrium (*Fsed*) in order to assess and/or constrain fits
- An attempt to use theoretical *Fsed* values to constrain the fitting of transfer to the data and the implications.

Approximate Representation of Transfer

Transfer between the water column and sediment is mainly driven by molecular diffusion, with adsorption limiting the rate of diffusion into the sediment. Hydrodynamic dispersion may also significantly influence this transfer and make it difficult to estimate *a priori*. For example, small eddy currents in the water column (due to gentle stirring or agitation to keep the water column well mixed) can result in faster transfer. Thus, the transfer processes may need to be estimated *a posteriori* instead to determine the apparent or effective value of this coefficient. However, this requires information on concentration gradients in sediment that are not generally available for water-sediment studies.

The work group therefore decided to try using reversible first-order kinetics as an approximate method of representing transfer. This approximation was fitted to the data of a water-sediment study run under conditions in which degradation did not occur, to see if could fit the empirical pattern of transfer. The results shown below in Figure A9-1 indicate that this approximation fits transfer quite closely under these conditions. Therefore, the work group decided that this approximate representation of transfer was acceptable as a first step.

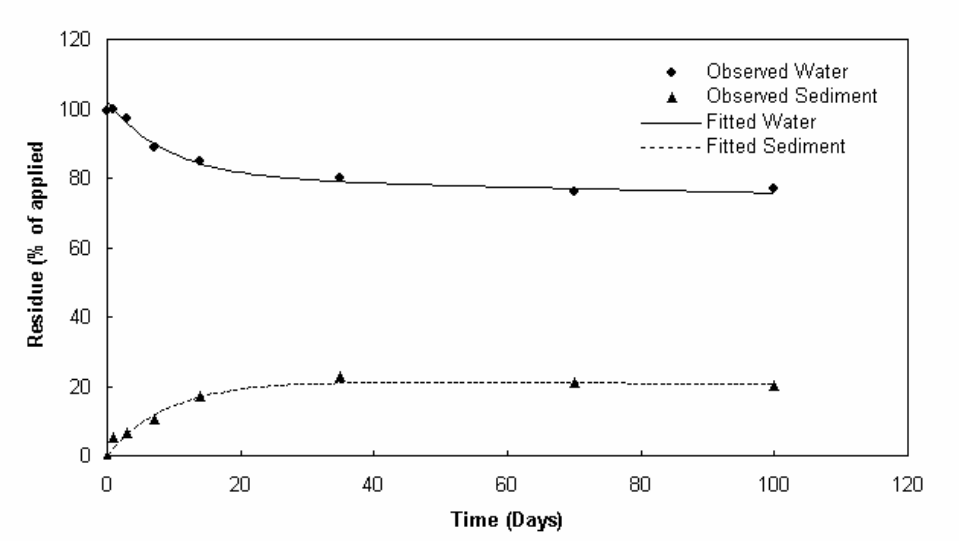


Figure A9-1. Fit of reversible first-order transfer to data for a moderately sorbing compound from a water-sediment study without degradation.

Methods for Calculating *Fsed* Values

The fraction of pesticide that transfers into the sediment at equilibrium (*Fsed*) can be calculated using Level P-II model parameters, using measurements from water-sediment studies run under conditions in which degradation did not occur, and using theoretical considerations of partitioning into sediments.

Modelled *Fsed* values from fitting the Level P-II kinetic model are a simple function of the transfer coefficients:

$$Fsed = r_{w\text{-}s} / (r_{w\text{-}s} + r_{s\text{-}w}) \qquad \text{(A9-1)}$$

Modelled values of *Fsed* should be related to the strength of pesticide adsorption to the sediment. However, in fitting the Level P-II kinetic model to a number of data sets, the *Fsed* values were sometimes thought to be physically implausible. While this is not a kinetic endpoint, incorrect transfer coefficients can have a significant impact on the estimated degradation rates in the water column and the sediment. In general, if modelled values of *Fsed* are over-estimated, this results in the water column degradation rate being under-estimated and the sediment degradation rate being over-estimated. And if modelled values of *Fsed* are under-estimated, this results in the reverse situation: water column degradation rate being over-estimated and the sediment degradation rate being under-estimated. Consequently, if the modelled values of *Fsed* are physically implausible, the degradation rates are also likely to be unrealistically fast or slow. The work group therefore decided to

examine how to assess and constrain modelled *Fsed* values, to confirm/ensure that estimated degradation rates are in a realistic range.

First, the modelled *Fsed* values could be checked against measured *Fsed* values, if a water-sediment study has also been conducted under conditions in which degradation does not occur, once equilibrium has been reached as shown above in Figure A9-1. However, such information will not always be available or possible, if it was not part of the study design or if the compound is subject to abiotic degradation. Thus, the work group considered whether it was possible to calculate theoretical *Fsed* values instead, using a combination of water-sediment system and pesticide properties.

Several starting assumptions had to be made in order to calculate theoretical *Fsed* values from the fundamental statement of mass balance that must be met, namely that:

$$Fsed = (Csed \times Vsed) / [(Cwc \times Vwc) + (Csed \times Vsed)] \quad \text{(A9-2)}$$

Where:

Csed is the total mass of pesticide in the sediment divided by the total volume of sediment

Vsed is the total volume of sediment

Cwc is the total mass of pesticide in the water column divided by the total volume of the water column

Vwc is the total volume of water column

First, if pesticide in the water column is assumed to be only in the liquid phase, i.e. there is no particulate matter in the water column, then

$$Cwc = C_l \quad \text{(A9-3)}$$

Where

C_l is the concentration of the pesticide in the liquid phase of the water column

Secondly, that at equilibrium, the concentration of the pesticide in the liquid phase of the sediment is the same concentration as that in the water column, then equation A9-2 can be re-written in terms of concentration in liquid phase and that adsorbed to sediment:

$$Fsed = (C_s \rho_b + C_l \theta)\ Vsed / [C_l Vwc + (C_s \rho_b + C_l \theta)\ Vsed] \quad \text{(A9-4)}$$

Where

C_s is the concentration of the pesticide adsorbed to the sediment

ρ_b is the dry bulk density of the sediment

θ is the volumetric water content of the sediment

Equation A9-2 can be re-written yet further since at equilibrium, the ratio of C_s over C_l is the adsorption coefficient, *Kd*:

$$Kd = C_s / C_l \qquad \text{(A9-5)}$$

Hence, the substitution of equation A1.9-5 into equation A9-4 and subsequent re-arrangement yields:

$$Fsed = (Kd\ \rho_b + \theta) / [(Vwc / Vsed) + (Kd\ \rho_b + \theta)] \qquad \text{(A9-6)}$$

Finally, in order to utilise equation A9-6 requires that the *Kd* of the compound is known, plus θ, ρ_b and ρ_d (the average particle density of the sediment). The value of *Kd* can either be measured directly as part of a water-sediment study, or estimated from the standard equation:

$$Kd = (Koc \times \%OC) / 100 \qquad \text{(A9-7)}$$

Where

Koc is the adsorption coefficient based on organic carbon

%OC is the percentage organic carbon

For agreement with Appendix 12, equations (A12-1) and (A12-2) should be used to calculate the values for ρ_b and ρ_d (the average particle density of the sediment), respectively, using the percentage clay and organic matter for the sediment, and from which θ can be estimated using the equation:

$$\theta = 1 - (\rho_b / \rho_d) \qquad \text{(A9-8)}$$

Assessment of Modelled *Fsed* Values

In order to assess the modelled *Fsed* values, a comparison can be made with the theoretical *Fsed* values in equation A9-6. However, this equation may over-estimate the amount of

transfer to sediment, by assuming that all the sediment effectively participates in transfer. In particular, for highly sorbing compounds, transfer into the sediment may be limited to the first few millimetres of sediment during experimental time-scales. Hence, for practical implementation, it was thought better to use an *operationally-defined* theoretical *Fsed* value, based on the effective depths of movement into sediment over typical experimental time-scales. Hence equation A9-6 was modified, by replacing *Vwc* and *Vsed* by the terms *Zwc* and Z_D, respectively, as the height of the water column and effective depth of sediment.

$$Fsed = (Kd\ \rho_b + \theta) / [(Zwc / Z_D) + (Kd\ \rho_b + \theta)] \quad \text{(A9-9)}$$

As a starting point, the value of Z_D can be estimated using diffusion theory and some of the chemical and system properties from (*cf*. Nye and Tinker, 1977):

$$Z_D = \{(2\ D_L\ f\ t) / [1 + (Kd\ \rho_b/\theta)]\}^{0.5} \quad \text{(A9-10)}$$

Where

D_L is the diffusion coefficient in free solution

f is the tortuosity factor

t is the experimental time scale (100 days as a default)

This equation assumes that a constant concentration is present at the top of the sediment and that the sediment is semi-infinite in depth. Hence, it maybe somewhat imprecise in estimating penetration into the sediment. Simulations of several cases when degradation is occurring indicated that this equation can both under or over estimate penetration into the sediment.

In addition, to calculate a range of operationally-defined *Fsed* values to compare with modelled *Fsed* values, some uncertainty needs to be introduced into some of the terms in equation A9-9). In order to minimise the number of terms that are varied, only *Kd,* ρ_b, and Z_D are considered here, since:

- Kd measurements are normally available, although mostly for soils rather than sediments, and will vary significantly;
- ρ_b measurements can be estimated, although estimation methods are for soils rather than sediments, and will vary significantly;
- Z_D estimates can normally be made, and simulations indicated a significant lack of precision in these estimates;
- *f* and θ values are determined by ρ_b in conjunction with the particle density (ρ_s) of sediments, with the latter not exhibiting large variations; and

- D_L and *f* values can be determined generically, as 0.432 cm^2/day and $\theta^{0.5}$, respectively, as given in Nye and Tinker (1977), and are considered not to be the more dominant sources of uncertainty.

For *Kd* values, a suitable range of values appears to be from 2 times the mean *Kd* value to 0.5 times the mean *Kd* value. For ρ_b values, the recommended range of values is from 1.25 times the mean ρ_b value to 0.75 times the mean ρ_b value. A range from 2 times the mean Z_D value to 0.5 times the mean Z_D value was used, based on the results of the above simulations. This may be somewhat strict, but the variability from all three terms is expected to provide a suitable range of acceptable *Fsed* values when substituted into equation A9-9. Note that if Z_D exceeds the depth of the sediment, then Z_D should be set to the sediment depth, since it cannot diffuse below this lower boundary by definition.

Table A9-1 below gives some examples of theoretical *Fsed* values for a series of compounds from weakly sorbing to strongly sorbing in sediment comprising 10 percent clay and 10 percent organic matter (assuming in a mean bulk density of 1.42 and a particle density of 2.48). The envelope of acceptable *Fsed* values is quite small for strongly sorbing compounds and then rises to a maximum size for medium sorbing compounds before contracting again for weakly sorbing compounds. The acceptable *Fsed* values are mainly determined by uncertainty in *Kd* for highly sorbing compounds, by uncertainty in ρ_b for weakly sorbing compounds, and a combination of uncertainty in *Kd*, ρ_b, and Z_D for medium sorbing compounds.

Table A9-1. Mean and range of theoretical Fsed values typical for a range of compounds with a water column depth of 6 cm and a sediment depth of 2 cm.

Range of Theoretical Fsed Values	*Fsed* values for different mean *Kd* values				
	***Kd* = 0.1**	***Kd* = 1**	***Kd* = 10**	***Kd* = 100**	***Kd* = 1 000**
Mean	0.16	0.38	0.76	0.91	0.97
Lower Limit	0.11	0.27	0.47	0.74	0.90
Upper Limit	0.21	0.54	0.88	0.97	0.99

Constraining Modelled *Fsed* Values

An attempt was made to constrain the modelled *Fsed* values during fitting not to exceed the range of theoretical *Fsed* values, to obtain more realistic estimates of degradation rates. A very crude method of implementing this constraint procedure was used for a range of 6 parent compounds (14 data sets in total). The conclusion of this exercise was that

constraining transfer results in more realistic estimates of degradation rates, particularly those that are initially estimated to be zero. However, the crude method of implementing this constraint procedure does not justify its use. Further improvements could justify such a method in future, particularly by comparing the predicted effective depths of movement into sediment with that calculated from measured *Fsed* values. The latter can be calculated by re-arranging equation (A9-6) and using measured *Fsed* values from water-sediment studies that are run under conditions in which degradation does not occur.

Conclusions

The first conclusion of this appendix is that the theoretical *Fsed* values can be used to assess whether modelled *Fsed* values are acceptable, and thus that the estimated degradation rates have an acceptable reliability.

The second conclusion is that theoretical *Fsed* values cannot be used at present to constrain modelled *Fsed* values to an acceptable range. In the absence of its implementation, the work group decided to adopt the default approach outlined in Figure 10-3 of Chapter 10.

Reference

Nye, P. H., and Tinker, P. B. 1977. Solute movement in the soil-root system. Studies in Ecology Volume 4. Blackwell Scientific Publications

APPENDIX 10: DERIVATION OF MODELLING ENDPOINTS, PARTICULARLY WHEN NO DEGRADATION APPEARS TO OCCUR IN THE WATER COLUMN OR SEDIMENT

The Problem

Deriving modelling endpoints can be problematic when degradation does not appear to occur in the water column or sediment when fitting the Level P-II model to the data (unless it can be shown with other data that this is realistic for the compound / dataset being evaluated). A potential problem arises because the parameters are not independent, and so the degradation rate in the degrading compartment maybe over-estimated since to compensate lack of for degradation in the other compartment. If these degradation rates are used as modelling endpoints, then the PEC values may be too high in the compartment with no apparent degradation, but too low in the other compartment. In practice, this is unlikely to cause significant differences in the calculated PEC values using FOCUS surface water scenarios as long as the overall fits to the water column and sediment are good, due to the system balancing itself and the upscaling and residence time effects of the FOCUS surface water bodies. The following analysis of the problem was conducted, to ensure modelling endpoints can be derived that will not result in PEC values that are too low for the compartment in which the degradation rate is apparently over-estimated.

Potential Approaches to Solve the Problem

The work group's first approach (termed Tier 2A in this appendix) to derive modelling endpoints was to use the system half-life for the compartment in which degradation appeared to occur, together with worst-case default half-life value of 1000 days for the compartment with no apparent degradation:

This approach was considered to be conservative, since the system half-life would be longer than the estimated half-life for that compartment. However, there was no consensus that this approach would always be conservative, since the system half-life could be shorter than the actual half-life for the compartment in which all the degradation appears to occur. Whilst this is theoretically possible, in practice it is unlikely to have a significant impact on the calculated PEC values. In cases where the slower degradation in one compartment can be confirmed / justified, Tier 2A will clearly provide a conservative assessment. For example, degradation data from an anaerobic soil study may be useful in showing that k_s approaching zero is a best approximation, or substantial degradation being found in a water hydrolysis study would support that degradation in the water column is likely to dominate and that k_s approaching zero is a realistic estimate.

The work group's second approach (termed Tier 2B in this appendix) to derive modelling endpoints was the reverse of Tier 2A. That is, to use the worst-case default half-life value of 1000 days for the compartment in which all the degradation appeared to occur, but to counter-balance this by using the system half-life for the compartment with no apparent degradation. This additional approach was considered as a conservative way to address the concern over Tier 2A, but in itself can cause problems with the data.

However, the consequence of using both approaches is that risk assessments would always need to be run with two sets of degradation inputs for FOCUS Step 3 and above. Such a consequence is undesirable due to the additional effort required and potentially unrealistically conservative. Some examples of simulated and actual data sets were thus used to determine whether:

- The use of both approaches was necessary at all; and
- There was a simple way to eliminate the use of one approach

Examples Based on Simulated Data Sets

Data sets were simulated using the Level P-II model with the degradation half-lives set using two cases[16]:

- Case 1: 25 days in the water column and 10 000 days in the sediment; or
- Case 2: 1000 days in the water column and 25 days in the sediment

The simulated data sets were intended to approximate a wide range of situations that could potentially occur, by using different fractions of substance that would transfer into sediment when transfer reaches equilibrium. This fraction is called the theoretical equilibrium *Fsed* value and is described in detail in Appendix 9. Three *Fsed* values (0.20, 0.70 and 0.90 occurring in the sediment) were used to provide a preliminary examination of the Tier 2A and Tier 2B approaches.

In order to simulate the data sets for the different *Fsed* values, first the value of one of the two transfer parameters ($r_{w\text{-}s}$ and $r_{s\text{-}w}$) on which *Fsed* values depend (*cf*. Appendix 9) had to

[16] The simulated data sets were therefore identical to the initial Level P-II model fit. All of the cases in this appendix were run when different default values were being considered for water and sediment. The same default value of 1000 days is currently being considered for water and sediment. However, changing the default value from 10 000 days to 1000 days would have essentially no impact on the results.

be fixed. The most logical choice was to set the value of $r_{w\text{-}s}$, so the value of $r_{s\text{-}w}$ was calculated by re-arranging the equation given in Appendix 9 to:

$$r_{s\text{-}w} = r_{w\text{-}s} \, (1/Fsed - 1)$$

The value of $r_{w\text{-}s}$ increased as *Fsed* increased to represent more rapid initial transfer to sediment for more strongly sorbing compounds. This was based on an observed trend in fitted $r_{w\text{-}s}$ values using actual data sets with the Level P-II model. Various aspects of dissipation for the simulated water-sediment data are summarised on Table A10-1. Note that only the first-order *degradation* rates for the water column and the sediment are fixed and that the *dissipation* rates for the water column and *degradation* rates for the total system (neither are first-order) are derived from the simulation. For these examples, the $r_{w\text{-}s}$ values were assumed to be 2.77, 0.277 and 0.0277 for the high, medium and low Fsed hypothetical compounds, resulting in $r_{s\text{-}w}$ values of 0.308, 0.119, and 0.111, respectively.

Table A10-1. Summary of various aspects of dissipation for the simulated data sets.

Case	DegT50 (days)		DT50 / DT90 (days) wc			DegT50 / DegT90 (days) system		
	wc	sed	*Fsed* = 0.20	*Fsed* = 0.70	*Fsed* = 0.90	*Fsed* = 0.20	*Fsed* = 0.70	*Fsed* = 0.90
1	1000	25	100 / 400	3.1 / 62	0.26 / 1.9	140 / 440	39 / 120	28 / 93
2	25	10 000	19 / 93	2.7 / 130	0.26 / 2.0	30 / 110	81 / 280	240 / 820

The Tier 2A and 2B approaches were thus tested using the default half-lives (1000 days in the water column or 10 000 days in sediment), together with the system half-lives in the other compartment (calculated as the system DT90 / 3.32)

The results of the Tier 2A and Tier 2B approaches can be seen in Figures A10-1 to A10-3 (shown as the “fitted model” by fixing degradation parameters to the Tier 2A or Tier 2B approach and the transfer parameters to those used in the initial Level P-II fit). Amounts in the water column or sediment calculated with the initial assumptions for DegT50 (Case 1 = 1000 days in water and 25 days in sediment; Case 2 = 25 days in water and 1000 days in sediment) are shown as symbols and are referred to as “observed “ in Figures A10-1 to A10-3. Several conclusions can be drawn from these results:

- Both the approaches are always conservative compared to the “observed” data, if albeit ranging from modelled values very close to the “observed” data to modelled values much higher than the “observed” data (this depends upon the sensitivity to whether degradation occurs primarily in the water column or the sediment).

- One of the approaches (Tier 2A or the Tier 2B) appears to be more conservative for both the water column and sediment compartments, rather than one approach for the water column and the other approach for the sediment. Hence, modelling endpoints

may be derived by running the Level P-II model with the Tier 2A and 2B options, to determine which is the more conservative.

- The Tier 2B approach appears to be more conservative than Tier 2A for:

    - Case 1-type compounds (no apparent degradation in the water column) at higher *Fsed* values, particularly for the sediment, since the Tier 2B approach switches the lack of degradation to the sediment where the major fraction transfers to after application. For the compounds here, the amount in the water column is less affected by this switch, since partitioning into sediment is the primary route dissipation from the water column; and
    - Case 2-type compounds (no apparent degradation in the sediment) at lower *Fsed* values, particularly for the water column, since the Tier 2B approach switches the lack of degradation to the water column where the major fraction remains after application. For the compounds here, the amount in the sediment is less affected by this switch, since partitioning is then primary process controlling the amount in sediment.

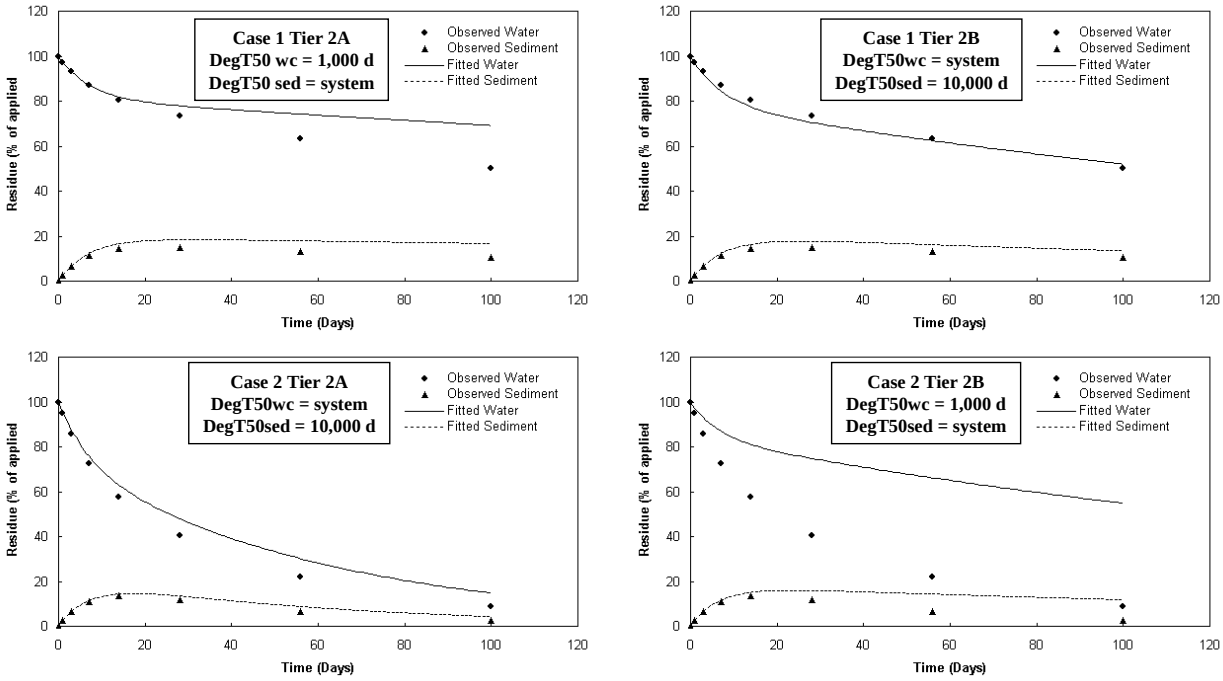


Figure A10-1. Comparison of Tier 2A and Tier 2B versus simulated data for Fsed = 0.20.

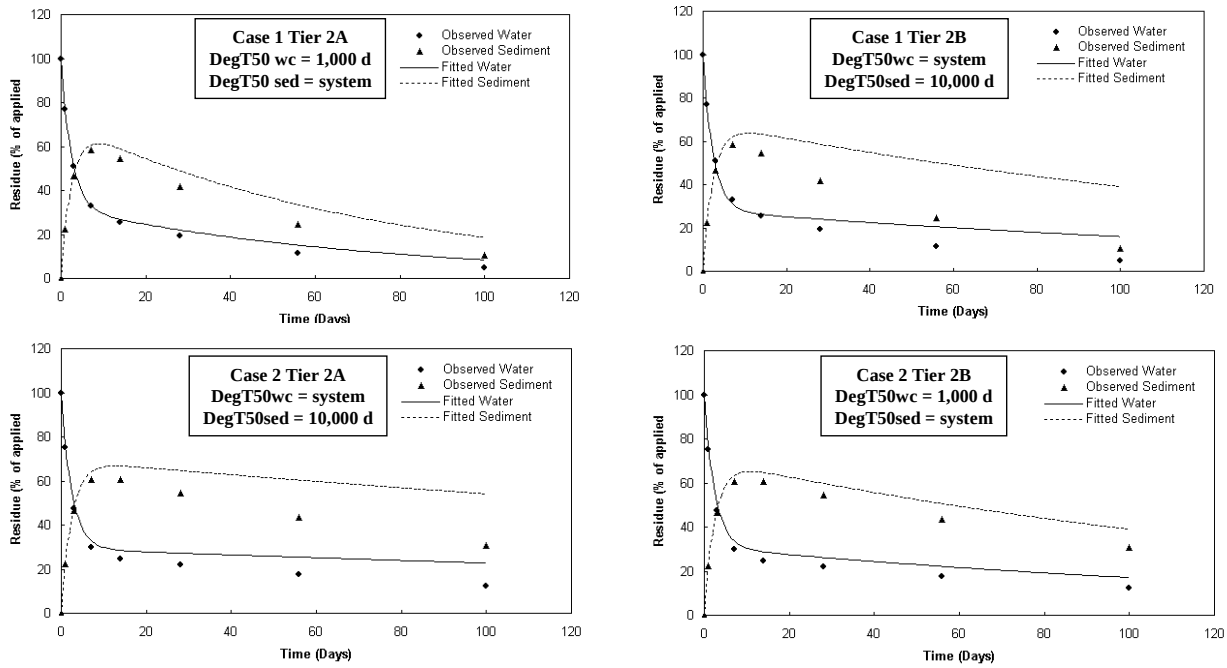


Figure A10-2. Comparison of Tier 2A and Tier 2B versus simulated data for Fsed = 0.70.

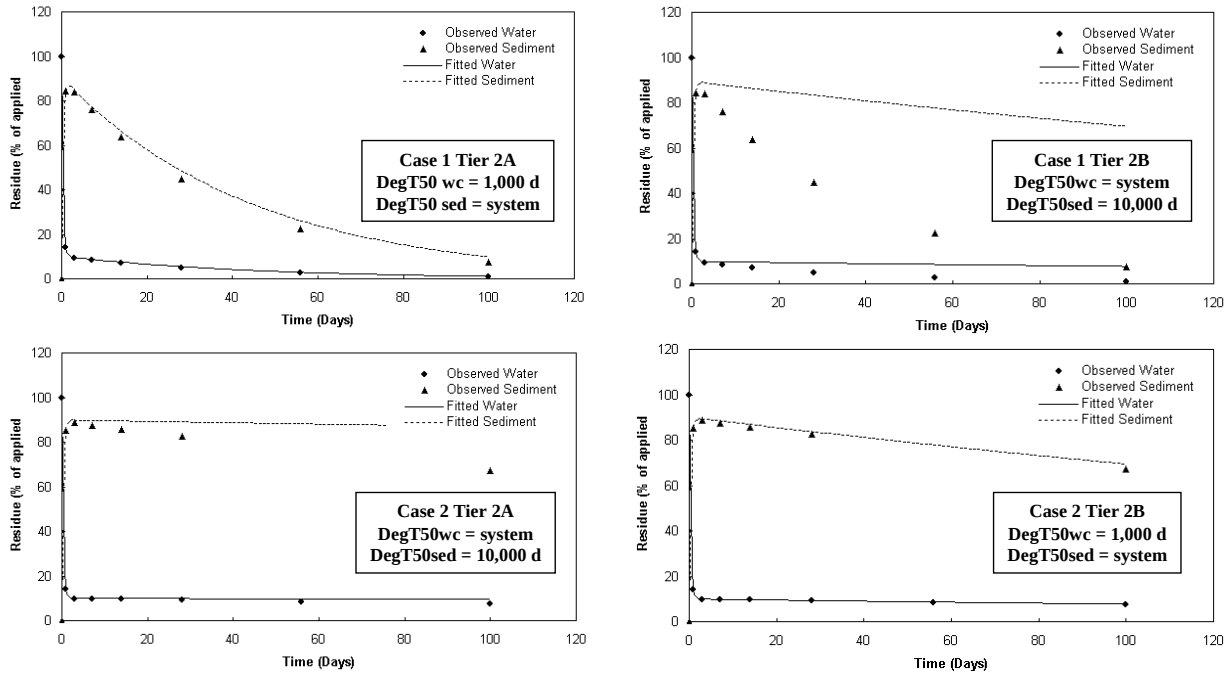


Figure A10-3. Comparison of Tier 2A and Tier 2B versus simulated data for Fsed = 0.90.

Examples Based on Actual Data Sets

After examining the simulated data sets, an exercise was conducted with some actual data sets to understand the implications better, by comparing the results from the initial Level P-II fit with those for Tier 2A and 2B using the Level P-II model and TOXSWA simulations. The aim was to get four data sets that covered a wide range of situations, namely:

- Two Case-1 type compounds (no apparent degradation in the water column): one weakly sorbing and the other strongly sorbing
- Two Case-2 type compounds (no apparent degradation in the sediment): one weakly sorbing and the other strongly sorbing

However, this exercise had to be restricted to three data sets, since no weakly sorbing Case-2 type compounds were found in the development and testing of Level P-II approach. Various aspects of dissipation from the initial fitting the Level P-II model to these data sets are summarised in Table A10-2 and shown graphically in Figure A10-4.

Table A10-2. Summary of various aspects of dissipation for the Level P-I and initial Level P-II fitting of three data sets.

Compound	Koc	%OC	DegT50 (days)		DT50 / DT90 wc (days)	DegT50 / DegT90 system (days)
			wc	sed		
2	900	6.8	1000	33	11 / 59	54 / 150
3	76 000	5.4	3.0	10 000	0.27 / 14	6.6 / 22
6	50	2.0	1000	2.1	18 / 63	21 / 66

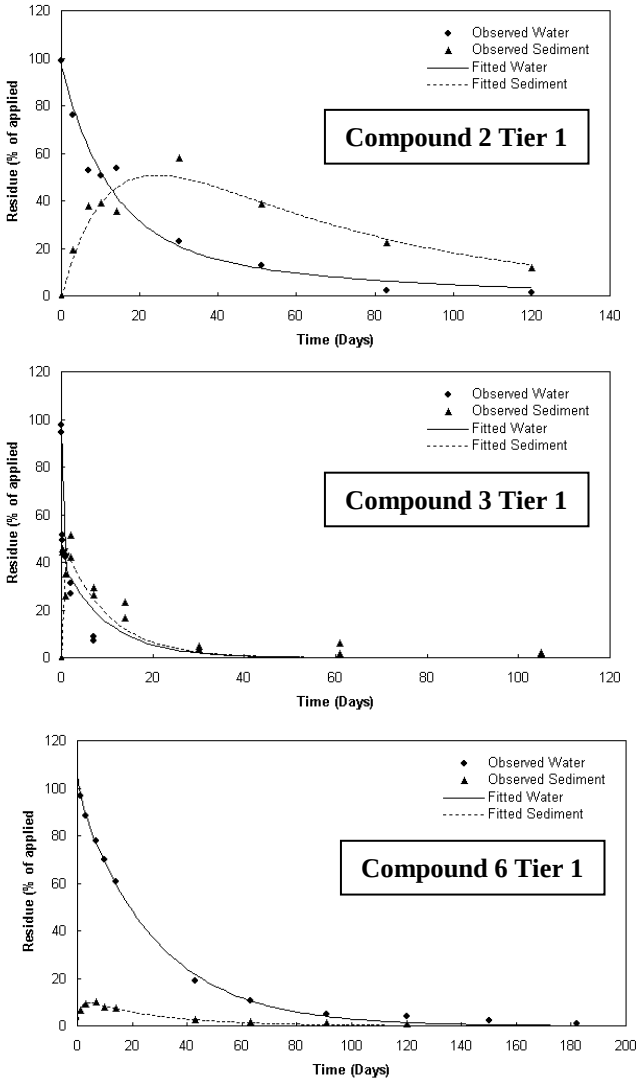


Figure A10-4. Initial Level P-II fits to the three data sets as indicated on the graphs

Level P-II model analysis

The implications of the Tier 2A and 2B approaches were first examined using the Level P-II model using the default half-lives (1000 days in the water column or 10 000 days in sediment), together with the system half-lives in the other compartment (calculated as the system DT90 / 3.32). The results of using the various combinations of default and system half-lives are shown in Figure A10-5, indicating that the Tier 2A and 2B approaches are more conservative as follows:

- Tier 2A for the strongly sorbing compound (Compound 3) with no apparent degradation in the sediment
- Tier 2A for the weakly sorbing compound (Compound 6) with no apparent degradation in the water column
- Tier 2B for the strongly sorbing compound (Compound 2) with no apparent degradation in the water column

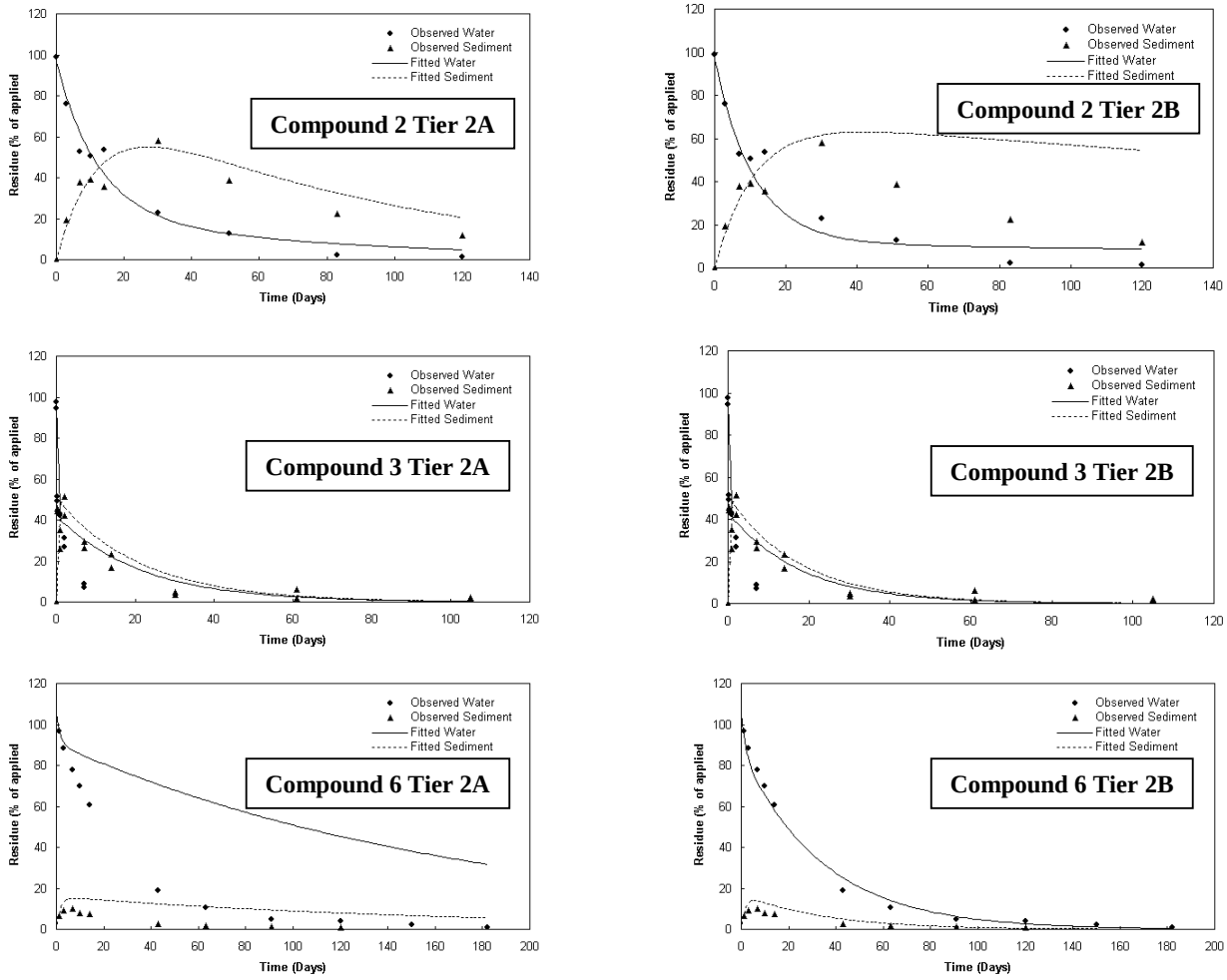


Figure A10-5. Tier 2A and 2B predictions ("fitted values") compared with the three data sets as indicated on the graphs

The conservatism of the Tier 2A assessments was also evaluated by determining the optimised DT50 value for the 'non-degrading' compartment when the 'degrading' compartment was fixed to the system DT50. Comparison of this value with the assumed default would then indicate how appreciably different the actual half-life would need to be for Tier 2A not to be conservative.

Compound 2: Fixing the sediment DT50 to the system DT50 of 54 days and refitting gives an optimised $DegT50_{wc}$ of 69 days. Therefore, for PECsed to be more conservative than at Tier 2A would require that the 'actual' $DT50_{wc}$ was <69 days rather than the default 1000 days.

Compound 3: Fixing the water column DT50 to the system DT50 of 6.6 days and refitting gives an optimised $DegT50_{sed}$ of 65 days. Therefore, for the PEC_{sw} to be more conservative

than at Tier 2A would require that the 'actual' $DT50_{sed}$ was <65 days rather than the default 10000 days.

Compound 6: Fixing the sediment DT50 to the system DT50 of 21 days and refitting gives an optimised DegT50wc of 21 days. Therefore, for the PEC_{sed} to be more conservative than at Tier 2A would require that the 'actual' $DT50_{wc}$ was <21 days rather than the default 1000 days.

TOXSWA analysis

To compare the effects of the Tier 2 assumptions on the final PEC_{sw} and PEC_{sed} values with the Tier 1 results, a number of TOXSWA simulations were run for the test compounds with ditch, stream and pond scenarios and either 1 or 2 applications.

Compound properties for the soil were kept constant for all evaluations (first order degradation rate corresponding to a half-life of 10 days), with spring applications at 1000 g/ha to a winter cereal crop (for the 2-application scenarios a minimum interval of 14 days was used). The D6 scenario was chosen for evaluation of the Ditch scenarios, with R1 being chosen for the pond and stream runs.

TOXSWA restricts half-life values to a maximum of 1000 days for both the water and sediment compartments and therefore, a maximum sediment half-life of 1000 days has been used in these evaluations where appropriate. This assumption has negligible impact on the final results, due to the short timescales of 100 days for PEC calculations and also the average residence times in the FOCUS water bodies (0.1, 5 and 150 days for the stream, ditch and pond scenarios respectively).

Tables A10-3 to A10-5 show a summary of the 2-application scenario runs for Compounds 2, 3 and 6 respectively (the multiple application scenario should, in theory, be a worst-case to identify any differences). The results show no major differences for the calculated PEC_{sw} values between the initial Level P-II fit and Tiers 2A & B. Results for the other remaining test compounds as well as the 1-application scenarios also show no significant effects. Calculated PEC_{sed} values showed more significant effects than found for PEC_{sw}, but in general still varied by less than 2.5-fold for all evaluations (initial Level P-II fit to Tiers 2A&B).

The results in Tables A10-4 and A10-5 show that for Compounds 3 and 6, Tier 2A always gave the highest PEC_{sed} and PEC_{sw} values. The results for Compound 2 in Table A10-3 were not as straightforward. Tier 2B always was always significantly higher for PEC_{sed} and

usually for PEC_{sw}. The exception was for PEC_{sw} values for 1-14 days where Tier 2A was up to ten percent higher than Tier 2B. This small difference means that the two approaches are essentially equivalent in this region given the uncertainty associated with the estimates.

Table A10-3. Summary of TOXSWA output for Compound 2 (Ditch 2 apps).

Time (days)	PEC_{sw} (µg/L)			PEC_{sed} (µg/kg)		
	Initial P-II	Tier 2A	Tier 2B	Initial P-II	Tier 2A	Tier 2B
0	5.64	5.64	5.65	10.65	11.20	12.23
1	5.17	5.17	5.11	10.53	11.08	12.13
2	4.86	4.87	4.73	10.22	10.79	11.88
4	4.23	4.24	4.00	9.30	9.91	11.15
7	2.61	2.63	2.40	7.79	8.47	10.00
14	0.50	0.52	0.49	5.22	5.98	8.10
21	0.13	0.15	0.16	3.73	4.51	7.00
28	0.06	0.07	0.09	2.77	3.53	6.28
42	0.02	0.02	0.04	1.60	2.28	5.34
50	0.01	0.01	0.03	1.19	1.81	4.96
100	0.00	0.00	0.01	0.22	0.49	3.60

Table A10-4. Summary of TOXSWA output for Compound 3 (Ditch 2 apps).

Time (days)	PEC_{sw} (µg/L)			PEC_{sed} (µg/kg)		
	Initial P-II	Tier 2A	Tier 2B	Initial P-II	Tier 2A	Tier 2B
0	5.09	5.23	5.10	19.32	44.78	25.51
1	3.63	2.23	2.20	19.30	44.64	24.91
2	2.47	1.66	1.63	19.25	44.29	23.61
4	1.08	1.14	1.05	19.13	43.27	20.24
7	0.22	0.78	0.60	18.91	41.47	15.06
14	0.02	0.41	0.16	18.42	37.47	6.62
21	0.02	0.26	0.05	17.95	34.22	2.72
28	0.02	0.18	0.01	17.48	31.64	1.10
42	0.01	0.11	0.00	16.60	27.71	0.15
50	0.01	0.09	0.00	16.13	26.00	0.05
100	0.01	0.03	0.00	13.57	19.38	0.00

Table A10-5. Summary of TOXSWA output for Compound 6 (Pond 2 apps).

Time (days)	PEC_{sw} (µg/L)			PEC_{sed} (µg/kg)		
	Initial P-II	Tier 2A	Tier 2B	Initial P-II	Tier 2A	Tier 2B
0	2.92	2.94	2.75	1.13	2.52	2.15
1	2.88	2.91	2.66	1.10	2.52	2.15
2	2.85	2.88	2.57	1.08	2.52	2.14
4	2.78	2.83	2.41	1.04	2.51	2.14
7	2.69	2.75	2.19	0.99	2.50	2.12
14	2.49	2.59	1.71	0.92	2.42	2.05
21	2.30	2.44	1.33	0.85	2.34	1.96
28	2.13	2.31	1.04	0.79	2.25	1.85
42	1.81	2.04	0.64	0.65	2.12	1.65
50	1.65	1.91	0.47	0.59	2.07	1.54
100	0.90	1.21	0.10	0.44	1.69	1.07

Conclusions

- The examples with simulated and actual data show that one of the two default approaches is generally more conservative with respect to comparisons of predicted versus measured values in the water-sediment approach initial Level P-II fit.
- When the compartment with the faster degradation rate can be identified, the default approach setting the faster degrading compartment to the Level P-1 system half-life and the slower degrading compartment to 1000 days is an appropriate default.
- When the compartment with the faster degradation rate cannot be determined, then two cases (system half-life in one compartment and a half-life of 1000 days in the other compartment) need to be evaluated. A comparison of the predictions will generally identify that one of the cases produces higher estimates of both the water and sediment values compared to the measured values in the water sediment study as confirmed in the majority of cases of a more detailed analysis of additional FOCUS scenarios simulated with TOXSWA. When discrepancies were found, they were small. Therefore, the approach appears to be of sufficient accuracy given the general uncertainty associated with PEC_{SW} calculations.

These conclusions have been used in the development of the flow chart for Level P-II (Figure 10-3 in Chapter 10).

APPENDIX 11: CORRECTION PROCEDURES TO ACCOUNT FOR DISSIPATION BY VOLATILISATION

Introduction

Chapter 10 on water-sediment studies notes that the degradation kinetics were valid for non-volatile compounds that only undergo losses from the water-sediment system by degradation. However, the kinetics can be validly applied to slightly volatile compounds, if volatile losses correction procedures are used to account for dissipation by volatilisation. These procedures are outlined here for parent compounds for SFO and FOMC kinetics, with some outlines of how they may be derived for HS and DFOP kinetics. The correction procedures for all these types of kinetics assume that the volatile losses of parent were adequately identified and quantified in the volatile trapping systems. Each time that these correction procedures are used, a justification should be made for their use. With metabolites, this approach may also apply, though a justification for its use must be made which accounts for when the metabolite is formed in an experiment.

Correction Procedures for SFO Kinetics

Conceptually, the correction procedures are most straightforwardly derived by considering a parent compound that is subject to an overall rate of loss from the water-sediment system by degradation and volatilisation, and that each loss process is described by SFO kinetics. In this case, there are three SFO rate constants that can be used to describe different aspects of the loss process: k_{TOT}, k_{VOL} and k_{DEG} for the total overall loss from the water-sediment system, and the losses by volatilisation and by degradation, respectively. Assuming that:

$$k_{TOT} = k_{VOL} + k_{DEG} \qquad \text{(A11-1)}$$

then k_{DEG} can be estimated simply from the difference between these two parameters by re-arranging the above equation.

The value of k_{TOT} that is estimated by fitting SFO kinetics to data for the amount of parent remaining in the water-sediment system, i.e., the standard fit to system data as described in Chapter 10.

The value for k_{VOL} can be estimated by fitting SFO kinetics to data for the amount of volatile losses of parent from the water-sediment system as follows. First, SFO kinetics need to be fitted to the cumulative volatile loss from the water-sediment system, equivalent to the build up in the volatile traps. Secondly, defining the eventual build up in the traps as V_0, and V as

the instantaneous amount at any time t after the beginning of the experiment, then for SFO kinetics, the equation that needs to be fitted to the volatility data is

$$V = V_0 [1 - \exp(-k_{VOL}t)] \quad \text{(A11-2)}$$

The assumption of SFO kinetics must be demonstrated to hold to the extent that the above equation provides an acceptable fit to the data. A key aspect to obtaining an acceptable fit is that the build up of volatile losses in the traps follows the *shape* dictated by the equation.

Correction Procedures for FOMC Kinetics

The correction procedures are derived in a similar way to those for SFO kinetics, namely for a parent compound that is subject to an overall rate of loss from the water-sediment system by degradation and volatilisation, and that each loss process is described by FOMC kinetics. The underlying *shape* of these loss processes is also assumed to be the same. Hence the value of the α parameter for volatile losses will be the same for the overall rate of loss from the water-sediment system, so the assumption that the shape is similar must be tested in order to apply the correction procedures validly. Provided that this assumption holds, then different aspects of the loss process are only affected by the value of the location parameter β. β_{TOT}, β_{VOL} and β_{DEG} are defined to account for the different loss rates from the total overall loss from the water-sediment system, and those by volatilisation and by degradation, respectively. Therefore, :

$$\beta_{TOT} = \beta_{VOL} + \beta_{DEG} \quad \text{(A11-3)}$$

then β_{DEG} can be estimated simply from the difference between these two parameters by re-arranging the above equation.

The value of β_{TOT} is that estimated by fitting FOMC kinetics to data for the amount of parent remaining in the water-sediment system, i.e., the standard fit to system data as described in Chapter 10.

The value of and if β_{VOL} can be estimated by fitting FOMC kinetics to data for the amount of volatile losses of parent from the water-sediment system,

The value for β_{VOL} can be estimated by fitting FOMC kinetics to data for the amount of volatile losses of parent from the water-sediment system as follows. First, FOMC kinetics need to be fitted to the cumulative volatile loss from the water-sediment system, equivalent to the build up in the volatile traps. Secondly, defining the eventual build up in the traps as V_0, and V as

the instantaneous amount at any time t after the beginning of the experiment, then for FOMC the kinetic equation that needs to be fitted to the volatility data is

$$V = V_0 [1 - (t/\beta_{VOL} + 1)^{-\alpha}] \quad \text{(A11-4)}$$

The assumption of FOMC kinetics must be demonstrated to hold to the extent that the above equation provides an acceptable fit to the data. A key aspect to obtaining an acceptable fit is that the build up of volatile losses in the traps follows the *shape* dictated by the equation.

Note that the correction procedure for the FOMC kinetics only applies to systems with a single application and that the integral form must be used for metabolites.

Correction Procedures for HS and DFOP Kinetics

Correction procedures can be derived in a similar way to those for SFO and FOMC kinetics with similar caveats on their validity, mainly by assuming that some aspect of the underlying *shape* of these loss processes is the same, that is for HS kinetics that the time for the breakpoint should remain the same; while for DFOP kinetics that the ratio of the amounts in each compartment should remain the same. Given that these assumptions hold empirically, then the first-order rate constants in these types of kinetics should be able to be corrected in the same way as for SFO kinetics, namely by using the equation:

$$k_{TOT} = k_{VOL} + k_{DEG} \quad \text{(A11-5)}$$

APPENDIX 12: EXAMPLES OF FITTING A WATER-SEDIMENT EXPERIMENT TO TOXSWA USING THE PEST-OPTIMISATION PACKAGE

Introduction

Section 10.3.4 indicates that, as an alternative, water-sediment studies can be fitted to TOXSWA. This appendix presents guidance on the parameterisation of TOXSWA for this purpose and an example of such a fit for one water-sediment experiment. To perform the fitting procedure, TOXSWA 1.2 (the version preceding FOCUS_TOXSWA v.1.1.1) was coupled to the PEST optimisation programme (Doherty, 2000). A detailed instruction for the optimisation of TOXSWA using PEST is given by Beltman and Adriaanse (2005).

Two optimisation options are applied: (i) optimisation of DegT50 with fixed K_{OC} values, and (ii) optimisation of both DegT50 values and K_{OC}. The first option is the default procedure whereas the second option can be considered if the first option produces unacceptable results. In the second option, the K_{OC} can be optimised but it is restricted to 0.5 to 2 times the average K_{OC} derived from the dossier (similar to Appendix 9).

The selected example water-sediment experiment also fitted to the two-compartment model of Box 10-2. The fitted DegT50 for the water was 0.56 d and that for the sediment was 10 002 d (indicating no degradation in sediment over experimental period of about 100 d). However, the resulting fit did not pass the Fsed test: the modelled Fsed was 0.85 and the theoretical Fsed range as derived from Appendix 9 was 0.94 to 0.98. Thus this experiment was considered to be an appropriate example of using TOXSWA.

Procedure for TOXSWA runs

Input data characterising the compound and the water-sediment system

The water-sediment experiment was conducted with an example compound, whose K_{OC} was reported to be 76 000 L/kg. The water solubility was 7.5 mg/L at 25°C and the saturated vapour pressure was 0.17 µPa at 20°C. The depth of the water layer was 6 cm and that of the sediment was 2.5 cm. The concentration of suspended solids was not measured in the study. Thus the default concentration of suspended solids of 15 mg/L was used, and its organic matter content was assumed to be equal to that of the sediment (Adriaanse *et al*.,

2002). The clay content of the sediment was 3.9% and its organic carbon content was 0.9%. TOXSWA requires an organic matter content, which was obtained via multiplying the organic carbon content by 1.724, which gives 1.55%. This multiplication factor agrees with FOCUS (2003), and is not the factor recommended by Adriaanse *et al*. (2002). TOXSWA also needs the dry bulk density of the sediment, which may be estimated via the equation:

$$D = \frac{1}{0.603 + 0.003975\,C + 0.00207\,O^2 + 0.01781\ln(OM)} \qquad \text{(A12-1)}$$

where D is the dry bulk density (g cm^{-3}), C is the percentage clay and OM is the percentage organic matter. Equation A12-1 was derived by Wösten (1997) using data from loamy and clay soils. This resulted in D = 1.58 g cm^{-3}. Moreover, TOXSWA needs the volume fraction of water of the sediment, which can be estimated from its porosity. To do so, the density of the solid phase of soil (D_s in g cm^{-3}) was estimated using a relationship derived by Wösten (1997):

$$D_s = \frac{100}{\frac{O}{1.47} + \frac{C}{2.88} + \frac{100 - O - C}{2.66}} \qquad \text{(A12-2)}$$

The result was D_s = 2.63 g cm^{-3}. Then the porosity, θ, can be calculated as $1 - (D/D_s)$ which gave θ = 0.40.

The sorption to sediment and suspended solids was described with a Freundlich adsorption isotherm with a value of the Freundlich exponent *n* of 0.9. Also by default, the diffusion coefficient of the substance in the liquid phase was assumed to be 43 mm^2/d (Adriaanse *et al*., 2002).

Thickness of numerical compartments

In the calculations the thickness of the numerical compartments in the sediment was 0.03 mm for the top 0.24 mm increasing to 5 mm for the bottom layer (see Table A12-2 at the end of this appendix for the detailed description, lesewb in wbnu.inp). Such thin compartments should be used to obtain convergence of the numerical solution of TOXSWA if the K_{OC} of a compound exceeds about 30 000 L/kg. The calculation was repeated with thinner compartments to demonstrate that these compartments were thin enough. The user must check if convergence of the numerical solution has been obtained.

Optimisation of K_{OC} and degradation rates in water and sediment

In the optimisation procedure with PEST, equal weight was given to the water and sediment results. This was done by giving all measurements of the water layer a weight equal to the

number of measurements of the sediment layer and by giving all measurements of the sediment layer a weight equal to the number of measurements of the water layer. In the second optimisation option, (where the K_{OC} was optimised as well), the K_{OC} range was restricted to 38 000 – 152 000 L/kg (0.5 to two times the reported average).

The error percentage for the simulation compared to the measurements is calculated as prescribed in Chapter 6. So, for both water and sediment layers, the average of the measurements at each point in time has been used.

Output from TOXSWA used in fitting procedure

The output of TOXSWA used for fitting was the total concentration in the water layer, hence the mass dissolved plus the mass adsorbed to the suspended solids. This implies that it is implicitly assumed that the suspended material was not separated from the water sample before analysis. For compounds with high K_{OC} values, like this example compound, the mass adsorbed to suspended solids can not be ignored, especially if the organic carbon content of the suspended solids is considerable. For compounds with small K_{OC} values this distinction can be safely ignored.

Results

The sorption coefficients and degradation rates obtained are presented in Table A12-1 and graphs are shown in Figure A12-1. The χ^2 error percentages are indicated in the figures.

Table A12-1. Degradation rates coefficients and K_{oc} values obtained via TOXSWA-PEST optimisation for the example water-sediment experiment. Degradation half-lives are given in brackets.

	Optimisation of	
	Only Degradation Rates	***Both* K_{OC} and Degradation Rates**
K_{OC}	-	80 769 L/kg
Rate coefficient in water	0.826 d^{-1} (0.84 d)	0.852 d^{-1} (0.81 d)
Rate coefficient in sediment	0.00117 d^{-1} (590 d)	0.00175 d^{-1} (395 d)

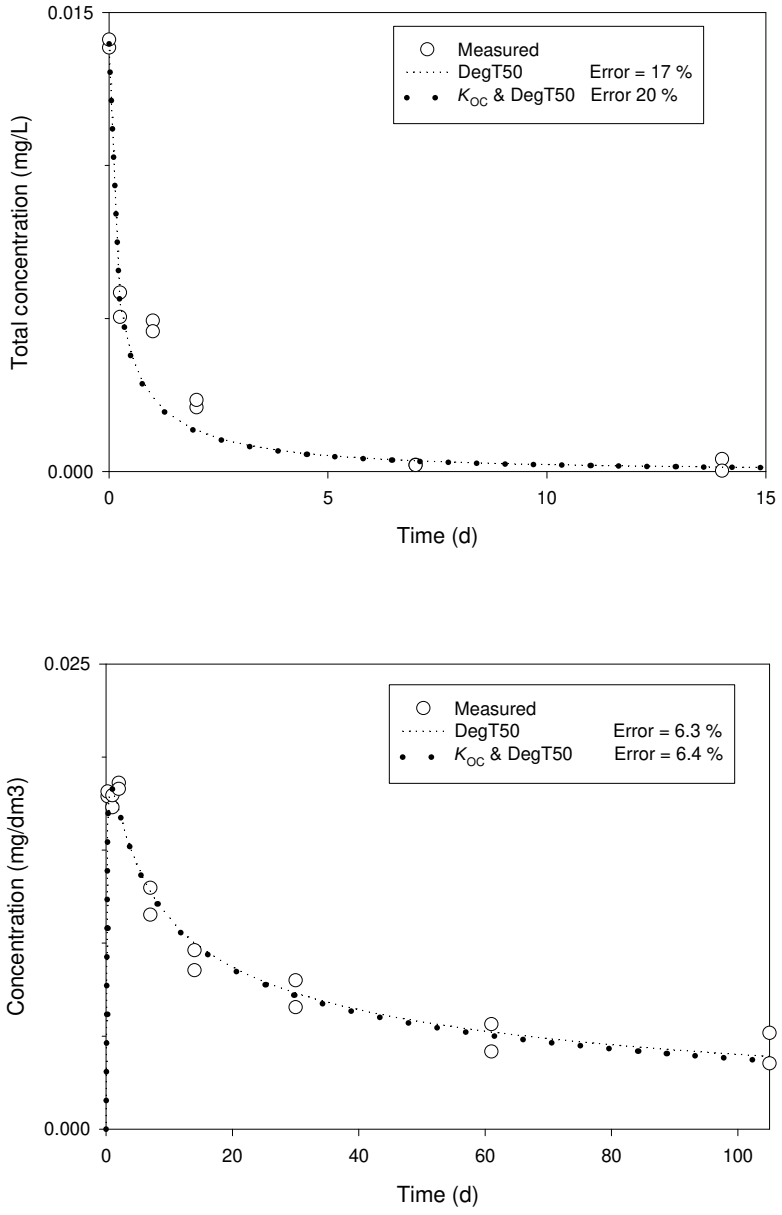


Figure A12-1. Comparison of measured and simulated concentrations as a function of time in the first water-sediment study with the example compound. Top graph is the water layer and bottom graph is the sediment layer. The total concentration in the water layer corresponds to the mass dissolved plus the mass adsorbed to suspended solids. All calculations were carried out with TOXSWA. Concentrations are shown for the two optimisation options (optimisation of only degradation coefficients and of both degradation coefficients and K_{OC}). Note that the axes in the two graphs have different scales.

Figure A12-1 shows that the degradation coefficients values fitted using TOXSWA-PEST resulted in a good description of the decline in the water and an excellent description of the concentration in the sediment. Adding the optimisation of the K_{OC} thereafter did not further improve the simulation, probably because the fitted K_{OC} differs less than 10% from the reported K_{OC} (Table A12-1).

In the water layer the error is smallest for the simulation with the level P-II degradation rates, but in sediment the error is highest. The simulations with TOXSWA-PEST optimised degradation rates show for the water layer an error that is about twice the error of the simulations with the level P-II degradation rates. However, for sediment it is about 1/5 of the error of the simulations with the level P-II degradation rates. The error increases when both the degradation rates and the K_{OC} are fitted, because the number of degrees of freedom decreases.

The chi-square errors indicated in Figure A12-1 show that the errors for the water layers were 19-22% whereas they were much lower for the sediment (6%). For the water layer, the error of the fit of both the degradation rates and the K_{OC} is lower because the number of degrees of freedom is lower (although the fit is almost exactly the same).

Table A12-1 shows that the sediment DegT50 values from the two fitting procedures differ considerably. Fitting both K_{OC} and degradation rates results in a K_{OC} value that differs less than 10% from the reported K_{OC} and a degradation rate in water that is close to the one obtained in the procedure when only the degradation rates were fitted. Nevertheless, the degradation half-life in sediment changed from 590 to 395 days. This large change probably can be attributed to the relative insensitivity of the simulated concentrations to the value of the degradation coefficients at these high degradation half-lives. This explanation is consistent with the wide 95% confidence intervals of these degradation coefficients (i.e. corresponding to degradation half-lives from -172 to 1352 days for the degradation half-life of 590 days and from -80 to 869 days for the degradation half-life of 395 days).

The result obtained with level P-II parameters in Figure A12-1 is acceptable for FOCUS Step 3 calculations that aim at assessment of PEC in surface water. If the aim would be to assess PEC in sediment, the level P-II parameters may not be accurate enough.

For compounds with high K_{OC} values, like this example compound, sorption to suspended solids may be important. To illustrate the impact of including suspended solids in the system, additional optimisations were conducted with realistic concentrations of suspended solids of 1 mg/L and of 50 mg/L, and using the reported K_{OC} of 76 000 L/kg. The fitted degradation half-lives for the water layer were 0.87 and 0.83 days, and for sediment 588 and 644 days. The organic matter content of the suspended solids was rather low, 1.55%. When the organic matter content is higher, the degradation rates are expected to be stronger influenced by the presence of suspended solids.

As described in the introduction of this appendix, the two-compartment model resulted in a fitted degradation half-life of about 0.6 days in the water and of 10 002 days in the sediment. Hence, the degradation half-lives for the water layer from TOXSWA and from the two-compartment model were quite similar (0.6 versus 0. 8 d). For the sediment, the TOXSWA fits indicated evidence of degradation in the sediment whereas the two-compartment model did not detect any degradation (a degradation half-life of 10 002 days from an experiment on a time scale of 100 days implies that degradation could be ignored).

References

Adriaanse, P.I., Leistra, M., Vink, J.P.M., Brouwer, W.W.M., Tas, J.W., Linders, J.B.H.J., Pol, J.W., 2002. Estimating transformation rates of pesticides, to be used in the TOXSWA model, from water-sediment studies. Alterra Report 023, Alterra, Wageningen, The Netherlands, 129 pp.

Beltman, W.H.J. & P.I. Adriaanse, 2005. Optimisation of degradation rates from water-sediment studies using TOXSWA and PEST. www.pesticides.alterra.nl (select ->Research theme->Fate in soil, water and air->Products) (in preparation).

Beltman, W.H.J. & P.I. Adriaanse, 1999. User's manual TOXSWA 1.2.; simulation of pesticide fate in small surface waters., DLO Winand Staring Centre, Techn. Doc. 54, Wageningen, the Netherlands

Doherty, J., 2000. Visual PEST (user's manual). Watermark Numerical Computing, Corinda, Australia.

FOCUS. 2003. FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC. Report of the FOCUS working group on surface water scenarios, EC document reference SANCO/4802/2001-rev.2, 245 pp. URL: http://europa.eu.int/comm/food/plant/protection/evaluation/focus_en.htm.

Wösten, J.H.M. 1997. Bodemkundige vertaalfuncties bij SC-DLO. State of the art. Report 563. DLO Winand Staring Centre, Wageningen, the Netherlands.

Table A12-2. Overview of all parameter values needed for simulation of the water-sediment studies presented per TOXSWA input file. See Annex 1 of Beltman and Adriaanse (1999) for explanation of the parameters.

Para-meter	TOXSWA input	Units	Value	Justification
wlnu.inp				
	betawl	-	0.5	default
	thetawl	-	1.	default
	deltwl	s	600.	default calculation time step
	ttot	d	105.	from study, duration of study
	xdit	m	0.05	default
	xfb	m	0.	default
	xeb	m	0.	default
	nxsedit	-	1	default
	nxsefb	-	0	default
	nxseeb	-	0	default
	lesefb	m	0.	default
	lesedit	m	0.05	default
	leseeb	m	0.	default
wlpa.inp				
b	wibot	m	0.05	default
s_1	sisl	-	0.00001	default
h_w	wdhfl	m	0.	default
ℓ	leplot	m	999.	dummy
ss	coss	g m^{-3}	15.	from study, else default
$m_{om,wb}$	raomss	-	0.0155	from study, organic matter content of sediment
DW	dwmp	g DW m^{-2}	0.	default
wlst.inp				
c^*	castwl	g m^{-3}	0.01398	from study, initial total concentration
c^a	coair	g m^{-3}	0.	default

Table A12-2 (continued). Overview of all parameter values needed for simulation of the water-sediment studies with presented per TOXSWA input file. See Annex 1 of Beltman and Adriaanse (1999) for explanation of the parameters.

Para-meter	TOXSWA input	Units	Value	Justification
wbnu.inp				
	betawb	-	0.5	default
	thetawb	-	1.	default
	deltwb	s	600.	default calculation time step
	zwb	m	0.025	from study
	zebb	m	0.	default
	nzsewb	-	23	thin segments
	nzseebb	-	0	default
	lesewb	m	8 * 0.00003 2 * 0.00006 2 * 0.00012 3 * 0.00030 2 * 0.00075 2 * 0.00200 1 * 0.00300 3 * 0.00500	thin segments
	leseebb	m	0.	default
wbpa.inp				
ρ_b	bdwb	kg m^{-3}	23 * 1580.	from study (via Eqn A10-1)
ε	por	-	23 * 0.40	from study (via Eqn A10-1 and A10-2)
λ	tor	-	23 * 0.34	calculated from porosity (see Beltman and Adriaanse, 1999)
$m_{om,ss}$	raomwb	-	23 * 0.0155	from study
L_{dis}	ldis	m^2 d^{-1}	0.15	dummy
wbst.inp				
c_b*	castwb	g m^{-3}	0.	default

Table A12-2 (continued). Overview of all parameter values needed for simulation of the water-sediment studies presented per TOXSWA input file. See Annex 1 of Beltman and Adriaanse (1999) for explanation of the parameters.

Para-meter	TOXSWA input	Units	Value	Justification
hy.inp				
u	u	m d^{-1}	0.	default
h	wdh	m	0.06	from study
E_x	kds	m^2 d^{-1}	10.	dummy
q	qseif	m^3 m^{-2} d^{-1}	0.	default
c_{lb}	colot	g m^{-3}	0.	dummy
T	te	K	293.	from study
sl.inp				
	op_slus	-	1	default
	op_slud	-	0	default
	op_slur	-	0	default
	op_slmd	-	0	default
	op_slmr	-	0	default
	ntslus	-	1	default
	tslus	d	0.	default
	mslus	g m^{-2}	0.	default
	stxslus	m	0.	default
	enxslus	m	0.05	default

Table A12-2 (continued). Overview of all parameter values needed for simulation of the water-sediment studies presented per TOXSWA input file. See Annex 1 of Beltman and Adriaanse (1999) for explanation of the parameters.

Para-meter	TOXSWA input	Units	Value	Justification
opout.inp				
	op_input	-	1	default
	op_icwlhy	-	0	default
	op_icwb	-	0	default
	op_wlmb	-	1	default
	op_wlmbnodenr	-	1	default
	op_wbsconodenr	-	1	default
	op_wbmbnodenr	-	1	default
	op_wbmball	-	1	default
	op_dbnodenr	-	1	default
	op_dbdit	-	1	default
	op_ecnodenr	-	1	default
	deltout	d	0.25	minimum time interval between measurements
	nwbsy	-	1	default
	iwbsy	-	1	default
	ktop	-	23	total nr of segments in sediment

Table A12-2 (continued). Overview of all parameter values needed for simulation of the water-sediment studies presented per TOXSWA input file. See Annex 1 of Beltman and Adriaanse (1999) for explanation of the parameters.

Para-meter	TOXSWA input	Units	Value	Justification
su.inp				
	dt50wl	d	0.84	fitted
E	aetf	J mol^{-1}	55 000.	default
$K_{om,ss}$	kdomssdit	m^3 kg^{-1}	44.08	compound property
$c_{om,ss}$	coobkomss	kg m^{-3}	0.001	default
n_{ss}	exfrss	-	0.9	compound property, or default (used here)
K_{mp}	kdmpdit	m^3 kg^{-1}	0.	dummy
M	mamol	g mol^{-1}	418.9	compound property
k_l	klq	m d^{-1}	1.55	calculated from molecular mass (see Beltman and Adriaanse, 1999)
k_g	kga	m d^{-1}	149.	calculated from molecular mass (see Beltman and Adriaanse, 1999)
P	psat	Pa	1.7E-7	compound property
	tepsat	K	293.	compound property
ΔH_P	mepsat	J mol^{-1}	95000.	default
c_{sol}	cosol	g m^{-3}	7.5	compound property
	tesol	K	298.	compound property
ΔH_{sol}	mesol	J mol^{-1}	27000.	default
D_w	kdfw	mm^2 d^{-1}	43.	default
	dt50wb	d	422	fitted
$K_{wb,ss}$	kdomwb1	m^3 kg^{-1}	44.08	compound property
$c_{wb,ss}$	coobkomwb	kg m^{-3}	0.001	default
n_{wb}	exfrwb	-	0.9	compound property, or default (used here)

APPENDIX 13: OVERVIEW OF SOFTWARE PACKAGES

ACSL Optimize

Berkeley Madonna

GraphPad PRISM

Kinetica

Matlab

ModelMaker

ModelManager

Statistica

Tablecurve 2D

Topfit

ACSL Optimize

1 General information

Name	ACSL Optimize
Version	1.2 (1996)
Category	general purpose package
Model developer	AEgis Technologies Group, Inc. 631 Discovery Drive Huntsville, AL 35806, USA (256) 922-0802 (voice) (256) 922-0904 (fax) web: http://www.aegistg.com/
Company	(distribution in Germany) Dr. Ingrid Bausch-Gall BAUSCH-GALL GmbH Wohlfartstr. 21 b D-80939 Muenchen Tel: +49 / 89 / 3232625 oder ++49 / 89 / 3221150 e-mail: Ingrid.Bausch-Gall@Bausch-Gall.de web: www.Bausch-Gall.de
Price	??

2 Documentation

Manual	available as hardcopy, part of product
Language	English
Clarity	average
Description of concepts	fair
Tutorial	available, but not very helpful
Help-function	available
Help line	?
References	ACSL Optimize Version 1.2, MGA Software, Concord, Massachusetts, USA.
Internal benchmark dataset	?
Tightness of version control	?
Availability of examples / source code	available
Training	courses are offered
Known bugs	suspected error in calculation of r^2 for weighted data sets

ACSL Optimize (continued)

3 System considerations

Hardware requirements	standard PC
Operating system	Win95, Win98, WinNT. WinXP?
Software requirements	no specific software requirements

4 User friendliness (easy, moderate, laborious)

Selection of model	extremely flexible, but requires training
Selection of statistics	moderate
Selection of optimisation method	easy
Selection of object function	selection easy, loglikelyhood automatically chosen
Input of data	by hand, from file, (ASCII) scripts possible
Output of results	to file (ASCII), screen
Graphical output	yes, user defined graphs, exportable as bitmap
Statistical output	yes, detailed output automatically
Output of input	yes
Output of model	yes, detailed output automatically
Input of user-defined model	all models have to be user-defined
Advanced use support	default values provided, user-defined possible
Possibilities for automatic handling of multiple datasets	easy, if corresponding scripts are written by user
Archiving	no

5 Functionality (compartments)

Number of substances	flexible
Transformation scheme	user-defined (flexible)
Parameter estimation in multi-component systems	sequential or parallel

6 Functionality (kinetic models)

Built-in	none
User defined	everything possible

ACSL Optimize (continued)

7 Functionality (statistics)

Goodness of fit	no, only r^2 given
Confidence interval	yes
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	no test but other useful (essential) information, such as parameter variance and correlation
Optimisation of experiments	yes

8 Endpoints (for selected kinetic models)

	differentiation for parents and metabolites is possible
DT50, DT90	?
Rate coefficients	yes
Formation fractions	yes

9 Tips (specific for package)

Sensitivity to initial parameter settings	no
Are default settings for estimation procedure OK?	yes
Speciality	ACSL can handle different error models (= weighting schemes) from 100% absolute to 100% relative error and any “mixture” in between; the error model can also be adjusted by optimisation

Berkeley Madonna

1 General information

Name	Berkeley Madonna
Version	8.0.1 (2000)
Category	general purpose differential equation solver (incl. graphs)
Model developer	R.I. Macey, G.F. Foster
Company	website: www.berkeleymadonna.com
	contact: madonna@kagi.com
Price	individual licenses cost $299 per user. Quantity discounts are available for 5 to 14 licenses ($199 per user) and 15 or more licenses ($129 per user). ($99 for owners of Modelmaker, SAAMII, STELLA/ithink and PowerSim)

2 Documentation

Manual	user's guide pdf-file
Language	English
Clarity	average
Description of concepts	poor in software package
Tutorial	yes, sufficient
Help-function	yes, adequate
Help line	not direct
References	no
Internal benchmark dataset	no
Tightness of version control	tight, commercial package
Availability of examples / source code	examples available, only few relevant for degradation kinetics. Source code not available.
Training	no information
Known bugs	

Berkeley Madonna (continued)

3 System considerations

Hardware requirements	PC and Macintosh
Operating system	Win95, Win98, Windows NT (tested), Windows XP (tested) no info on other platforms Macintosh OS X
Software requirements	JAVA, specific version (for using flowchart editor)

4 User friendliness (easy, moderate, laborious)

Selection of model	typed in or copied from clipboard; option to import from STELLA files graphical input of model, flowchart (when Java is loaded),
Selection of statistics	no statistics
Selection of optimisation method	not specified
Selection of object function	user definable
Input of data	various possibilities including manually or import from TXT or CSV files
Output of results	on paper, export to TXT file
Graphical output	yes, limited possibilities to edit graph
Statistical output	no
Output of input	possible
Output of model	optimised parameters only
Input of user-defined model	yes, in form of differential equations
Advanced use support	limited
Possibilities for automatic handling of multiple datasets	possible (no experience)
Archiving	simple save option

Berkeley Madonna (continued)

5 Functionality (compartments)

Number of substances	not limited
Transformation scheme	not limited
Parameter estimation in multi-component systems	possible

6 Functionality (kinetic models)

Built-in	models may be taken from examples
User defined	any set of differential equations
Weighting	no information
Transformation	possible

7 Functionality (statistics)

Goodness of fit	no
Confidence interval	no
Degrees of freedom	no
Identification of outliers	no
Further statistical tests	no
Optimisation of experiments	no

8 Endpoints (for selected kinetic models)

Any model	all parameters are given (also fixed parameters)
DT50, DT90	to be calculated by user
Rate coefficients	given
Formation fractions	given

9 Tips (specific for package)

Sensitivity to initial parameter settings	slightly (as far as tested)
Are default settings for estimation procedure OK?	choice between fast and more accurate (the latter is recommended

GraphPad PRISM

1 General information

Name	GraphPad PRISM
Version	2.01 (June, 1996) (Latest version 4.0)
Category	generic parameter estimation package (incl. graphs)
Model developer	GraphPad Software, Incorporated
Company	GraphPad Software, Inc. 5755 Oberlin Drive, #110 San Diego, CA 92121 USA Tel: 800-388-4723 (in U.S.) or 858-457-3909 (outside U.S.) Fax: 858-457-8141 Email: sales@graphpad.com, support@graphpad.com, or orders@graphpad.com
Price	all prices in US dollars
	Prism: one copy $495.00, academic $445.50, student – qualifications $371.25 2 to 5 copies– multi-copy options $371.25 each 6 to 10 copies– multi-copy options $321.75 each upgrades $149.00 each

2 Documentation

Manual	yes, downloadable pdf file. Also for statistics.
Language	English
Clarity	clear, comprehensive and with references
Description of concepts	all concepts described in some detail; references to more comprehensive literature
Tutorial	yes, comprehensive
Help-function	yes, very comprehensive
Help line	yes
References	via website GraphPad Com
Internal benchmark dataset	available through tutorial
Tightness of version control	tight, commercial package
Availability of examples / source code	examples in tutorial; source code not available; principles described in manuals, help and via library
Training	yes, organised upon demand, customised
Known bugs	

GraphPad PRISM (continued)

3 System considerations

Hardware requirements	PC and Macintosh
Operating system	Win95, Win98, Windows NT (version 2, tested) Version 3: Win 95, Win 98, Win ME, NT 4, Win 2000, or XP, 5MB free on hard disk. (Prism 3 and 4 are not available for Windows 3.1) Macintosh OS 8.1 or higher
Software requirements	none; some features only work with Excel

4 User friendliness (easy, moderate, laborious)

Selection of model	easy (Windows based), both built-in and user-defined models
Selection of statistics	easy (Windows based)
Selection of optimisation method	Levenberg-Marquardt method, no other options
Selection of object function	choice between actual and relative ($1/Y^2$) Sum of Squares
Input of data	various possibilities: manually copied from file (for example, spreadsheet) by cut and paste import from TXT, CSV, DAT or PRN files automatically in batch mode (not tried)
Output of results	summary or detailed output possible on paper export to file TXT, CSV, DAT or PRN
Graphical output	Yes, editing of graphs is easy, output to file possible in various formats (WMF, BMP, PCX, TIF, GIF
Statistical output	yes, adjustable by user
Output of input	possible
Output of model	full / summary of chosen model generated in output details of optimisation parameters, etc.
Input of user-defined model	yes, explicit function of one independent variable only; functions stored and available for later use.
Advanced use support	optimisation parameters user-defined (this might be different for each of the parameters)
Possibilities for automatic handling of multiple data sets	automatic calculation for different data sets within one project further possibilities using macros (no experience)
Archiving	everything is archived in a dedicated workbook

GraphPad PRISM (continued)

5 Functionality (compartments)

Number of substances	1
Transformation scheme	not applicable
Parameter estimation in multi-component systems	not applicable

6 Functionality (kinetic models)

Built-in	one site site binding (hyperbola) two site binding sigmoidal dose-response sigmoidal dose-response (variable slope one site competition two site competition Boltzmann sigmoid one phase exponential decay two phase exponential decay one phase exponential association two phase exponential association exponential growth power series polynomial equations sine wave Gaussian distribution
User defined	possible, restricted to explicit 2 dimensional (X,Y or X,T functions
Weighting	limited (version 3 has more options than version 2)
Transformation	possible

7 Functionality (statistics)

Goodness of fit	yes
Confidence interval	yes
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	t-test 1-way, 2 way ANOVA runs test residue analysis comparison of models
Optimisation of experiments	no

GraphPad PRISM (continued)

8 Endpoints (for selected kinetic models)

SFO	initial concentration, rate coefficient (including confidence limits
DT50, DT90	SFO, etc
Rate coefficients	SFO, etc
Formation fractions	SFO, etc

9 Tips (specific for package)

Sensitivity to initial parameter settings	slightly (as far as tested)
Are default settings for estimation procedure OK?	choice between fast and more accurate (the latter is recommended

Kinetica

1 General information

Name	Kinetica
Version	4.2
Category	Kinetica™ is a fitting and simulation tool actually developed and established for pharmacokinetic models
Model developer	
Company	InnaPhase Corporation, http://www.innaphase.com/ contact: Simon Davis, European Technical Support Scientist Cell phone : +44 7980 832 666 Telephone : +44 1494 582 080 Facsimile : +44 1494 582 454/+1 801 991 7145 e-mail: sdavis@innaphase.com
Price	Kinetica is available as both standalone (node) and network(floating) license - commercial pricing is listed below; KSTD Kinetica Standard Edition $3500 KSTDM Kinetica Annual Maintenance $800 KSTD Kinetica Network Edition $4500 KSTDM Kinetica Annual Maintenance $1000 demonstration version on website: http://www.innaphase.com/support_downloads_kinetica.html;

2 Documentation

Manual	?
Language	English
Clarity	?
Description of concepts	?
Tutorial	?
Help-function	yes
Help line	?
References	yes, through website
Internal benchmark dataset	?
Tightness of version control	tight
Availability of examples / source code	examples mainly for pharmacokinetic models/ source code for some models available
Training	yes
Known bugs	?

Kinetica (continued)

3 System considerations

Hardware requirements	PC
Operating system	presumably Win95 or later
Software requirements	?

4 User friendliness (easy, moderate, laborious)

Selection of model	graphical interface can be used to implement a compartment model; additionally, the models can be established with BASIC computer language;
Selection of statistics	easy (statistics are given in a report file)
Selection of optimisation method	easy (Marquardt estimation procedure possible)
Selection of objective function	?
Input of data	by hand, from file, from spreadsheet (copy and paste possible), compatible with common software
Output of results	easy, optimised parameters, graphs and statistics can be automatically transferred to a WORD or EXCEL file
Graphical output	yes
Statistical output	yes, basic statistical output
Output of input	?
Output of model	?
Input of user-defined model	yes
Advanced use support	?
Possibilities for automatic handling of multiple datasets	yes
Archiving	?

Kinetica (continued)

5 Functionality (compartments)

Number of substances	flexible
Transformation scheme	user-defined (flexible)
Parameter estimation in multi-component systems	sequential or parallel
Weighting of data	yes

6 Functionality (kinetic models)

Built-in	yes
User defined	yes

7 Functionality (statistics)

Goodness of fit	need to be included in model manually as variable
Confidence interval	?
degrees of freedom	need to be included in model manually as variable
identification of outliers	?
further statistical tests	ANOVA Latin Square unbalanced Block non-parametric Tests linear regression estimating power descriptive statistics & Ssummary tables
Optimisation of experiments	automatically

8 Endpoints (for selected kinetic models)

DT50, DT90	need to be included in model manually as variable
Rate coefficients	yes
Formation fractions	would need to be included in model manually as variable

9 Tips (specific for package)

Sensitivity to initial parameter settings	depends on data set
Are default settings for estimation procedure OK?	yes

Matlab

1 General information

Name	MATLAB
Version	version 6.5, version 7.0
Category	generic parameter estimation package
Model developer	The MathWorks (www.mathworks.com)
Company	The MathWorks, Inc. 3 Apple Hill Drive Natick, MA 01760-2098, USA
Price	single-user licenses: 2650 Euro for MATLAB, 1250 Euro for Optimization Toolbox; discounts for multi-user licences; "Software Maintenance Service" contract (480 Euro per year) needed to get free updates and access to help line

2 Documentation

Manual	documentation in printed and/or online form
Language	English
Clarity	clear and comprehensive
Description of concepts	detailed description
Tutorial	yes
Help-function	yes
Help line	covered by "Software Maintenance Service" (yearly fee)
References	www.mathworks.com/documentation
Internal benchmark dataset	no
Tightness of version control	very tight (commercial package)
Availability of examples / source code	large number of general examples, but none specific to pesticide kinetics
Training	large number of courses
Known bugs	

3 System considerations

Hardware requirements	standard PC or MAC
Operating system	Windows, UNIX, Linux, Macintosh OS X
Software requirements	no additional software required

Matlab (continued)

4 User friendliness (easy, moderate, laborious)

Selection of model	easy (manual input of equations)
Selection of statistics	see below, “Statistics”
Selection of optimisation method	easy
Selection of object function	easy
Input of data	by hand, from file, from spreadsheet; fully compatible with common software
Output of results	to the screen, export only via “copy and paste”
Graphical output	yes, editing of graphs
Statistical output	no
Output of input	no
Output of model	no
Input of user-defined model	yes
Advanced use support	optimisation parameters user-defined
Possibilities for automatic handling of multiple data sets	no
Archiving	no

5 Functionality (compartments)

Number of substances	flexible
Transformation scheme	user-defined (flexible)
Parameter estimation in multi-component systems	parallel or user-defined

6 Functionality (kinetic models)

Built-in	no specific kinetic equations built in
User defined	yes, possible

7 Functionality (statistics)

Goodness of fit	no specific output of statistics (may be included in MATLAB’s “Statistics Toolbox” [850 Euro] that was not available for testing); programming of statistical output is possible
Confidence interval	
Degrees of freedom	
Identification of outliers	
Further statistical tests	
Optimisation of experiments	

Matlab (continued)

8 Endpoints (for selected kinetic models)

DT50, DT90	SFO, etc
Rate coefficients	SFO, etc
Formation fractions	SFO, etc

9 Tips (specific for package)

Sensitivity to initial parameter settings	not tested
Are default settings for estimation procedure OK?	not tested
Speciality	a MATLAB compiler (3750 Euro) is available that allows to create executables for specific kinetic models that could be distributed

ModelMaker

1 General information

Name	ModelMaker
Version	4.0
Category	generic parameter estimation package
Model developer	
Company	A.P. Benson Soane Point 6-8 Market Place Reading Berkshire RG1 2EG UK tel: +44 8702 417 018 fax: +44 8702 417 023 email: enquiries@apbenson.com webpage: www.apbenson.com
Price	ModelMaker 4 for Commercial Users £199 €320 $290 ModelMaker 4 for Academic Users £149 €240 $220 ModelMaker 4 Download Only Students £80 €128 $115 ModelMaker 4 Manuals £45 €72 $65

2 Documentation

Manual	yes (order from website or call)
Language	English
Clarity	confusing
Description of concepts	optimisation routines, statistics, etc
Tutorial	no
Help-function	yes
Help line	no
References	
Internal benchmark dataset	available
Tightness of version control	loose
Availability of examples / source code	yes, there is a number of examples as tutorial (however not very helpful for estimation of degradation parameter)
Training	yes, courses are offered for ModelMaker particularly aimed at Environmental Modelling and Risk Assessment
Known bugs	in version 3.0 R^2 is not always correctly reported; the bug does not occur in version 4

ModelMaker (continued)

3 System considerations

Hardware requirements	PC
Operating system	presumably Win95 or later
Software requirements	Excel useful for preparation of input data, additional statistical analysis and graphing (e.g. residuals)

4 User friendliness (easy, moderate, laborious)

Selection of model	easy
Selection of statistics	easy (all statistics are given in the report file) In MM4.0 the correlation matrix is not given!
Selection of optimisation method	easy (Marquardt and Simplex possible)
Selection of objective function	no selection possible (minimizes sum of least squares)
Input of data	by hand, from file, from spreadsheet (copy and paste possible), compatible with common software
Output of results	easy optimisation process and statistics listed in ASCI file (report.txt); optimised values and graphs given in tables and figures are easy to copy
Graphical output	yes
Statistical output	yes, basic statistical output (no correlation and covariance matrix in MM4.0 but in MM3.0.4)
Output of input	no
Output of model	no
Input of user-defined model	yes
Advanced use support	choice of optimisation parameters
Possibilities for automatic handling of multiple datasets	no
Archiving	Available in MM4.0

ModelMaker (continued)

5 Functionality (compartments)

Number of substances	very flexible
Transformation scheme	user-defined (flexible)
Parameter estimation in multi-component systems	*???*
Weighting of data	yes, absolute, relative and individual weighting possible

6 Functionality (kinetic models)

Built-in	no
User defined	yes

7 Functionality (statistics)

Goodness of fit	yes, r^2 value, F-test
Confidence interval	standard error
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	
Optimisation of experiments	not automatically

8 Endpoints (for selected kinetic models)

	possible differentiation for parents and metabolites
DT50, DT90	need to be included in model manually as variable
Rate coefficients	yes
Formation fractions	would need to be included in model manually as variable

9 Tips (specific for package)

Sensitivity to initial parameter settings	depends on data set
Are default settings for estimation procedure OK?	yes

ModelManager

1 General information

Name	MODELMANAGER (EK)
Version	Version 1.1
Category	Specific purpose
Model developer	Cherwell Scientific (now Family Genetix)
Company	http://www.modelmanager.com
Price	$1300 but multiple copies at reduced rates

2 Documentation

Manual	1 manual available
Language	English
Clarity	satisfactory and comprehensive
Description of concepts	concepts not fully described
Tutorial	no, covered by manual
Help-function	yes, somewhat limited; covered by manual
Help line	yes, e-mail and phone, but not very helpful
References	user manual
Internal benchmark dataset	none, but tested against SAS during development with Zeneca
Tightness of version control	since only one commercial version exists, non-issue
Availability of examples / source code	source code not provided (commercial package)
Training	some training courses offered
Known bugs	a number of minor bugs, but usually does not affect calculations.

3 System considerations

Hardware requirements	PC
Operating system	>Windows 95
Software requirements	need Excel

ModelManager (continued)

4 User friendliness (easy, moderate, laborious)

Selection of model	easy
Selection of statistics	easy
Selection of optimisation method	easy
Selection of object function	easy (unweighted least squares, log weighting (equivalent to 1/fitted value^2, and weighting by data points equivalent to 1/data value^2)
Input of data	easy
Output of results	easy
Graphical output	easy
Statistical output	easy
Output of input	easy
Output of model	easy
Input of user-defined model	not possible without very advanced knowledge – basically needs manufacturer to do it
Advanced use support	now redundant
Possibilities for automatic handling of multiple datasets	up to 10 data sets
Archiving	easy

5 Functionality (compartments)

Number of substances	1 substance and up to 2 breakdown products 1 substance with transfer/degradation between/in compartments
Transformation scheme	5 set schemes are given; lag phases can be included for parent degradation using hockey-stick kinetics
Parameter estimation in multi-component systems	sequential, simultaneous and parameter fixation

6 Functionality (kinetic models)

Built-in	all functionality is built in
User defined	no

Modelmanager (continued)

7 Functionality (statistics)

Goodness of fit	yes (R^2, Adjusted R^2 and Error Mean Square)
Confidence interval	yes
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	F-test
Optimisation of experiments	not explicitly, but can be used by expert user in this framework

8 Endpoints (for selected kinetic models)

DT50, DT90	provided automatically…plus DT75
Rate coefficients	yes
Formation fractions	yes

9 Tips (specific for package)

Sensitivity to initial parameter settings	generally ok – depends on kinetics (DFOS has simulated annealing to get around its poor response surface and fix on a local instead of a global minimum)
Are default settings for estimation procedure OK?	generally ok

Statistica

1 General information

Name	STATISTICA
Version	Version 6.0
Category	general purpose package (stats and graphics)
Model developer	Statsoft (www.statsoft.com)
Company	headquarters in Tulsa (USA); offices in 21 countries.
Price	Statistica Base (895 Euro) + Advanced Linear/Non-Linear Models (445 Euro) = 1340 Euro large discounts for multi-licences and academia

2 Documentation

Manual	3 manuals available
Language	>20 languages
Clarity	clear and comprehensive
Description of concepts	very detailed description + references provided for more details
Tutorial	some tutorials; No tutorial for non-linear estimation
Help-function	yes; very comprehensive
Help line	free technical support available provided that the user has the latest version
References	STATISTICA user manuals
Internal benchmark dataset	two examples of non-linear estimation are provided (many more in linear/non linear models)
Tightness of version control	very tight (commercial package)
Availability of examples / source code	examples are provided and explained; source code not provided (commercial package)
Training	large number of standard courses + possibility of bespoke courses
Known bugs	the package has been thoroughly tested (commercial package); patches readily posted on the web where necessary.

3 System considerations

Hardware requirements	PC and MAC
Operating system	>Windows 95
Software requirements	no additional software required

Statistica (continued)

4 User friendliness (easy, moderate, laborious)

Selection of model	model first needs to be written as a user-specified equation.; it can then be saved and re-used easy selection
Selection of statistics	easy (Windows based)
Selection of optimisation method	easy (drop down menu)
Selection of object function	easy (use least squares or define your own objective function)
Input of data	by hand, from file, from spreadsheets, from databases, from clipboard
Output of results	to the screen plus in an output window in the universal RTF format
Graphical output	yes, editing possible
Statistical output	yes, editing possible
Output of input	yes if desired (the user selects the desired output)
Output of model	equation given plus a full summary of optimisation (includes history of iterations)
Input of user-defined model	yes
Advanced use support	two modes, one easy mode where defaults are used, and a complex mode where much tweaking with the optimisation can be done
Possibilities for automatic handling of multiple data sets	possible *yes or no* ease of using this option (for instance: necessity to write macros …)
Archiving	everything is archived in a dedicated workbook

5 Functionality (compartments)

Number of substances	
Transformation scheme	parent/metabolite schemes can only be simulated if the equation can be written in analytical form
Parameter estimation in multi-component systems	sequential, parallel or user defined option

6 Functionality (kinetic models)

Built-in	none of the common degradation equations are built in, but these can be typed once and be saved
User defined	yes

Statistica (continued)

7 Functionality (statistics)

Goodness of fit	yes
Confidence interval	yes
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	F-test, t-test, ANOVA, confidence limits, correlation and covariance between parameters, residues analysis (normal probability plots, half-normal probability plots, residues vs. predicted, histograms)
Optimisation of experiments	comprehensive module on DOE (Design of Experiments)

8 Endpoints (for selected kinetic models)

DT50, DT90	not provided automatically
Rate coefficients	optimised parameter
Formation fractions	optimised parameter or fixed

9 Tips (specific for package)

Sensitivity to initial parameter settings	not tested
Are default settings for estimation procedure OK?	*?*

Tablecurve 2D

1 General information

Name	Tablecurve 2D
Version	4.06 (1996)
Category	generic parameter estimation package
Model developer	Jandel Scientific,
Company	According to manual: Jandel Scientific, 2591 Kerner Blvd San Rafael, CA 94901 (415) 453-6700 (415) 453-7769 Apparently transferred to SPSS: http://www.spss.com/ supported by Cranes software?
Price	?

2 Documentation

Manual	available as hardcopy, part of product
Language	English
Clarity	OK
Description of concepts	OK for the experienced user, bad for the new user
Tutorial	yes
Help-function	yes, but same as manual
Help line	apparently not any longer
References	
Internal benchmark dataset	no
Tightness of version control	tight
Availability of examples / source code	examples enclosed, source code not available
Training	no
Known bugs	none

Tablecurve 2D (continued)

3 System considerations

Hardware requirements	PC Intel 386 or later, Math coprocessor
Operating system	Win95 and above
Software requirements	none required

4 User friendliness (easy, moderate, laborious)

Selection of model	easy for built-in models, relatively easy for typing user-defined models
Selection of statistics	easy
Selection of optimisation method	easy
Selection of object function	?
Input of data	copied from Excel or Lotus or text file
Output of results	to Excel and Lotus, as dat or prn or txt files
Graphical output	yes, editing of graphs easy, various formats
Statistical output	yes, editing of lay-out possible
Output of input	input data can be exported
Output of model	full output can be obtained or selected parts, chosen by the user.
Input of user-defined model	possible
Advanced use support	optimisation parameters can be user-defined
Archiving	not automatically, must be requested

5 Functionality (compartments)

Number of substances	1
Transformation scheme	not applicable

Tablecurve 2D (continued)

6 Functionality (kinetic models)

Built-in	all built-in models only exist in the integrated form of the equation: first order, half order, 2^{nd} order, 3^{rd} order, variable order, simultaneous first and second order first order sequential two component first order two first order independent two second order independent first and second order independent
User defined	yes, possible, can be saved

7 Functionality (statistics)

Goodness of fit	r^2, adjusted r^2, F-statistic, Fit std. error
Confidence interval	yes
Degrees of freedom	yes
Identification of outliers	yes
Further statistical tests	t-test, ANOVA, error bars, residue analysis, comparison of models
Optimisation of experiments	no

8 Endpoints (for selected kinetic models)

DT50, DT90	must be asked for manually after each run
Rate coefficients	given for all models
Formation fractions	not applicable

Topfit

1 General information

Name	TopFit
Version	2.0.0
Category	specific parameter estimation package
Model developer	G. Heinzel, R. Woloszczak, P. Thomann
Company	R. Woloszczak at Schering AG, Berlin; Tel. +49 30 4681-1259
Price	freeware

2 Documentation

Manual	available; published as *Heinzel G., Woloszczak R., Thomann P.: TopFit 2.0, Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC. Gustav Fischer Verlag Stuttgart, 1993*
Language	English
Clarity	user manual is reasonably clear; technical manual with strong focus on underlying mathematics
Description of concepts	detailed description
Tutorial	not available
Help-function	limited context help available
Help line	no
References	see above, “Manual”
Internal benchmark dataset	no
Tightness of version control	(no further development)
Availability of examples / source code	examples available
Training	no
Known bugs	

3 System considerations

Hardware requirements	standard PC
Operating system	MS-DOS (runs under Windows NT)
Software requirements	no specific software required

Topfit (continued)

4 User friendliness (easy, moderate, laborious)

Selection of model	moderate
Selection of statistics	easy
Selection of optimisation method	-
Selection of object function	-
Input of data	by hand; simple ASCII input format
Output of results	to file; ASCII output file can easily be post-processed
Graphical output	yes, limited editing of graphs
Statistical output	yes, no editing of layout
Output of input	yes
Output of model	no
Input of user-defined model	yes
Advanced use support	?
Possibilities for automatic handling of multiple datasets	?
Archiving	no

5 Functionality (compartments)

Number of substances	flexible
Transformation scheme	user-defined (flexible)
Parameter estimation in multi-component systems	sequential or parallel

6 Functionality (kinetic models)

Built-in	only first-order and Michaelis-Menten kinetics
User defined	yes

Topfit (continued)

7 Functionality (statistics)

Goodness of fit	yes, expressed as B value: $B = 1 - \frac{\sum_{n} (y_{n,obs} - y_{n,calc})^2}{\sum_{n} y_{n,obs}^2}$ (n = number of data points)
Confidence interval	95th percentile confidence intervals
Degrees of freedom	yes
Identification of outliers	no
Further statistical tests	standard deviation, SSQ, t-test, parameter correlation
Optimisation of experiments	?

8 Endpoints (for selected kinetic models)

	possible differentiation for parents and metabolites
DT50, DT90	SFO, etc
Rate coefficients	SFO, etc
Formation fractions	SFO, etc

9 Tips (specific for package)

Sensitivity to initial parameter settings	no
Are default settings for estimation procedure OK?	yes