

Manual for the openGUTS standalone software (v.1.1)

Tjalling Jager*

March 1, 2021

This documents is part of the openGUTS project, and can be downloaded from <http://.openguts.info/>. The openGUTS project is made possible by funding from Cefic-LRI in project ECO39.2. Funding for the update to Version 1.1. was provided by Syngenta Crop Protection AG.

Contents

1	Introduction and workflow	3
2	Installation and quick-start guide	5
2.1	Installation	5
2.2	Quick start	5
3	General considerations	9
3.1	Data sets	9
3.2	Standard plotting format	10
4	Tab 1: Data input	11
4.1	Inputting data	11
4.2	Notes on input data	11
5	Tab 2: Calibration	13
5.1	General information	13
5.2	Fixing background hazard	13
5.3	Model output	13
5.4	Expert setting	14
5.5	LC _{x,t} calculation	15

*DEBtox Research, Stevensweert, The Netherlands (tjalling@debtox.nl, <http://www.debtox.nl/>).

6	Tab 3: Validation	17
6.1	General information	17
6.2	Fixing background hazard	18
7	Tab 4: Predictions	19
7.1	General information	19
7.2	Entering and inspecting profiles	19
7.3	Model predictions	20
7.4	Batch calculations	21
8	Saving and loading a project file, reporting	23
9	Final words	24
9.1	Some tips	24
9.2	Some workarounds	24
9.3	More information	25

1 Introduction and workflow

This document provides a short user manual for the openGUTS software. Version 1.0 of the standalone Windows version of the software was developed by WSC Scientific GmbH from the formulation and Matlab-based prototype as developed by DEBtox Research. Version 1.1 was developed by RIFCON GmbH, based on the modifications made to the Matlab version of openGUTS (in the update to Version 1.1).

This document focusses on how to operate the software; background documentation is available from the download page as well. The openGUTS design document contains the detailed background for the software, and there is a separate document that explains how to interpret the output of the model (in regular and irregular cases).

Note that openGUTS is distributed under the terms of the GNU General Public License, version 3. This document assumes knowledge about the GUTS concepts, following the formulation in the e-book [3].

General points:

- The workflow of openGUTS is based on the proposed workflow in the EFSA scientific opinion on TKTD models for use in aquatic risk assessment of pesticides [2]. However, (elements of this) workflow will be useful for many other purposes as well (including scientific research). For example, openGUTS also calculates classic $LC_{x,t}$ values (using the entire data set).
- The current version of the software only includes the simplest cases of the GUTS framework: GUTS-RED-SD and GUTS-RED-IT. These are the reduced models (toxicokinetics and damage dynamics combined) for the special cases of stochastic death (SD) and individual tolerance (IT).
- The software allows model calibration and model prediction for constant and time-varying exposure (using linear interpolation). Up to 5 data sets can be entered for simultaneous calibration.
- Special cases for fast and slow kinetics are *not* included (see the GUTS e-book [3], Appendix C). Instead, a wide default range for k_d is used, which will basically be the same for all data sets. This range will be sufficient for most applications (see also discussion in the interpretation document).
- The same input data format is used for the calibration and validation stages, with blocks for survival data and for defining the exposure scenario in the toxicity test. The prediction module requires exposure profiles to be specified in a simple two-column format (time and concentration). The Matlab version uses the same file format as the standalone, so input files can be used by both versions. However, the project files are *not* compatible.

The general workflow of the software is presented in Figure 1. A project file (extension .ogp) can be used to save all input data, the main text outputs from the calculations, and

the sample from parameter space used for error propagation. The project file can be saved and loaded to start working from a previous calibration.

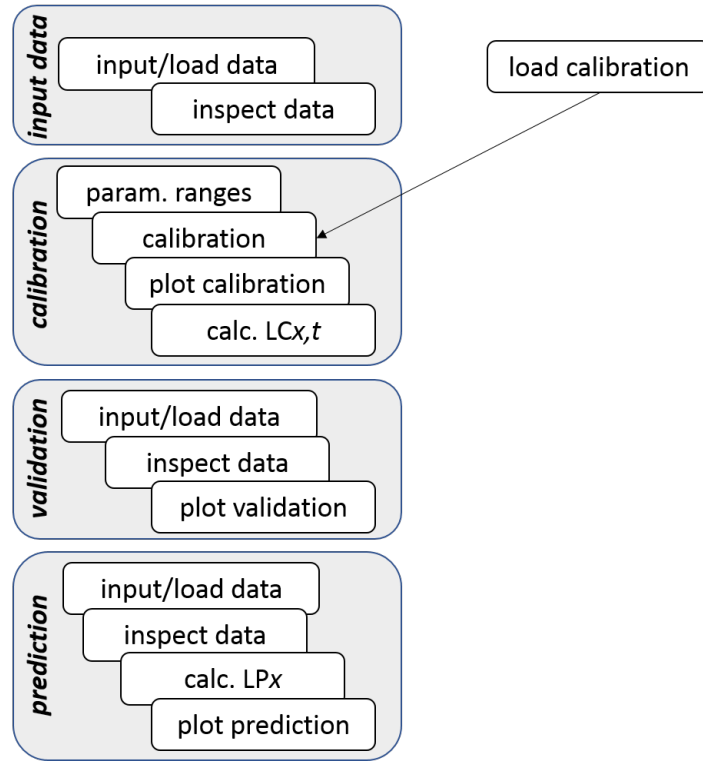


Figure 1: General workflow of the software. Blue fields indicate main tabs of the workflow of the software: input data, calibration, validation, predictions.

2 Installation and quick-start guide

2.1 Installation

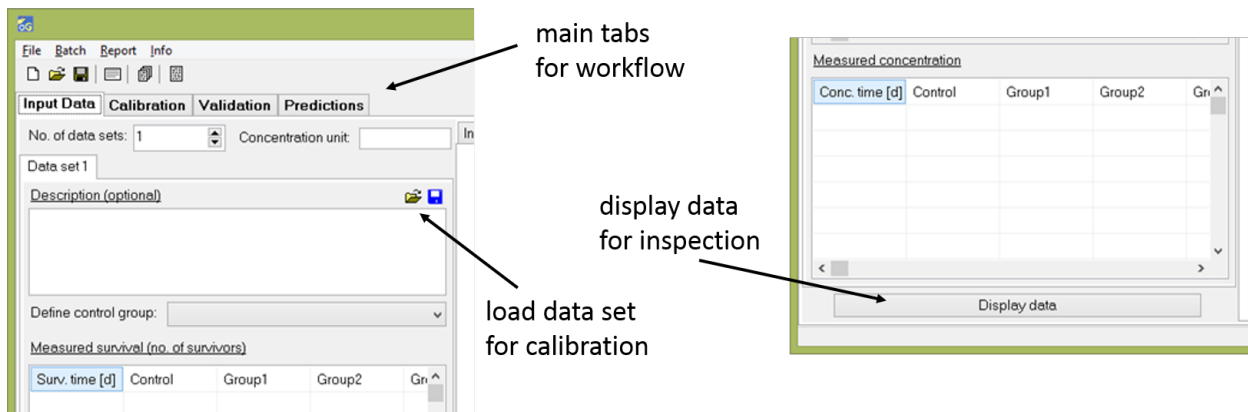
The installation file for the software can be downloaded from <http://openguts.info/download.html>. Run the Windows installer file `Setup_openGUTS.msi`, and the rest is rather self-explanatory. The installation location is free to choose (in contrast to v.1.0), but you do need to have administrator rights to your machine. Furthermore, installation may produce warnings from Windows or over-active virus scanners. Please note that any previous openGUTS installation must be completely removed prior to installing the new version 1.1. The installer also instructs you on that.

Example input files can also be downloaded from <http://openguts.info/download.html>. The quick start below makes use of these examples. These can be placed in any directory that you like to work in.

2.2 Quick start

For those that do not like to read manuals, this section offers a quick way to get acquainted with the software's operation. However, interpretation of the model output does require a more thorough understanding of what the software does. To this end, it is essential to consult the 'Interpretation of output' document from the openGUTS download page.

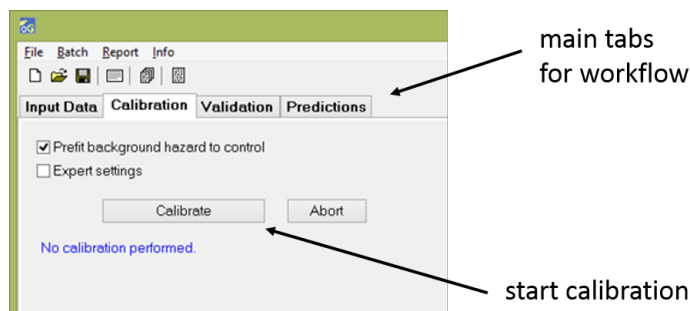
Tab 1: Data input. Locate and load the example file `propiconazole_constant.txt`, by clicking the 'file open' button on the tab for **Data set 1** (the files are in the directory `input_data` of the examples folder). Click the button **Display data** and check the graphs that are shown in the **Inspect data** tab on the right-hand side. These show the data set and serve as a visual check on the input data (especially handy for complex time-varying exposure scenarios).



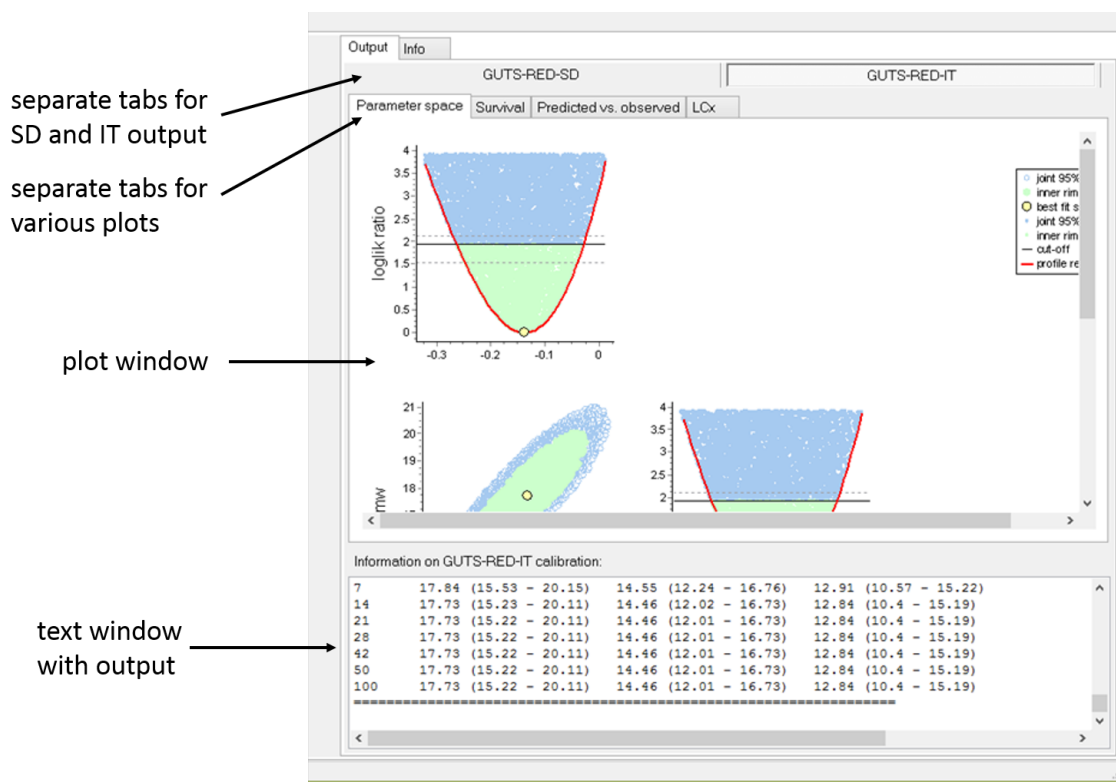
Tab 2: Calibration. Go to the tab **Calibration** and press the button **Calibrate**. The screens on the right will display the model output. Note the separate main tabs for the

two special cases GUTS-RED-SD and GUTS-RED-IT, and the sub-tabs for the various outputs. Detailed information on the fit (including the optimised parameter values with their 95% CIs, and LCx, t) is displayed in the text window (with a scroll bar) at the bottom.

By default, the box `Prefit background hazard to control` is checked. This will fix the background hazard to a value determined by a fit on the controls only (it is kept fixed while fitting the complete data set).



During the calibration, the progress is printed in the output text window. When the calibration is finished, graphs will appear in the plot window. Note the tabs for SD and IT output, and the sub-tabs for the various types of graphs.



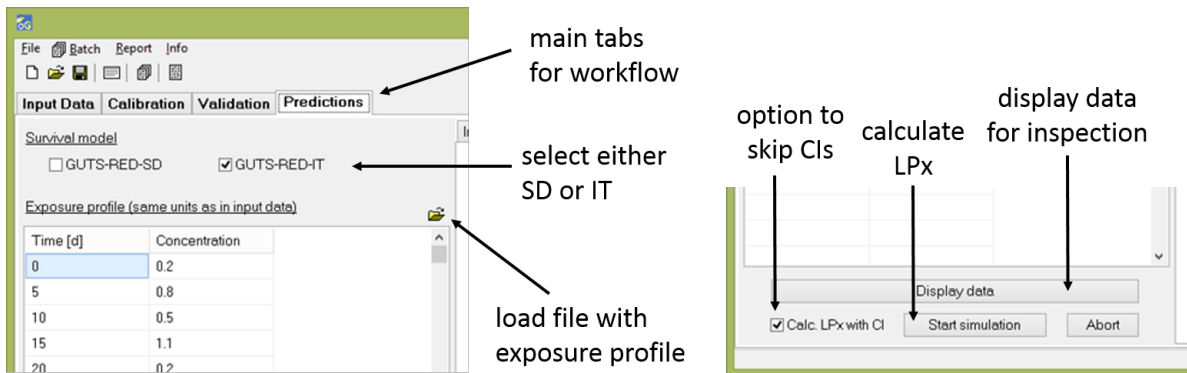
Tab 3: Validation. Go to the tab `Validation`. This screen is very similar to the `Input data` screen. From the example files distributed with the software, open the data

set `propiconazole_pulsed_linear.txt` for validation (also in the directory `input_data`). Click on `Display data` and check the graphs that are shown in the `Inspect data` tab on the right-hand side.

By default, the box `Fit background mortality to validation control group` is checked. This will fix the background hazard to a value determined by a fit on the controls from the validation data set only.

Press the button `Validate`. The output will appear on the right-hand side of the window, in the same form as for the calibration data (a plot window and a text output window).

Tab 4: Predictions. Go to the tab `Predictions`. Open an exposure profile (e.g., `test1.txt`, in the directory `input_profile`). Click on the button `Display data` and check the graphs that are shown in the `Inspect data` tab on the right-hand side. Select whether to use the SD or IT results, and make sure the option `Calc. LPx with CI` at the bottom of the screen is turned off. Calculations with CIs are considerably more time consuming. For this small test profile, calculation time is still reasonable (probably less than a minute), but for a full FOCUS profile, the SD calculation may take several hours.



Press the button `Start simulation`. The output will appear on the right-hand side. The plot under the `Survival` tab has the same form as for the calibration and validation data. The plots are shown for the exposure profile as is ($MF=1$), and for the profile multiplied by the factors that lead to 10 and 50% mortality at the end of the profile (i.e., the LP10 and LP50). The tab `Multiplication factor-response` provides a sort of dose-response plot for the multiplication factor.

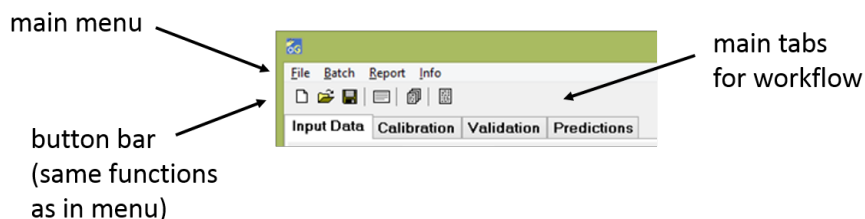
Finally: save and report. Now that you have finished an entire workflow in openGUTS, you can save a project file by pressing `File|Save` in the main menu (or the save button below that). This file has the extension `ogp` and has a plain text format. In the main menu `File|Open` (or the open button below the main menu) can be used to load a project file.

In the main menu, `Report|Create report` will construct a formatted MS Word document and a PDF with a report from the analyses performed. Make sure that MS Word is closed before trying to create a report.



3 General considerations

The top part of the screen has the standard-type Windows menu and button bar. Below that is the main series of tabs that relate to the four stages in the workflow of Figure 1.



3.1 Data sets

The openGUTS software is set up to allow calibration on data sets with constant and time-varying exposure. This requires two pieces of information for each treatment in a toxicity test: the observed survivors over time and the (measured or expected) exposure concentration over time. The exposure concentrations over time are linearly interpolated over the provided points.

Both the survivor block and the concentration block of input data need to be square and entirely filled. The first column contains the time points, and the first row contains identifiers for each treatment. Missing observations/information can be entered as a minus sign (-). The software will perform a number of checks on the input data and gives an error message if something is wrong (e.g., if the numbers of survivors are increasing in time).

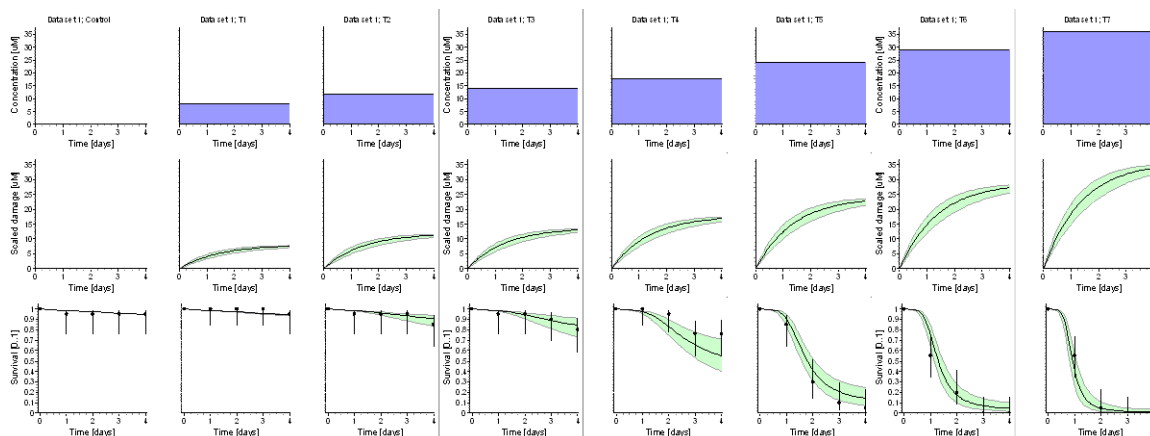
The exposure concentrations can be entered in any unit, but make sure that the same unit is consistently applied for all input data (the software performs some checks, but these are not foolproof). Note that the time vector of all data sets *must* be in days, otherwise the parameter ranges for calibration will be off.

The software allows simultaneous calibration on multiple data sets (up to 5). Just increase the **No. of data sets** and extra tabs will appear. Make sure all data sets are in the same concentration unit.

The input data format for the calibration and validation steps is the same, so that data sets can be interchanged if needed. The input format for the predictions stage is different, since that stage does not have survivor data. The predictions stage has only one block of numbers: a simple two-column (tab-delimited) text files, with time in the first row (these must be in days), and concentrations in the second (these must be in the same unit as that used for the calibration data set). These data points will also be linearly interpolated in the software. The example files on the download page provide a good overview of the data format, and the design document provides more detailed information.

3.2 Standard plotting format

The software applies a standard plotting format for exposure (top row), damage (middle row) and survival (bottom row) over time, for each treatment separately. The same format is used for all stages of the workflow.



Blue areas show the exposure profile over time (in this example: constant exposure). Green bands in the plot show 95% CIs on the model predictions. Survival data points are shown as fraction survival (between 0 and 1), and with an error bar. This error bar is the Wilson score interval, which is an indicator for how well the survival fraction can be estimated from this number of test individuals (higher number of individuals in the treatment will lead to smaller intervals).

4 Tab 1: Data input

4.1 Inputting data

The first step in the workflow is to enter data for calibration (or to load a saved project file from a previous calibration). The openGUTS software offers two ways to enter data.

Loading data from file. The software works with simple tab-delimited, plain-text input files. These files can be generated and modified by text editors or in software like Excel. The software comes with a set of example data files that illustrate various types of data sets. A simple strategy is to use these as template for your own data sets.

Use the input grids. The software offers input grids for the survival data and the exposure information (for the predictions stage only the latter). Information can be typed directly into the cells, but it is also possible to copy-paste from software like Excel. After entering all information (incl. concentration unit, and an optional description, for survival data sets), you can save the data in the standard text-file format.

Annotations for the screenshot:

- increase for simultaneous calibration on multiple data sets
- unit for reporting
- save data set shown in grids
- load data set for calibration
- select treatment for 'control'
- input grid survival data
- input grid exposure scenario
- display data for inspection

Surv. time [d]	Control	T1	T2	T3
0	20	20	20	20
1	19	20	19	19
2	19	20	19	19
3	19	20	19	18
4	19	19	17	16

Conc. time [d]	Control	T1	T2	T3
0	0	8.05	11.906	13.

4.2 Notes on input data

Treatment names and controls. Every column is a treatment, and the first cell should be the indicator (name) for that treatment. The treatments in the survivor block should

match those in the concentration block (the definition of the exposure scenario in the test). The name of the first column is fixed and cannot be changed (also do not change this in the text file as that will lead to errors). The names of the other columns are free to choose. It is a good idea to keep the name of the control as **Control**, and to use the first treatment for that purpose. However, the drop-down selection box allows you to select any treatment as the control (make sure that the correct treatment is selected there). Indicating the control is only needed to allow fixing the background hazard rate to the mortality observed in the control treatment.

Survival data. Make sure to enter absolute numbers of survivors, and *not* the fraction or percentage survival (or the mean of a number of replicates). This is essential for the statistics. Time points must be unique, and survivor observations must be whole numbers and cannot increase over time.

If the test design includes replicated treatments, it is strongly advised to sum the survivors over all replicates (use one column in the input blocks for the sum of all replicates for a treatment). For the statistical treatment, replication is irrelevant: combining replicates will yield the same results as entering all replicates as individual treatments (if the exposure profile is the same for all replicates). However, using many treatments will greatly increase calibration time (especially for time-varying exposure), as well as the number of graphs produced (which will also be more difficult to read).

Exposure data. The exposure concentrations over time are linearly interpolated over the provided points. If extrapolation is needed (i.e., the observations on survival are for time points beyond the range defined for the exposure concentrations), the last time point for the exposure concentration is used. That way, when working with constant exposure concentrations, it is sufficient to enter only a single row of exposure concentrations for time zero (see input data file `propiconazole_constant`).

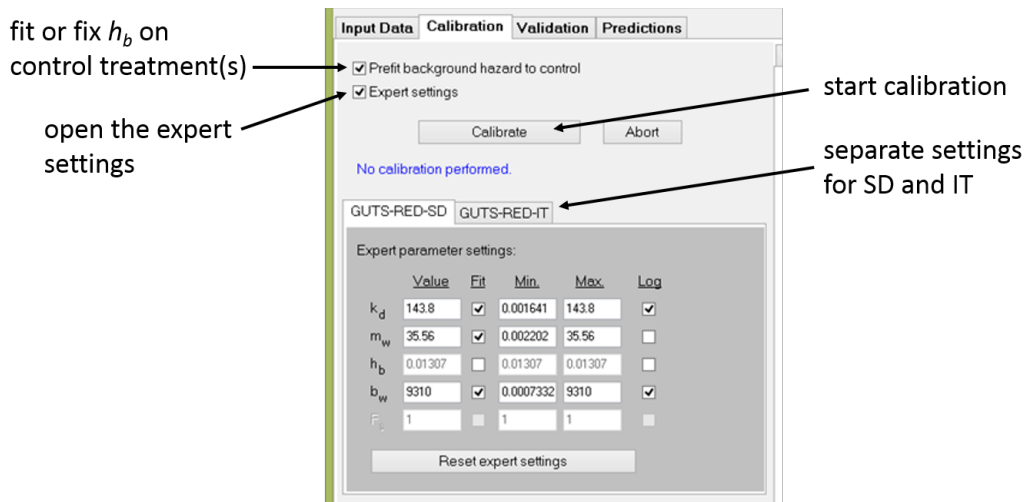
Entering more complex exposure profiles will take some getting used to, also because the software allows instant changes in exposure (i.e., hard pulses). The simplest way to get to grips with the data format is to study the example files provided, which demonstrate various exposure scenarios (see data file `propiconazole_pulsed_linear` for linear interpolation, and `propiconazole_pulsed_renewals` for hard pulses). If the change in concentration is indeed instantaneous (animals moved from one test container to another with a different concentration), the scenario definition with hard pulses is more accurate and slightly more efficient than adding additional time points as done in the first data set. More details are provided in the design document (on the openGUTS download page). Furthermore, it is always a good idea to use the button **Display data** in the software and perform a visual check on your exposure scenarios.

Exposure profiles. The data format for exposure profiles has no headers, and only one treatment is allowed. If more exposure profiles need to be analysed, each profile will need to be prepared as a separate text file. In that way, the same files can also be used for ‘batch calculation’ (running through many exposure profiles in sequence).

5 Tab 2: Calibration

5.1 General information

The openGUTS software applies a custom-designed algorithm to explore parameter space. This algorithm makes sure that the user does not have to worry about the technicalities of model optimisation (e.g., starting-value selection, checking for local minima, obtaining a representative sample for error propagation). Calibration is simply a matter of pressing a button.



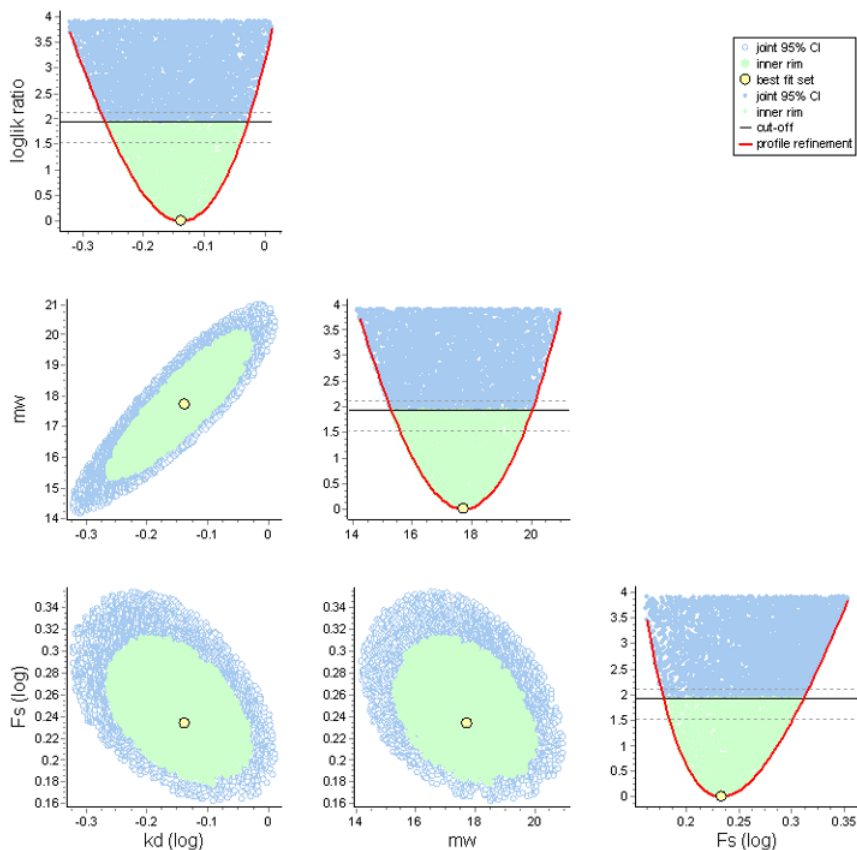
5.2 Fixing background hazard

In GUTS, background mortality is described by a constant background hazard rate (h_b). The software offers two options for dealing with estimation of this background hazard rate: fitting it to the observed survival in the control only, or fitting it together with the other parameters on the entire data set. Both options have their pros and cons, which are discussed in detail in the GUTS e-book [3] (Section 2.3.4 on Page 30).

Clicking the check box will fix the background hazard rate to the value already fitted on the control survival data. The control data set is the treatment that was indicated as **Control** from the drop-down menu on the tab **Input data**. If more than one data set is entered for calibration, the background hazard is determined from all control treatments simultaneously.

5.3 Model output

Graphs and other output will be produced in the right-hand side of the program window. Note the separate tabs for the SD and the IT fit, and the sub-tabs for the various graphs. The text window below provides the details of the model fit, the fitted parameters (with their CI), and the estimated LCx, t values.



The parameter-space plot provides important information about the optimisation and the identifiability of the model parameters from the data. In some cases, the parameter cloud will hit the minimum or maximum of the parameter search range.¹ This does not mean that the results cannot be used, but it does mean that these cases require a bit more scrutiny. Interpretation of these plots is explained in detail in the interpretation document.

5.4 Expert setting

In general, we *strongly* advise the user to work with the automatic settings of the calibration algorithm. However, in specific cases, expert users may want to change these settings. We don't expect that this will ever be needed in a regulatory setting for pesticides (in the context of [2]). Clicking the check box **Expert settings** opens the settings that will be used for calibration. Here, a user can fix a parameter to a certain value, change the search ranges, and change whether the parameter is to be fitted on log scale or not. Note that there are separate tabs for SD and IT, as (slightly) different settings apply to both models. Also note that the software requires you to fit at least two parameters (although you can set the ranges very tight). After calibration, the first column will be filled with

¹The Matlab version will show these boundaries in the parameter-space plot as blue lines. However, the standalone does *not* show them. The output in the text window will flag when the inner cloud (and thus the single-parameter CI) hits a min/max boundary.

the maximum-likelihood estimate for each parameter.

The parameter settings will reset back to the default values completely when closing the expert setting, changing the `prefit hb` setting, loading a new data set, starting a new project, and of course by clicking the `Reset expert setting` button. Leaving the expert setting open will lead to a flag in the output report, and there is an additional flag when the min-max ranges have been changed.

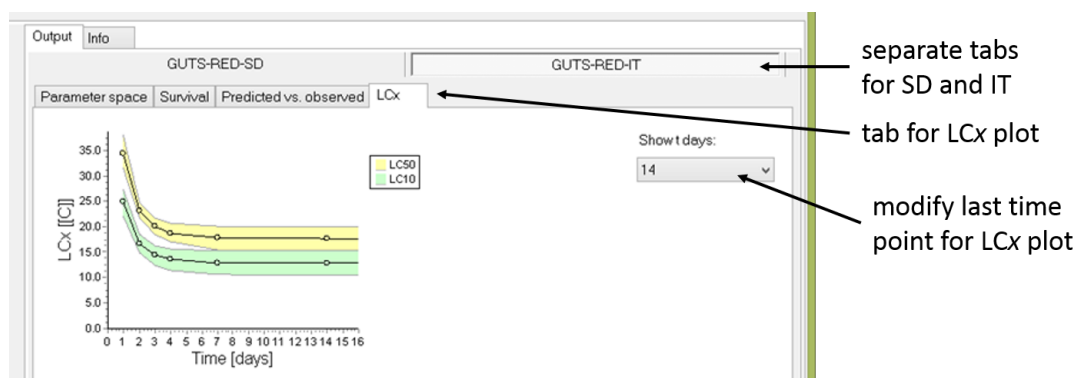
In case the optimisation algorithm spots ‘slow kinetics’ (low values of the dominant rate constant k_d , correlated to the value for the threshold m_w , see interpretation document), it automatically stops and restarts the algorithm with settings that work better in that case. Most importantly, the threshold m_w will be fitted on log-scale. This is not reflected in the expert settings, and a restart might not be triggered when a user changes the expert setting.

WARNING: The optimisation algorithm is extensively tested with the automatic settings, and has so far almost always worked properly, for a wide range of data sets. By manually changing the settings, it is certainly possible to make the algorithm perform poorly, or even fail completely.

Decreasing the search ranges is generally safe. This can be done if parameter space seems poorly sampled (see special cases in the interpretation document). Set the search ranges at least a bit wider than the CIs on the model parameters, and preferably a bit wider than the entire parameter cloud (blue points in the parameter-space plot). Keep k_d and b_w on log scale, unless their search range spans less than an order of magnitude.

5.5 $LC_{x,t}$ calculation

The classical $LC_{x,t}$ (for a range of effect levels x and time points t) is calculated in the calibration stage with 95% CIs. Even though it is technically a model prediction, this calculation does not rely on any form of input data and is quite rapid. Therefore, it is automatically performed after calibration. The $LC_{x,t}$ are displayed as a table in the text output window, and there is also a plot of LC10 and LC50 versus time under the output tab LCx (the range of the time axis can be set with a drop-down selection box).



the $LC_{x,t}$ values are calculated for a fixed range of x values (10, 20 and 50; only 10 and 50 are plotted) and t values (a range of typical values between 1 and 100 days). This

generally implies that LCx, t values are also calculated for time points beyond the range of the calibration data set. Furthermore, even when there is less than $x\%$ effect in a data set, LCx values will still be calculated. In both cases, it implies that these are extrapolations beyond the range of the data set, based on the calibrated model.

It should be noted that the LCx, t values are the concentrations that lead to $x\%$ effect, relative to the control, after t days of *constant* exposure to the toxicant. So even when the model is calibrated on data for time-varying exposure, the calculated LCx, t are predictions for the situation of constant exposure. In that way, the LCx, t values are (at least in theory) independent of the exposure scenario, and can be meaningfully compared between different test designs.

6 Tab 3: Validation

6.1 General information

The openGUTS software allows to use the calibrated models to make predictions for another exposure situation. This is done in the validation and predictions tabs. The fundamental difference between these tabs is that the validation tab expects survival data for the new exposure situation. The model prediction can thus be checked by comparison to independent data. For example, one can calibrate the SD and IT model to the data from a toxicity test with constant exposure, use the calibrated models to predict the survival probability (with CI) for a test with time-varying exposure, and compare that prediction to the observed survival in that experiment.

The screenshot shows the 'Validation' tab in the openGUTS software. It features a 'Description (optional)' field with a text area and a 'load data set for validation' button. Below this is a 'Define control group:' dropdown menu set to 'Control'. There are two data grids: 'Measured survival (no. of survivors)' and 'Measured concentration (same units as in input data)'. The survival grid has columns for 'Surv. time', 'Control', 'close puls', 'wide puls', and 'constant'. The concentration grid has columns for 'Conc. time', 'Control', 'close puls', 'wide puls', and 'constant'. At the bottom, there is a checkbox 'Fit background mortality to validation control group' and two buttons: 'Display data' and 'Validate'.

Annotations with arrows point to the following elements:

- save data set shown in grids (points to the save icon)
- load data set for validation (points to the load icon)
- select treatment for 'control' (points to the dropdown menu)
- input grid survival data (points to the survival data grid)
- input grid exposure scenario (points to the concentration data grid)
- perform validation and make plots (points to the 'Validate' button)
- display data for inspection (points to the 'Display data' button)
- fit or fix h_b on control treatment(s) (points to the checkbox)

To perform such a validation (or more strictly: output corroboration), enter or load a data set, press **Display data** to inspect the data set to see if it is the correct one (and entered correctly), and press the button **Validate**. The output will appear on the right-hand side of the window, in the same form as for the calibration data. The text window contains several goodness-of-fit measures (as proposed in [2]). Note that the software does not provide a conclusion on whether the correspondence is 'good enough'.

The input data format is the same for calibration and validation data. Therefore, the roles of two data sets can be reversed, or both can be used for a simultaneous calibration.

6.2 Fixing background hazard

As in the calibration stage, also for validation the background hazard rate can be fixed to a value fitted on the control of the validation data set. This is handy when the background mortality in the validation test differs from that in the calibration experiment. If this check box is not selected, the setting for the calibration is used: either fixed to the control of the calibration data, or fitted along in the calibration. Only in the latter case will you see CIs on the modelled survival probability in the control treatment.

7 Tab 4: Predictions

7.1 General information

The openGUTS software allows to use the calibrated model for predictions on new exposure profiles (i.e., other time series for the exposure concentration than used for calibration). This step can be useful for different purposes (e.g., test design), but the functionality of the software is geared towards assessing the toxicity associated with the output from fate models (e.g., FOCUS) or monitoring data series.

The software will not just produce a survival prediction for an exposure profile entered, but also calculates safety margins: by what factor do we need to multiply the exposure profile to yield 10 or 50% toxicant-induced mortality by the end of the profile [1, 2]. These factors are designated LP10 and LP50, which can be calculated with 95% CIs.

Survival model

GUTS-RED-SD GUTS-RED-IT

Exposure profile (same units as in input data)

Time [d]	Concentration
0	0.00002
0.041666667	0.00002
0.083333333	0.00002
0.125	0.00002
0.166666667	0.00002
0.208333333	0.00002
0.25	0.00002
0.291666667	0.00002
0.333333333	0.00002
0.375	0.00002
0.416666667	0.00002
0.458333333	0.00002
0.5	0.00002
0.541666667	0.00002
0.583333333	0.00002
0.625	0.00002
0.666666667	0.00002
0.708333333	0.00002
0.75	0.00002
0.791666667	0.00002

Display data

Calc. LPx with CI

select either SD or IT

load exposure scenario for predictions

input grid exposure scenario

display exposure scenario for inspection

calculate CIs (slow!) or not

perform LPx prediction and make plots

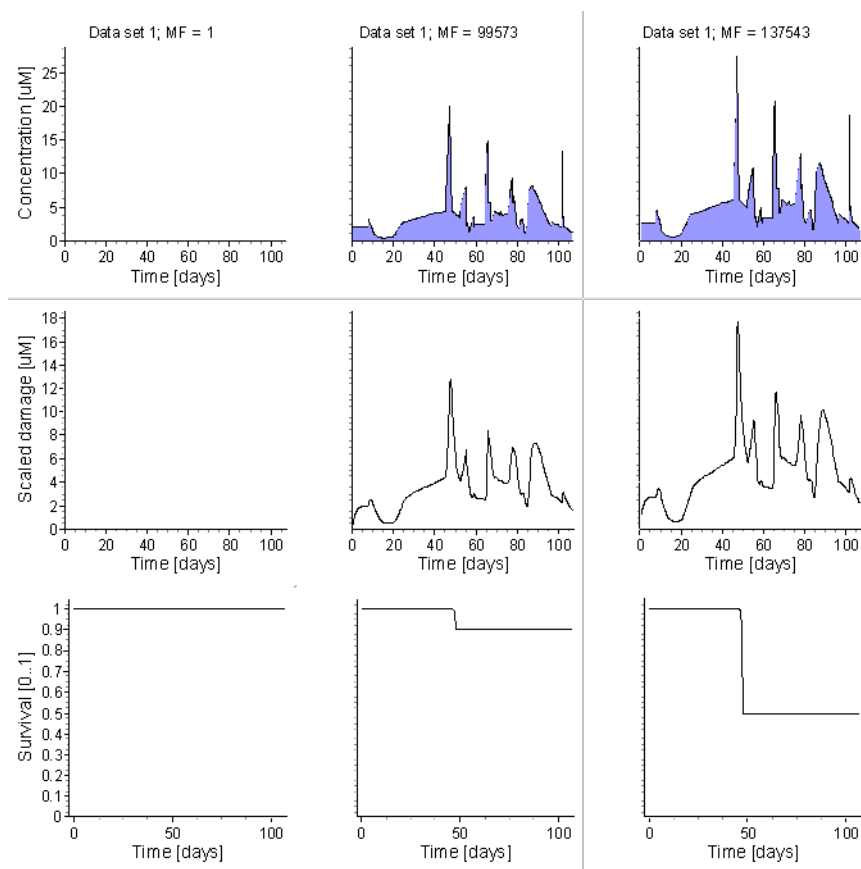
7.2 Entering and inspecting profiles

Enter or load a data set with an exposure profile, and press the button Display data to inspect the data set to see if it is the correct one (and entered correctly). Note that each data set can only contain one exposure profile. The profile is interpolated linearly, and the calibrated model is always run over the entire time series of the profile. Running the predictions over a part of the exposure profiles requires truncating the data set outside of the software (e.g., using Excel).

7.3 Model predictions

Select whether to make predictions with the calibrated SD or IT model. Also note the checkbox **Calc. LPx with CI**, which allows you to choose LPx calculations with or without CIs (these are the CIs for the LPx estimates; no CIs on the survival plots will be generated). The calculations with CIs will be considerably more time consuming (up to several hours for a FOCUS profile with the SD model). When calculating LPx with a CI, a progress bar will be shown; the calculation can be stopped with the **Abort** button.

Press the button **Start simulation** to start the predictions. The output will appear on the right-hand side of the window, in the same standard format as for the calibration and validation data (text window and plot window).



The plots show the damage and survival probability over time for the original profile (left column of panels), the profile times the LP10 (middle column of panels) and the profile times the LP50 (right column of panels).² Note that the survival plots only show the toxicant-related mortality (background hazard is set to zero). An additional plot is provided with survival (at the end of the profile) as function of the multiplication factor.

Only one set of output plots (either SD or IT) is shown on screen. However, if you calculated both SD and IT, both calculations will appear in an output report.

²The prediction plots are made with the rounded LPx values, so the final survival fraction in the plot may differ slightly from the expected 10 or 50%.

7.4 Batch calculations

Risk assessments for pesticides will often involve screening a large number of exposure profiles. To assist this procedure, the software offers calculations in ‘batch mode’. The user can select a range of exposure profiles (which have to be in the standard text format), and the software will run through them, calculate LP10 and LP50, and display the results.

Calculation of confidence intervals. By default, LP x batch calculations are performed without confidence intervals (CI). To include the estimation of CIs (new in v1.1), check the box **Calc. LP x with CI**.

On checking this box, sliders will appear that allow specifying the number of parameter sets (sample size) that shall be used to estimate the CI. The number can be varied from 100 (default) to the number of parameter sets selected during the calibration step. Alternatively, the number can be directly entered in the edit box. The edit box is turned red, until a valid number between 100 and the maximal number of parameter sets is entered. The sample sizes can be specified separately for GUTS-RED-SD and GUTS-RED-IT.

Estimating CIs from large samples (many parameter sets) is time consuming. Yet, with increasing sample size the accuracy of estimated CIs increases. Tests indicate that even small samples (around 100 parameter sets) are sufficient to reasonably approximate the confidence intervals.

The screenshot shows the 'Batch prediction - openGUTS 1.1' window. At the top, a list of files is shown, with arrows pointing to them labeled 'files selected for processing'. On the right, there are 'Add file', 'Delete file', and 'Clear' buttons, with an arrow pointing to them labeled 'add files for processing'. Below the file list, there are checkboxes for 'Do GUTS-RED-SD predictions' and 'Do GUTS-RED-IT predictions', with arrows pointing to them labeled 'select either or both SD or IT'. Below these are sliders and input boxes for 'Sample size for calculating CI', with an arrow pointing to them labeled 'sliders for sample size CIs'. At the bottom left, there is a 'Calc. LPx with CI' checkbox, with an arrow pointing to it labeled 'select calculation of confidence intervals'. At the bottom right, there is a progress bar and a file name '...apple_R2_stream.txt', with an arrow pointing to it labeled 'file currently processing and progress bar'. At the bottom, there is a table of results, with an arrow pointing to it labeled 'unsorted LPx values'.

File	LP10 (SD)	0.025 CI	16.42	16.71	15.71	LP50 (SD)	12.17	0.025 CI	14	0.975 CI	14.71	LP10 (IT)	18.81	0.025 CI	0.975 CI
apple_R1_pond	15.9	14.85	16.42	16.71	15.71	17.33	12.17	9.983	14	16.82	14.71	18.81			

Display of LP x . The estimated LP x values (including CIs) for each exposure profile are printed in the output screen, after calculation. The values are not sorted. However, the output table can be copied-pasted into Excel and sorted on any of the columns.

Batch prediction results can be included in an output report and in the project file. If you want to do so, please close the batch prediction window and follow the instructions

in Section 8. The results that are reported or saved are always the results from the last batch prediction. Therefore it is recommended to rename the files, if new batch prediction results shall be stored.

Closing the batch prediction window keeps the batch prediction results, which can be accessed after reopening the batch prediction window.

Example analysis. The software is distributed with the exposure profiles for propiconazole as used in the EFSA opinion on TKTD models [2]. These profiles are available in the directory `input_profile\efsa_so_scenario` from the example files. This allows the user to duplicate the case study used in that report. However, it should be noted that in the calibration data, exposure concentrations are in μM , whereas the exposure profiles are in $\mu\text{g/L}$. This was an error in the EFSA opinion, which we decided not to correct for the example data.

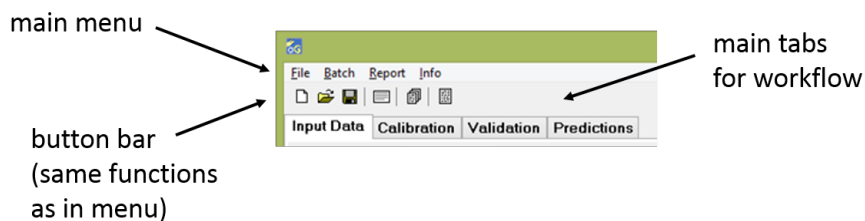
Known issues with batch processing.

- Note that choosing a new project does not erase the file names of the profiles used for your previous round batch processing. The results are cleared, however.

8 Saving and loading a project file, reporting

Once a calibration is performed, there is no need to repeat the calibration to make new validations or predictions. At any point in the analysis, you can save a project file, either from **file** in the main menu, or the disk symbol in the button bar. This creates a readable text file (extension `.ogp`), but it is strongly advised not to edit it. You can load a project file either from the **file** in the main menu, or the ‘file open’ symbol in the button bar. Loading will make the calibration results and parameter-space sample available for further validation and predictions. The outputs of the calibration (plot and text windows) will be reconstructed as much as possible from the `.ogp` file (which a.o. lacks the information for the red line in the parameter profile plots). Output for the validation and prediction stages is *not* reconstructed. However, results of the last batch prediction will still be available in the batch prediction window.

Loading a project file can take around one minute, because the calibration information must be restored. Also, loading the project file over a network can slow down the process. At the start of loading a project file, a window pops up that explains the loading progress. Please wait, until the window automatically closes.



You can create an output report from the **report** in the main menu, or the last symbol in the button bar. This will create an editable MS Word document and a pdf file with a formatted report of the analyses performed. Word needs to be closed before creating a report.

Known issues with project files.

- Project files (extension `.ogp`) saved in openGUTS v1.0 can also be loaded in openGUTS v1.1. However, loading a v1.0 project file will not erase any previous batch-calculation results (since batch information was not stored in v1.0).
- Do not save a project file *before* calibration. This leads to a corrupted `.ogp` file that cannot be loaded anymore.

9 Final words

9.1 Some tips

You can copy the plots by clicking on them with the alternate mouse button. Next, you can paste them in other applications, such as MS Word or PowerPoint. This is also practical to see the entire plot at a glance, since the screen (and the report) will cut off the larger plots.

The survival plots also have the option to copy the data, so that the user can make their own plots in any format that they like.³

9.2 Some workarounds

For the openGUTS software, we limited the functionality to what we think are the most common cases for model application. For more complex analyses, other software platforms will need to be used. The Matlab version (which acted as the software's prototype) is also available for download, and includes some additional functionality. Furthermore, since it is coded in Matlab, it allows easier modification to allow specific custom analyses. The openGUTS website contains links to other software implementations (<http://openguts.info/support.html>). However, some analyses can still be accommodated in the standalone software, with a little extra work.

Mimic the full model. To some extent, users can mimic the full models GUTS-SD and GUTS-IT by calculating the body residues over time themselves in sufficient time resolution (e.g., with a one-compartment model), and entering them as a time-variable exposure scenario for the reduced models in the software. This should work well enough, although the uncertainty in the body residues cannot be propagated through to the survival probability anymore.

Missing individuals. It is good to note that we can also accommodate experiments where individual animals went missing or were removed during the test (e.g., for body-residue analysis). The simple solution is to split up the treatment in two (which need a different identifier). The animals that are removed/missing at a certain point get their own treatment: they are counted as survivors as long as they are in the test, and as 'missing data point' after they have been removed/went missing. This is a bit of work when creating the input data set, though. Furthermore, the plots will be difficult to interpret in terms of goodness-of-fit (all mortality is assigned to the newly-split treatment without removal of animals).

Validating on LC50s. Users can also validate on LC50s (or LC x in general) by entering them as a data set with constant exposure and some fake data: e.g., 10 survivors at $t = 0$

³The model output is copied correctly, but the input data are not: the CIs for the survival data points are incorrect, and for time-varying concentrations in calibration/validation, the exposure values show nonsense.

and 5 survivors at the time point at which the LC50 was determined. Every LC50 would then need to be a new treatment. The only thing to note is that the CI on the data point will be meaningless. It would even be possible to calibrate the model on LC50s over time, although it is unlikely that these will contain sufficient information to identify all model parameters (and the CIs will again be meaningless).

9.3 More information

The openGUTS download page (<http://openguts.info/download.html>) contains a document that explains how to interpret the various graphs and text output that the software generates. It also holds a design document that provides technical background on the calculations. Furthermore, it contains a list of known issues with the current version, and options for further functionality that may be added to future versions. Please consult this list before reporting bugs.

For questions, comments and bug reports, we would appreciate if you use the discussion forum at <http://board.debttox.nl>. This makes sure that others can see your questions, and the answers provided, which hopefully minimises email traffic (and helps other that run into the same issues). However, please note that this is *not* a help desk. To post on the discussion board you need to register with an email address, and the admin (Tjalling) has to manually approve you. This is done to keep the spambots out. If you have problems registering just send an email to Tjalling and an account will be created for you.

References

- [1] R. Ashauer, P. Thorbek, J. S. Warinton, J. R. Wheeler, and S. Maund. A method to predict and understand fish survival under dynamic chemical stress using standard ecotoxicity data. *Environmental Toxicology and Chemistry*, 32(4):954–965, 2013.
- [2] EFSA. Scientific opinion on the state of the art of toxicokinetic/toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. *EFSA journal*, 16(8):5377, 2018.
- [3] T. Jager and R. Ashauer. *Modelling survival under chemical stress. A comprehensive guide to the GUTS framework*. Toxicodynamics Ltd., York, UK. Available from Leanpub, https://leanpub.com/guts_book, Version 2.0, 8 December 2018, 2018.