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TK-TD Model of *Lemna* Populations (MoLePo)
Version 1

User Manual

Authors

Judith Klein

Dr. Udo Hommen

Fraunhofer Institute for Molecular
Biology and Applied Ecology (IME)

Auf dem Aberg 1

57392 Schmallenberg, Germany

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Content

1. Introduction

This manual describes version 1.0 of the user friendly computer program MoLePo, which stands for "TK-TD model for *Lemna* populations". The program is developed by Udo Hommen and Judith Klein. The program is originally based on the *Lemna* model by Schmitt et al. 2013. MoLePo predicts growth of *Lemna* population in dependence of environmental conditions (temperature, global radiation, and phosphate and nitrate concentration) as well as concentration of up to two active substances.
The model itself, its implementation and testing is described in detail in a TRACE documentation (Klein & Hommen 2018).

Support:
MoLePo is available on request. In case of questions, comments or suggestions, please contact:

Judith Klein: judith.klein@ime.fraunhofer.de

Udo Hommen: udo.hommen@ime.fraunhofer.de

2. Installing MoLePo

MoLePo is provided as an installation package to create an executable local program file plus some example data sets. Open the file setup.exe and follow the instructions to install MoLePo. Please select first the language for the setup and decide on a few details of the installation:

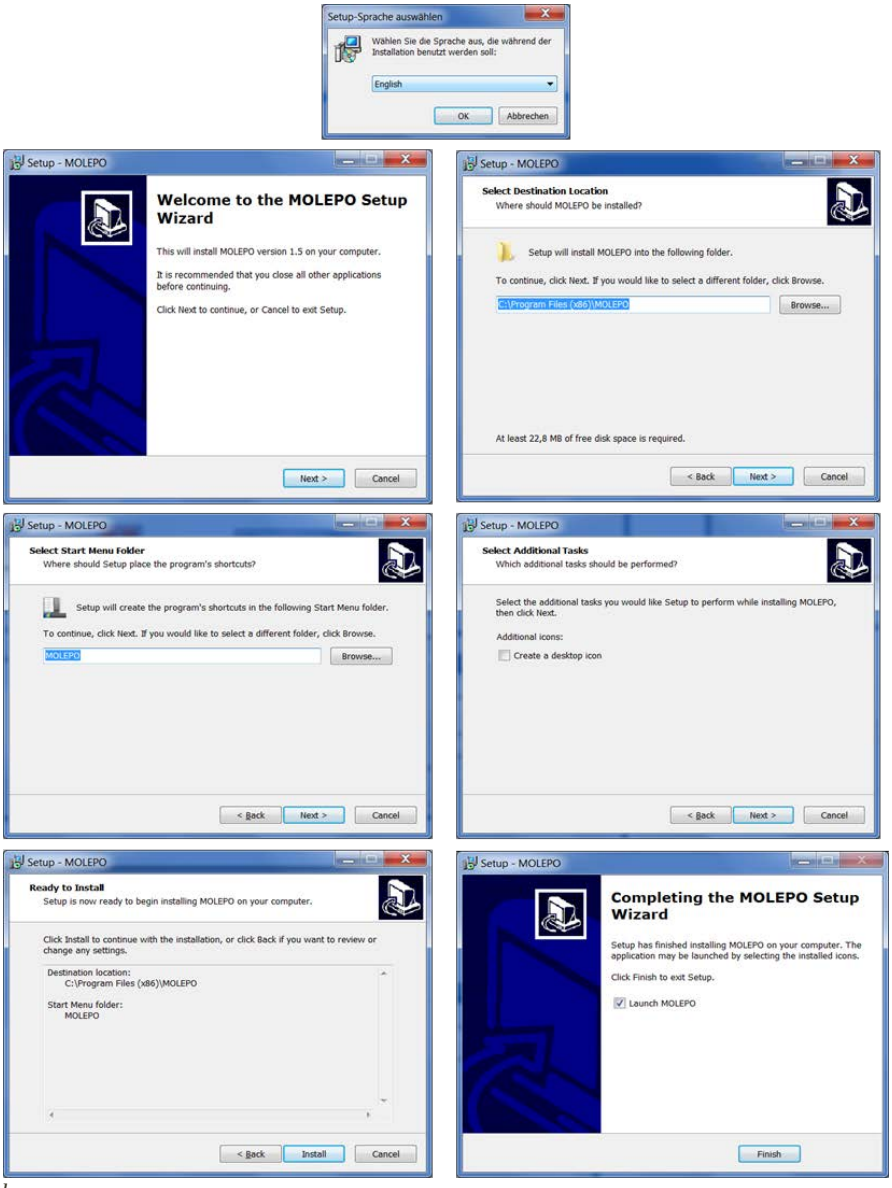


Figure 1: Installing the program

At the end of the setup, you can decide to launch MoLePo directly of or exit the program (Figure 1).

3. Working with MoLePo

The program offers three different procedures:

1. Calibration: Fitting the TK-TD model parameters using experimental data sets (growth inhibition tests in the laboratory),
2. Verification: Comparing model outputs with experimental data (usually from laboratory growth inhibition tests not used for calibration), and
3. Prediction: Simulation of not tested exposure patterns with increasing exposure magnitudes of the *Lemna* populations in the laboratory or the field.

In Figure 2, the start screen of the program is presented.

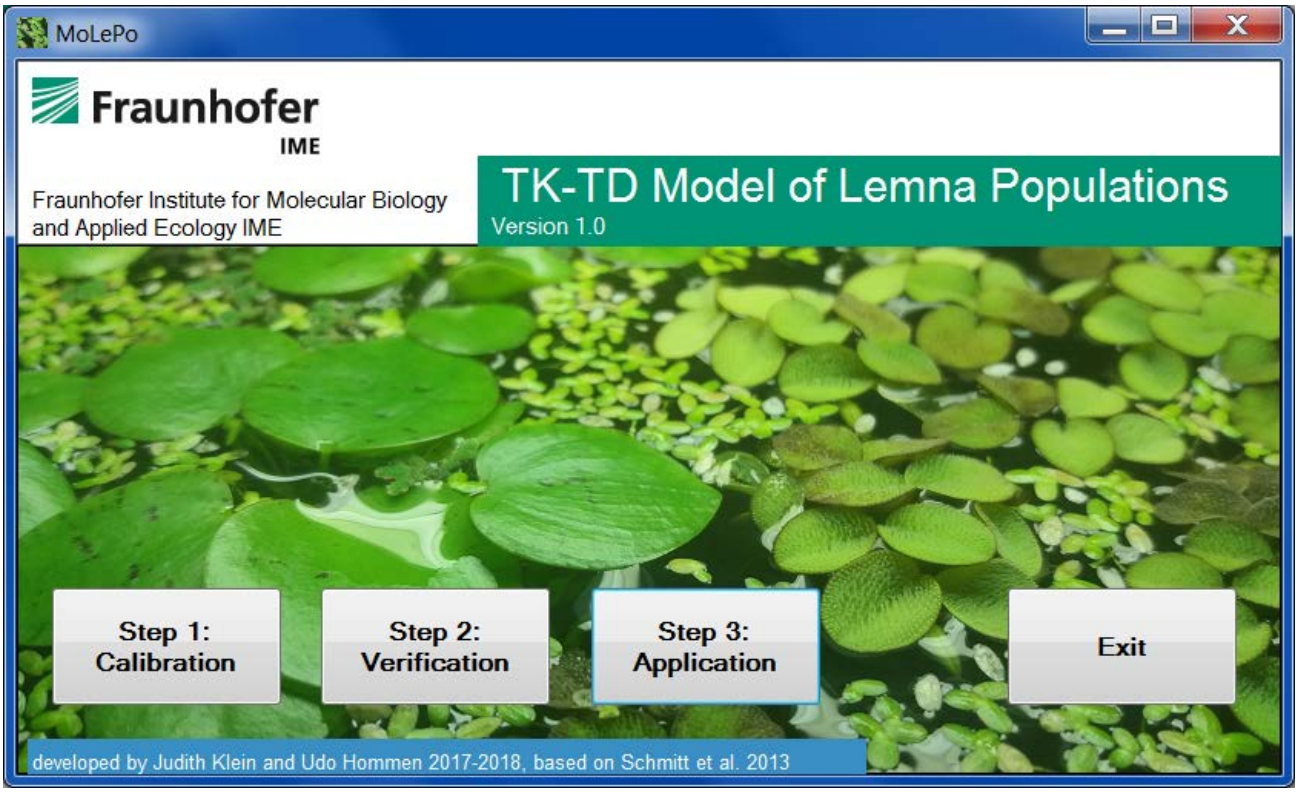


Figure 2: Start up window of the program TK-TD Model of Lemna Populations

3.1 Calibration

The aim of the calibration is to find the substance specific TK-TD parameters by minimizing the deviations between experimental data sets and model outputs.

The experimental data have to be provided as Excel files. It is possible to use more than one data set (i.e. growth inhibition test) as input for the calibration. Note that in the current version, only the mean frond numbers and biomasses per treatment level are considered (no use of the data per replicate).

Before the calibration can start, different settings have to be made, which are described in the following screenshots. By clicking at the calibration button, the following calibration form appears (Figure 3).

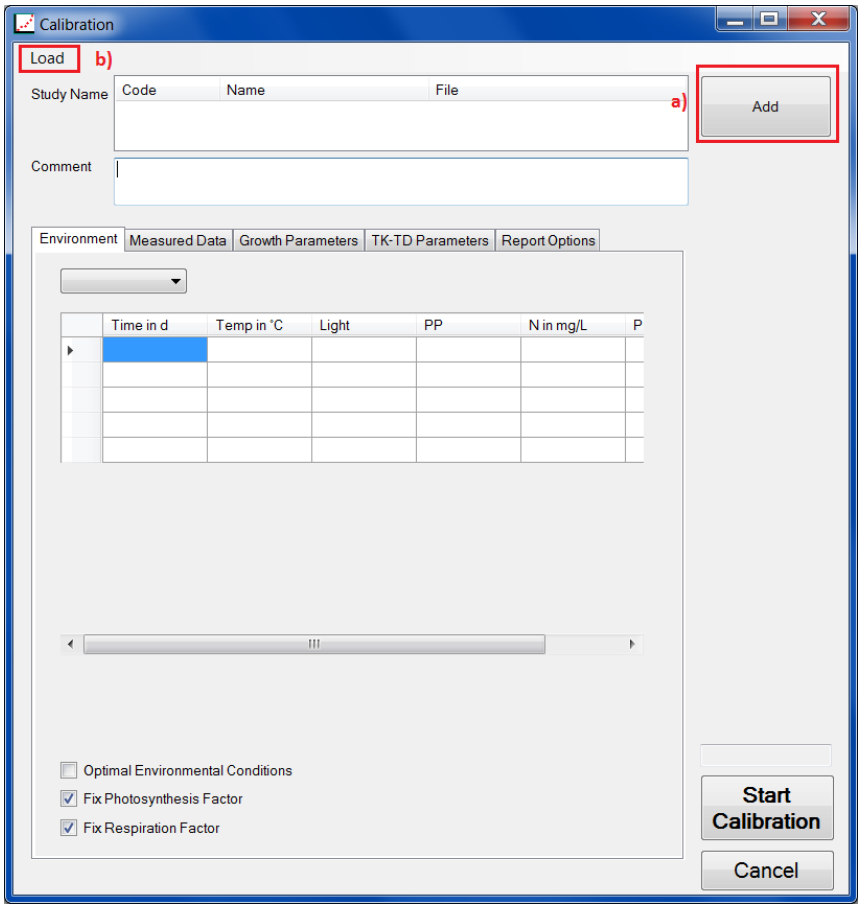


Figure 3: Calibration form

At first, we have to add experimental data. We can do so by clicking the add button and adding an Excel file including the data or clicking at Load and load a script file, that contains besides the experimental data also the calibration's settings (Figure 3) .

We add as example data sheet the data used by Schmitt et al. 2013. It is possible to add further studies by clicking at the add button. In this case, we only add one study by clicking at "add" and by choosing one study excel file.

The excel file is selected by browsing files on your computer. In Figure 33, an example of such an experimental data file is presented.

As the aim of the calibration is to find values for the TK-TD parameters, it is more suitable to use the growth rate observed in the control directly in the model instead of simulating the control growth from the experimental conditions. For this, it is not needed to enter the environmental conditions of the specific test if they can be assumed constant during the test but use a fixed photosynthesis and respiration rate.

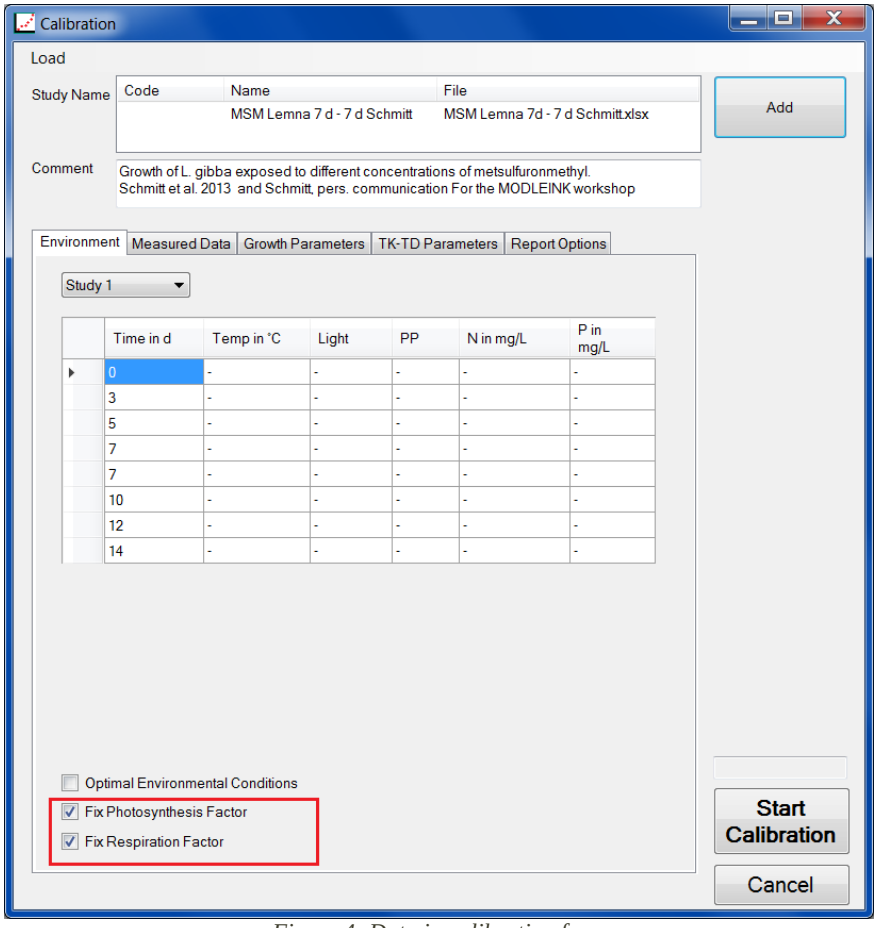


Figure 4: Data in calibration form

The program enters the information of the study file (environmental data and measured experimental data) in the program's surface. In the tab "measured data", one can see the external concentration, frond number and biomass value in time (Figure 4).
The program calculates yield and growth rate with the corresponding percentage inhibition with respect to the control. This is done using least squares as objective. If the biomass is reset at a certain day to a certain biomass the program calculates the growth rates for the respective time periods. The fitted growth rate of the control will be used by the model to calculate the photosynthesis rate of the control.

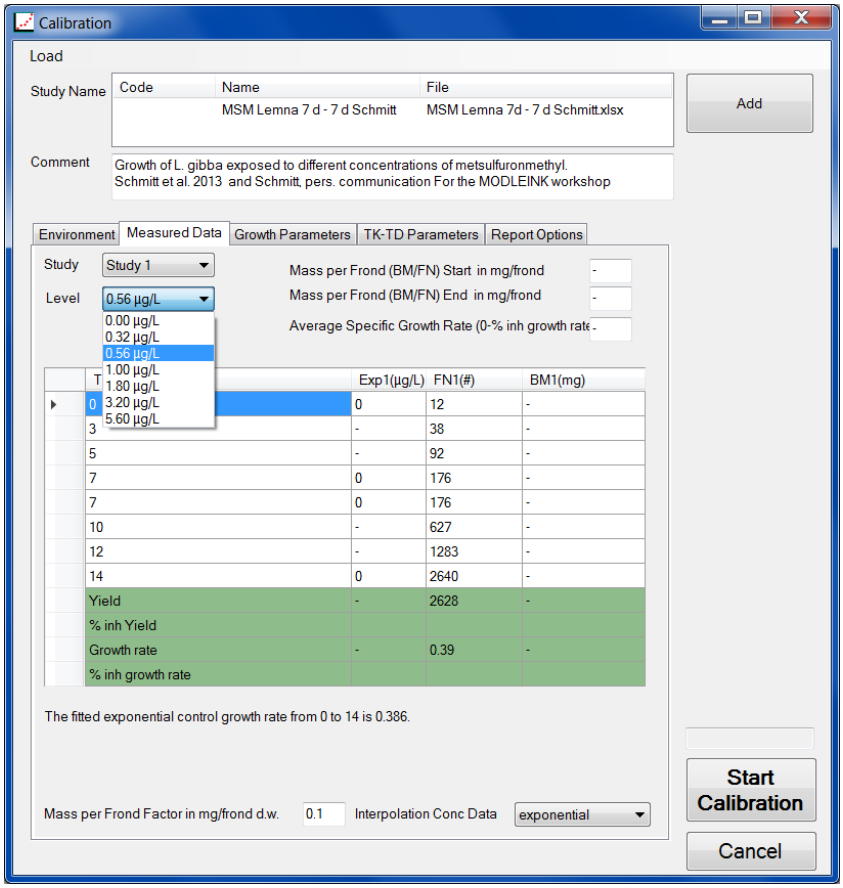


Figure 5: Measured data tab in calibration form

The program uses a fixed conversion factor from frond number to dry biomass. The dry biomass is calculated by multiplying the "Mass per Frond Factor" to the frond number (Figure 5). In this case, the conversion factor is 0.1 mg dry weight per frond, the value propose by Schmitt et al. 2013. However, based on study specific measurements, another value can be entered.

If frond number and biomass data is available the program calculates the mass per frond at the start point and the end point of the experimental test, such that the user can compare the constant standard mass per frond value (0.1 mg dry weight per frond) and the current entered experimental data.

To obtain external concentration data for all time points the program offers the probability to interpolate the data exponentially or linearly (Figure 5).

The next tab "Growth parameters" presents the growth related parameters (Figure 6).

The growth parameter values (in red) are the default parameters given in Schmitt et al. (2013).
In case of simulation of laboratory tests with constant environmental conditions and thus, a constant control growth rate, only the maximum photosynthesis rate (`kmax_photo`), the respiration rate (`kref_resp`), the initial biomass (`BM_0`) and the conversion factors `AperBM`, `massperfrond` and `BMw2BMd` are important.

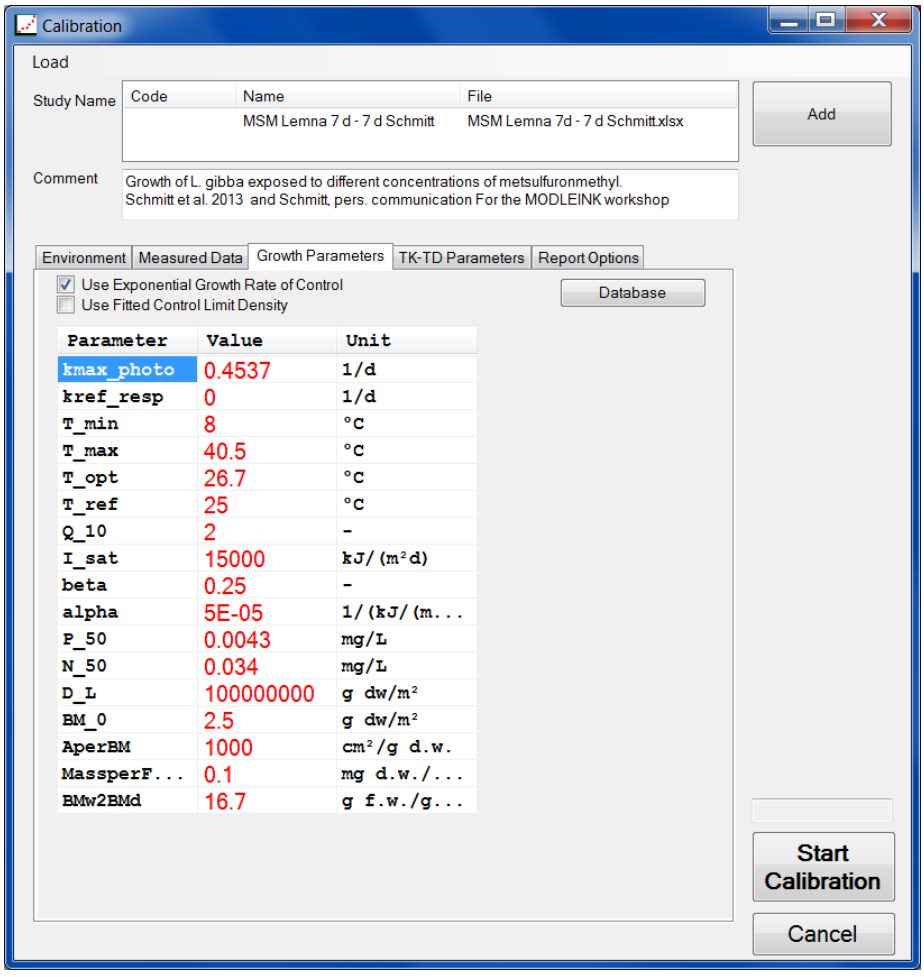


Figure 6: Growth parameters tab in calibration form

The checkboxes concerning the control growth rates indicate the type of control growth, e.g. exponential (the usual case in more recent tests) or logistic (e.g. in cases when the control growth becomes smaller at higher abundance). In the latter case, also a limit density is fitted from the control data.
In case, that you uncheck both boxes, the program uses the default values of `kmax_photo` and `kresp_ref` given in the data grid.

By clicking the Database button it is possible to save or load growth parameter sets (Figure 7).

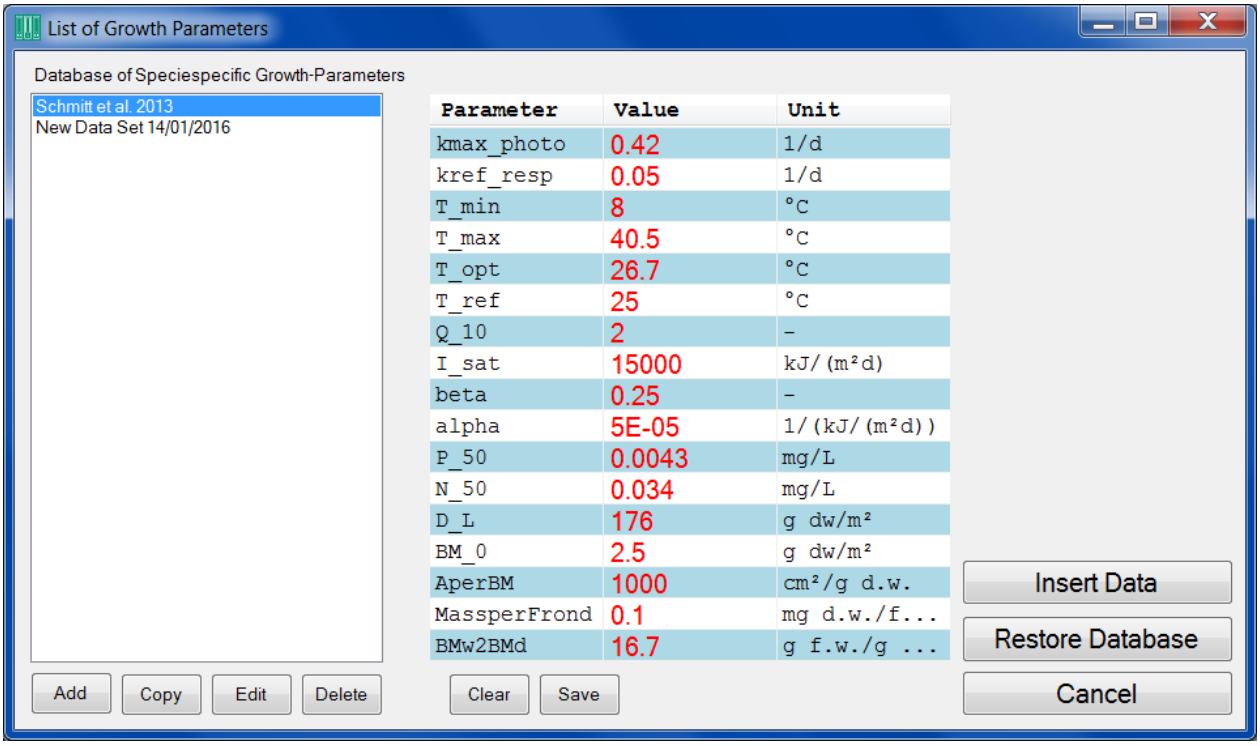


Figure 7: Data base of growth parameters

The next tab “TK-TD Parameters”, Figure 8, is about the substance specific TK-TD parameters and the calibration settings, e.g. which parameters should be calibrated, what are initial values and ranges for the parameters and what should be the objective function (e.g. the measure to be optimized during calibration).

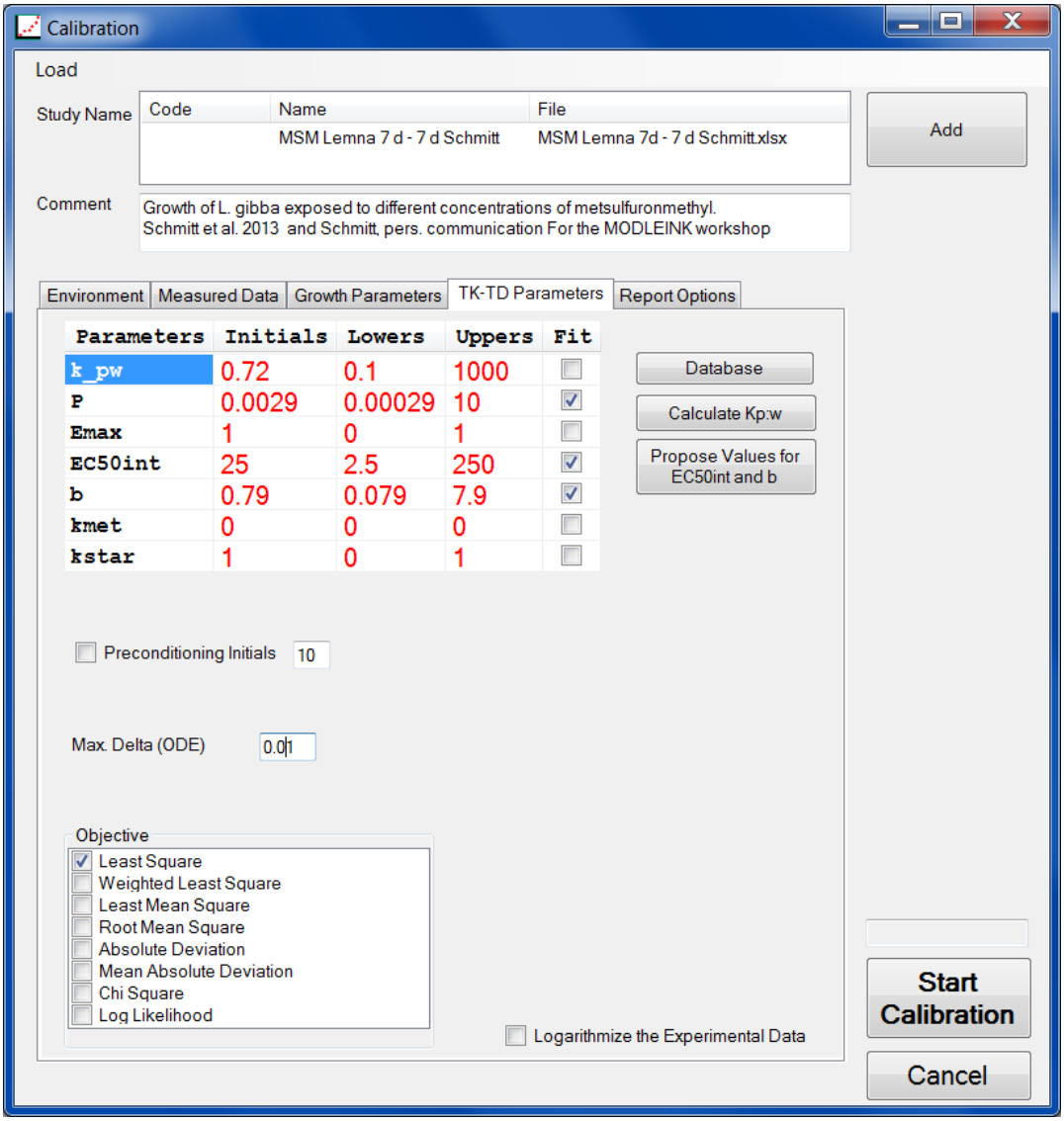


Figure 8: TK-TD parameters tab in calibration form

By clicking the "Calculate Kp:w" button, one can calculate the plant water coefficient and permeability by regression from the logKow of an active substance. We implemented the regression models by de Carvalho et al. 2007 for the plant water coefficient Kp:w and Heine et al. (2015) for permeability P (see TRACE documentation).

In this example, we insert in the calculator (Figure 9) for MSM a logKow of -1.87 (EFSA 2015).

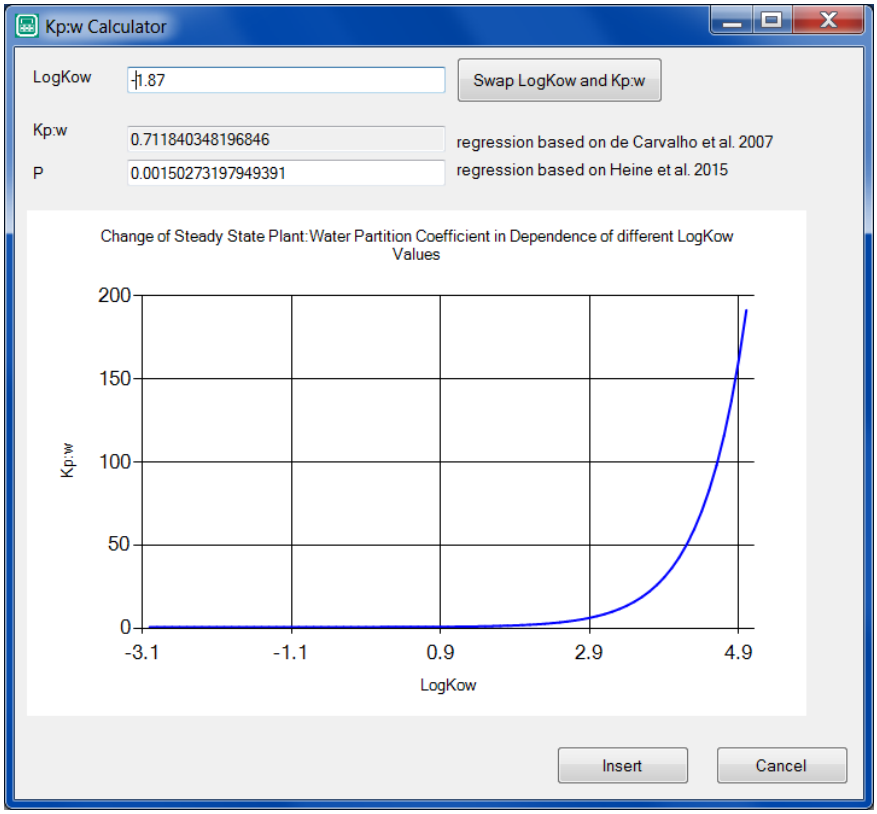


Figure 9: Kp:w Calculator

If the resulting Kp:w value is lower than 0.94, it is recommended to use then a Kp:w value of 0.94 since otherwise the internal unbound concentration could become higher than the external concentration (see TRACE documentation).

If you are not sure, which values are realistic, you can click the Propose button and the program makes suggestions for EC50 and slope b based on external concentrations (Dose response curve based on external concentration).
In addition to that you can save TK-TD parameters and load parameter values derived in other calibration runs by clicking the Database button. A similar window opens as concerning the growth parameters containing a database of all saved TK-TD parameter sets.

The preconditioning check box is to test several initial values to have greater possibility to find a good optimization solution. Instead of one initial parameter value set, the selected number of sets are tested with the parameters chosen randomly out of their ranges.

The “Max. delta (ODE)” check box is about the precision of solving the ordinary differential equation. It is valid for calculation of both, internal concentration and biomass, but a relative value.

The smaller the value, the more exact is the solution of differential equations but the longer the calibration takes.

In the objectives box, several measures for the deviation between model results and data are available. The most common measure is the least square. Below a list of all implemented objective functions is presented (Table 1: Implemented objective functions for calibration).

Let $N \in \mathbb{N}$ be the total number of data points, $O \in \mathbb{R}_+^N$ be the observations and $C \in \mathbb{R}_+^N$ be the calculations. Furthermore, let $\overline{O}, \overline{C} \in \mathbb{R}_+$ be the means of the respective data.

Table 1: Implemented objective functions for calibration

Objective	Formula
Least Square	$\|O-C\|_2^2 = \sum_{i=1}^{N} (O_i - C_i)^2$
Weighted Least Square	Let $w_i \in \mathbb{R}_+$, $\sum_{i=1}^{N} w_i \cdot (O_i - C_i)^2$
Least Mean Square	$\sum_{i=1}^{N} \frac{1}{N} (O_i - C_i)^2$
Root Mean Square	$\sqrt{\sum_{i=1}^{N} \frac{1}{N} (O_i - C_i)^2}$
Absolute Deviation	$\|O-C\| = \sum_{i=1}^{N} \|O_i - C_i\|$
Mean Absolute Deviation	$\|O-C\| = \sum_{i=1}^{N} \frac{1}{N} \|O_i - C_i\|$
Chi Square	$\chi^2 = \sum_{i=1}^{N} \frac{(C_i - O_i)^2}{O_i}$
Log Likelihood	$\ln(L) = \frac{N}{2} \cdot \ln\left(\sum_{i=1}^{N} (O_i - C_i)^2 \right)$

It is possible to log transform the data before calculating the objective function to down-weight larger deviations to be expected for the periods with high frond-numbers or biomass.

In the last tab “Report Options“, the design of the output can be specified (Figure 10).

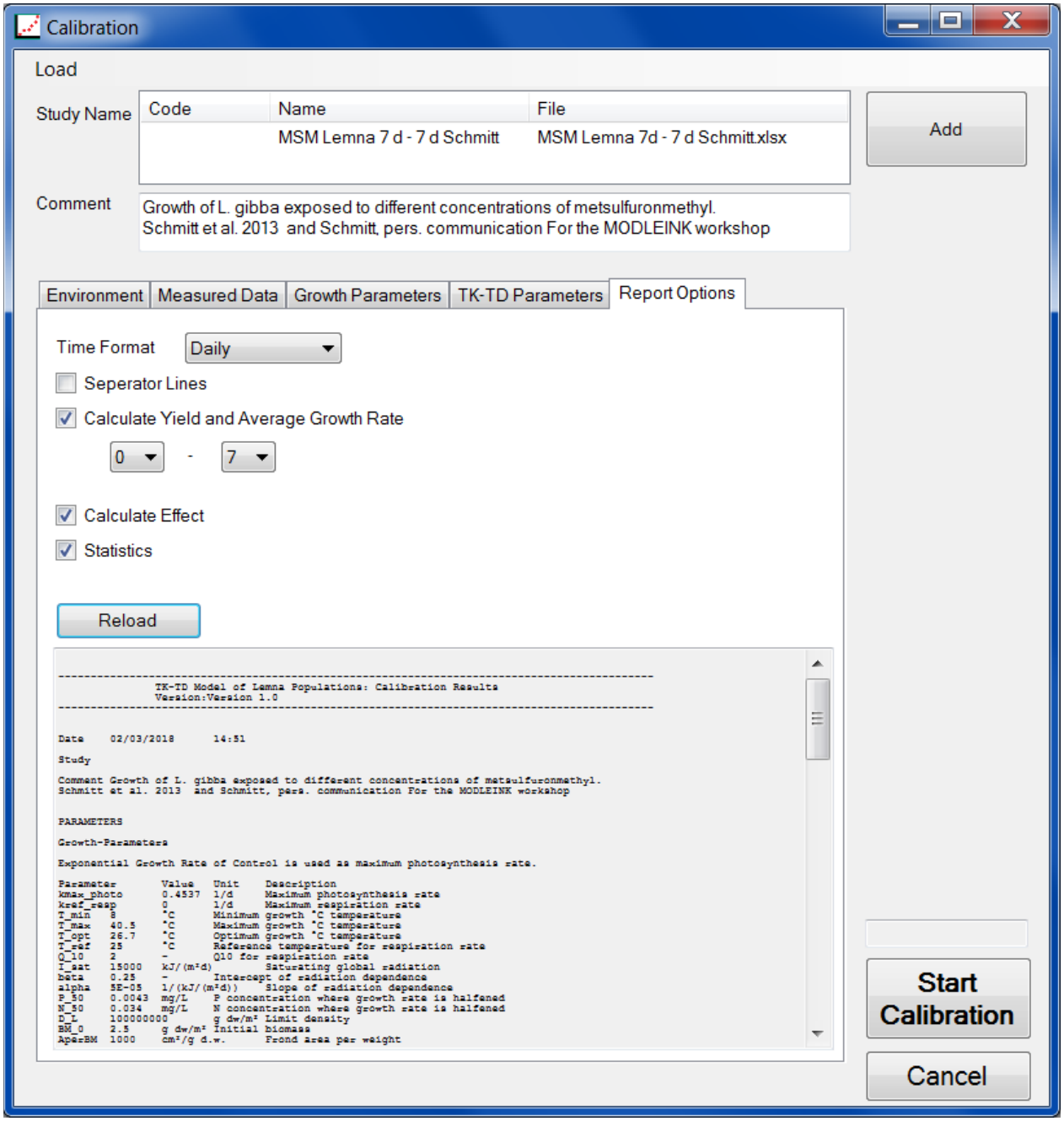


Figure 10: Report option of calibration

Clicking at Start Calibration starts the calibration procedure. The progress of the calibration is shown by the progress bar at the bottom. It is possible to stop the calibration by pressing the "Stop" button (Figure 11). Clicking at "Cancel" leads back to the initial start screen of the program and the calibration is cancelled.

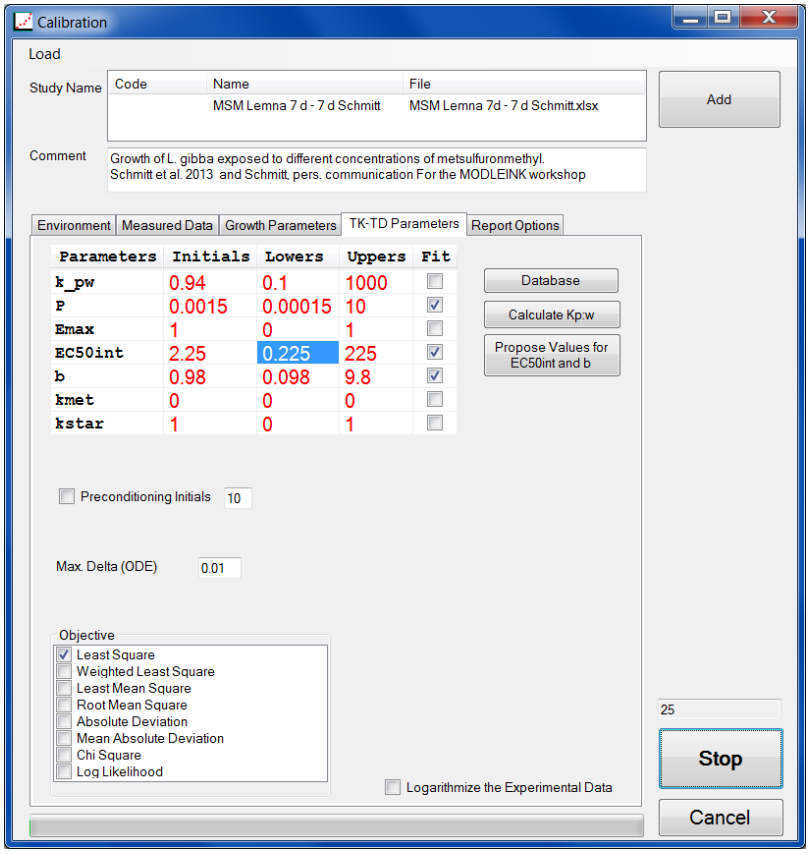


Figure 11: Calibration started

Above the "Stop" button the number of iterations of the algorithm finding the TK-TD parameters is given. Here, the screenshot was taken at iteration 25.

If the solver finds an optimal solution, a new window opens to show the results, in the form of a plot of the observed and modelled frond numbers (or biomass) over time, the set of the calibrated TK-TD parameter and the final value of the objective function (Figure 12).

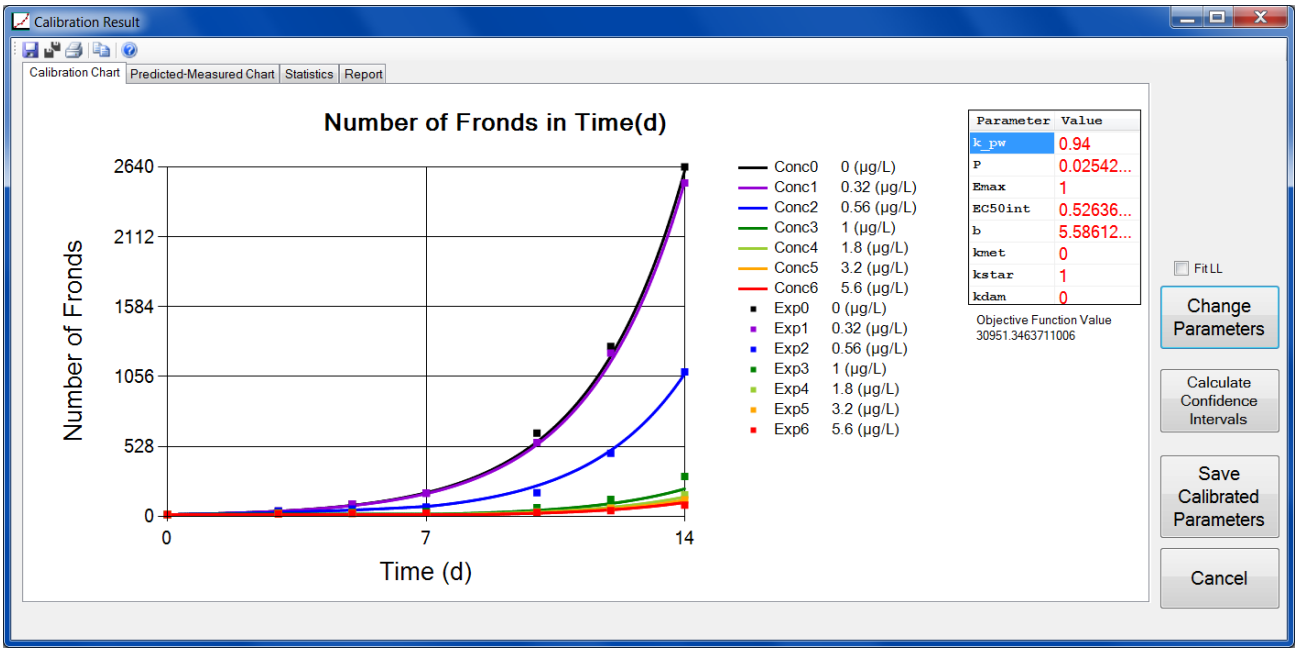


Figure 12: Calibration chart of calibration result form

It is also possible to regard the results in log scale view by clicking with the right mouse button at the plot.

The calculation of confidence intervals of parameters is done via profiling likelihood. In the program, two different possibilities for their calculation is given: with fitting the likelihood and without fitting the likelihood. The output of the calculation is a text report (txt file).
In general, the calculation is very time consuming. Thus, by clicking at "Calculate Confidence Intervals" a form appears showing the process of the calculation (Figure 13).

For more information, see Jager (2016); Meeker & Escobar (1995); Moerbeek et al. (2004).
Calculating the confidence interval of the optimal parameter p^* is finding the intersection of the approximate chi square distribution function $2 \cdot (L_{best} - L(p))$ and a critical chi square value $\chi^2_{df,1-\alpha}$ in an interval $[\underline{p}, p^*]$ respective$[p^*, \overline{p}]$:

$$2 \cdot (L_{best} - L(p) - \chi^2_{df,1-\alpha}) \approx 0$$

The approximate chi square distribution function is calculated based on the optimal likelihood value L_{best} and the current likelihood value $L(p) p \in [\underline{p}, p^*]$ respectively $p \in [p^*, \overline{p}]$.

The critical chi square value is based on the number of fix parameters (1). Using an alpha of 0.05 leads to a critical value of 3.841.

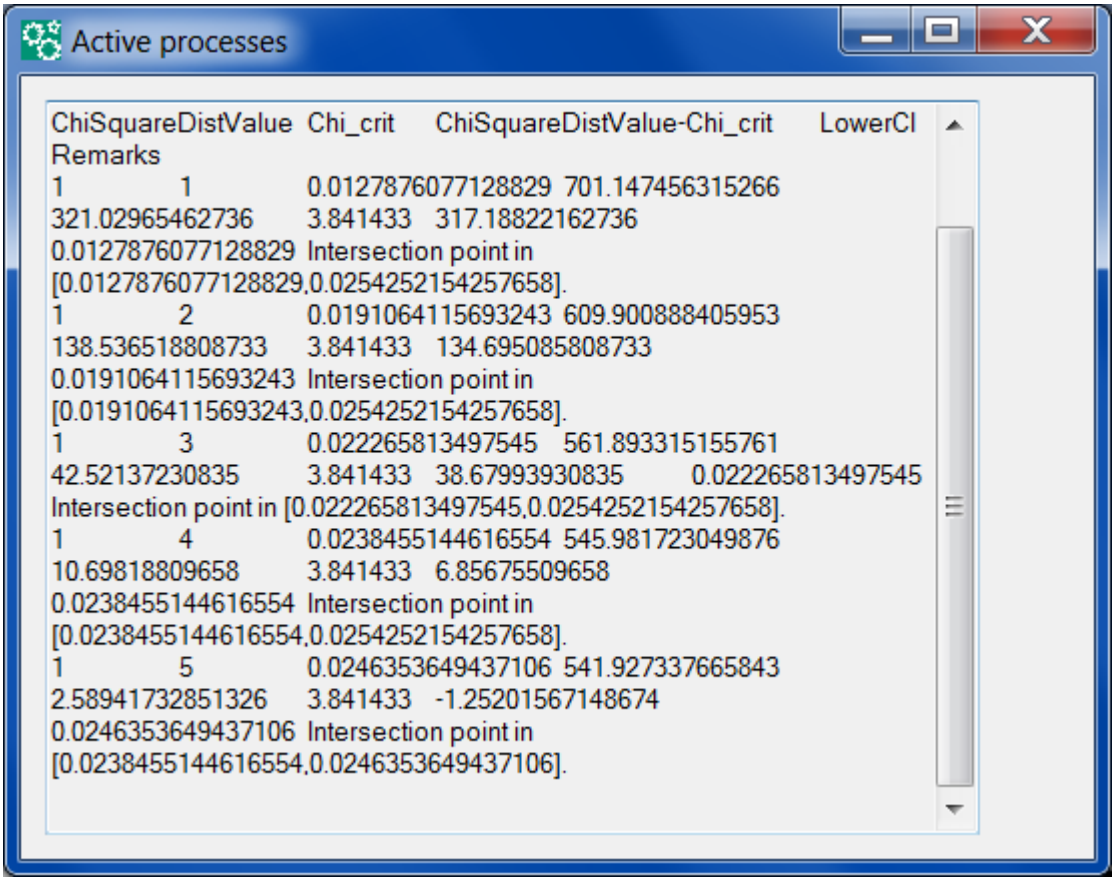


Figure 13: Active processes form appearing by clicking at "Calculate Confidence Intervals"

The second tab "Predicted-Measured Chart", Figure 14, contains a figure of prediction (x-axis) and measured data (y-axis) including initial values and control data.

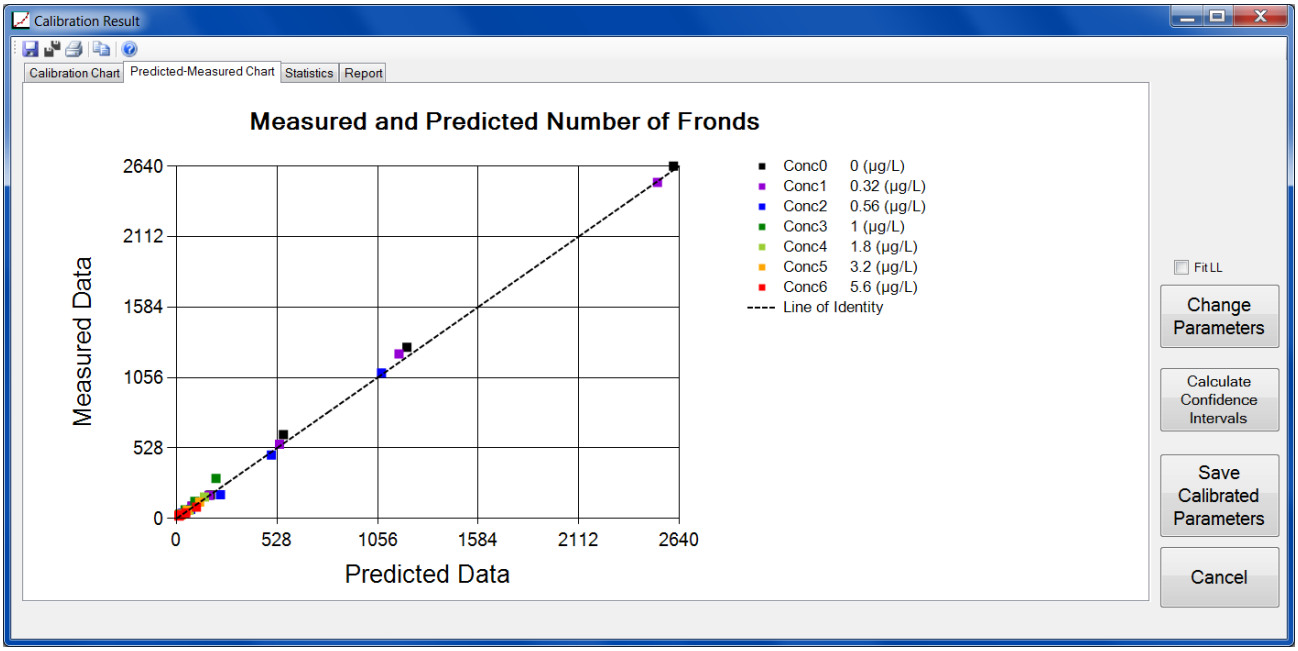
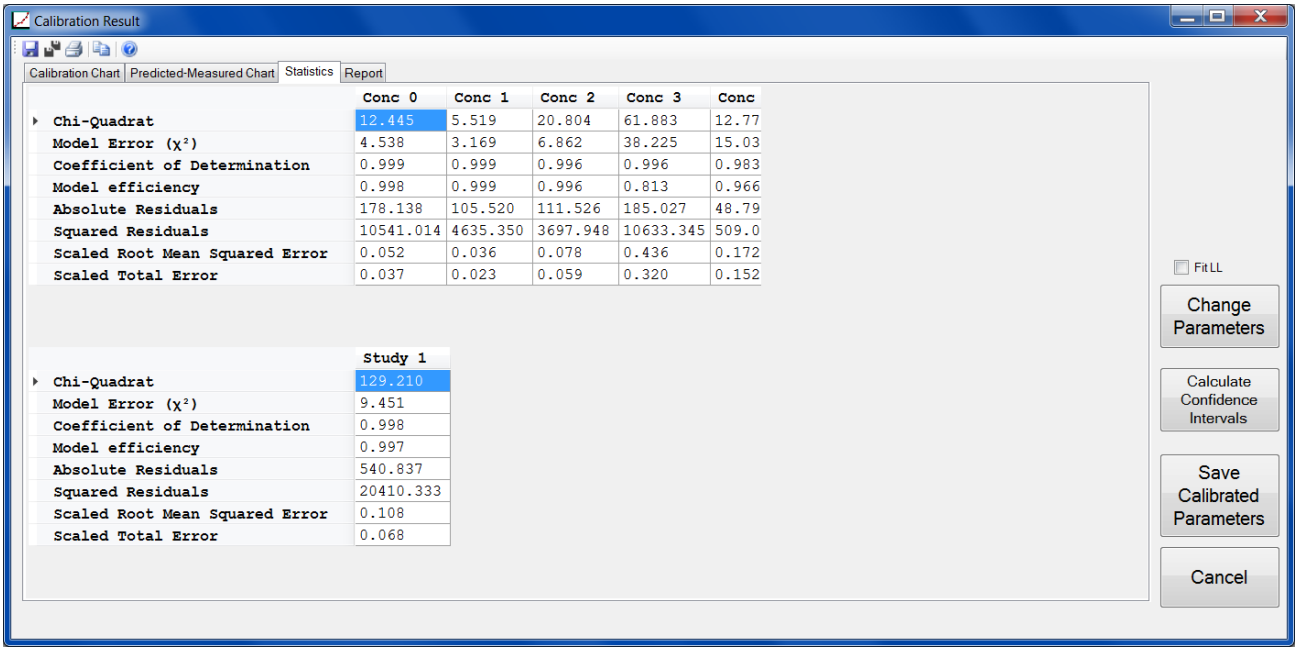


Figure 14: Predicted-Measured Chart of the calibration result form

The third tab, Figure 15, presents the statistical results based on frond number to provide additional information on the goodness of fit. For these statistical calculations, the initial values (t=0) are not considered. Furthermore, we do not consider the control data for the total study statistics as these are not affected by the TK-TD parameters. Details on the statistical calculations can be found in the TRACE documentation.



	Conc 0	Conc 1	Conc 2	Conc 3	Conc
Chi-Quadrat	12.445	5.519	20.804	61.883	12.77
Model Error (χ^2)	4.538	3.169	6.862	38.225	15.03
Coefficient of Determination	0.999	0.999	0.996	0.996	0.983
Model efficiency	0.998	0.999	0.996	0.813	0.966
Absolute Residuals	178.138	105.520	111.526	185.027	48.79
Squared Residuals	10541.014	4635.350	3697.948	10633.345	509.0
Scaled Root Mean Squared Error	0.052	0.036	0.078	0.436	0.172
Scaled Total Error	0.037	0.023	0.059	0.320	0.152

	Study 1
Chi-Quadrat	129.210
Model Error (χ^2)	9.451
Coefficient of Determination	0.998
Model efficiency	0.997
Absolute Residuals	540.837
Squared Residuals	20410.333
Scaled Root Mean Squared Error	0.108
Scaled Total Error	0.068

Figure 15: Statistical measurements of calibration result

However, an overview of the used statistics is given in Table 2. Again, let $N \in \mathbb{N}$ be the total number of data, $O \in \mathbb{R}_+^N$ the experimental observation and $C \in \mathbb{R}_+^N$ the corresponding calculated model prediction. The means are represented by $\bar{O} \in \mathbb{R}_+$ respectively $\bar{C} \in \mathbb{R}_+$.

Table 2: Statistical measurements to compare the correspondence of model prediction and experimental data

Statistics	Formula
Chi-Quadrat	$\chi^2 = \sum_{i=1}^{N} \frac{(C_i - O_i)^2}{O_i}$
Model error	The number $m \in \mathbb{N}$ denotes the degrees of freedom (number of measurements minus number of model parameters) and let be $\alpha \in (0,1)$. Let χ^2_{tab} be the tabulated $\chi^2_{m,\alpha}$. Usually a value of $\alpha = 0.05$ is chosen.

	$\epsilon = 100 \cdot \frac{1}{\overline{O}} \cdot \sqrt{\frac{1}{\chi^2_{tab}} \cdot \sum_{i=1}^{N} (C_i - O_i)^2}$
Coefficient of Determination	$r^2 = \left(\frac{\sum_{i=1}^{N} (O_i - \overline{O})(C_i - \overline{C})}{\sqrt{\sum_{i=1}^{N} (O_i - \overline{O})^2 \cdot \sum_{i=1}^{N} (C_i - \overline{C})^2}} \right)^2$
Model efficiency	$EF = 1 - \frac{\sum_{i=1}^{N} (C_i - O_i)^2}{\sum_{i=1}^{N} (O_i - \overline{O})^2}$
Absolute Residuals	$AR = \sum_{i=1}^{N} \lvert C_i - O_i \rvert$
Squared Residuals	$SR = \sum_{i=1}^{N} (C_i - O_i)^2$
Scaled Root Mean Squared Error	$SRMSE = \frac{1}{\overline{O}} \sqrt{\frac{1}{N} \sum_{i=1}^{N} (C_i - O_i)^2}$
Scaled Total Error	$STE = \frac{\sum_{i=1}^{N} \lvert C_i - O_i \rvert}{\sum_{i=1}^{N} O_i}$

The fourth tab contains a written report containing all necessary information on the input, settings of the calibration and the model prediction (Figure 16).

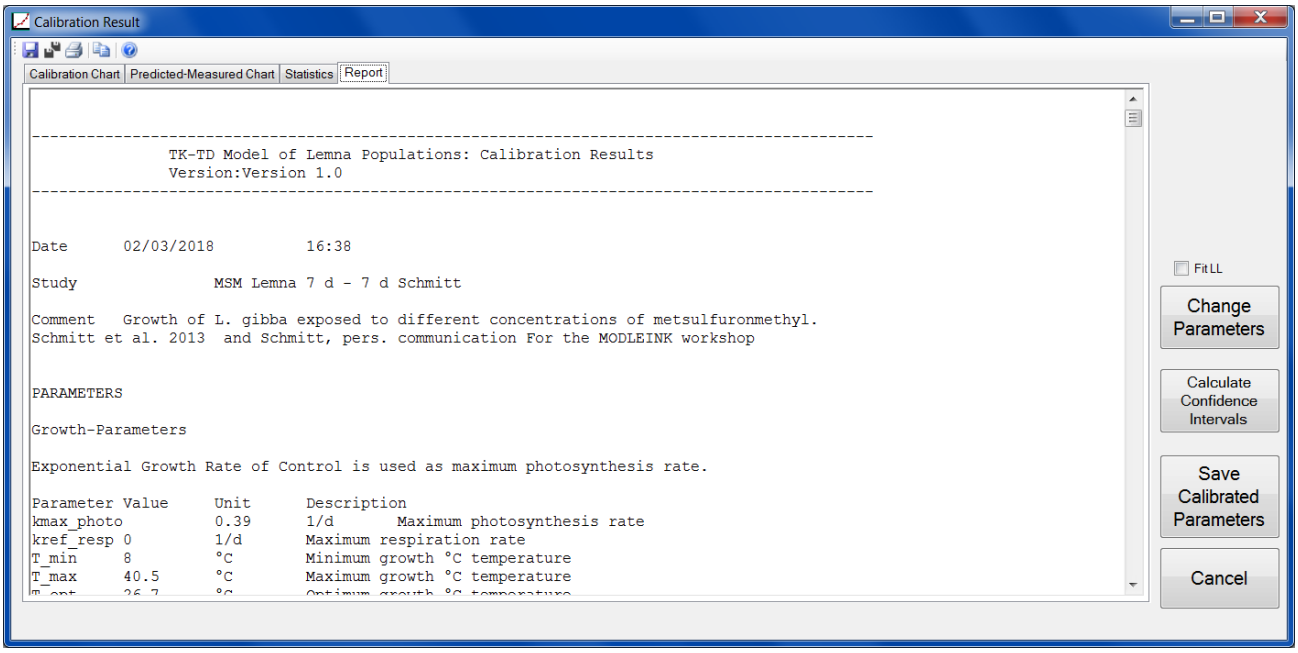


Figure 16: Text report of calibration

It is possible to copy the results, separately or all simultaneously, or to save and print them using the symbols at the top. The file format is dependent on the result file: the charts are saved as jpeg, the statistics and text report in txt.

3.2 Verification

The aim is to verify the TK-TD model by comparing model prediction to experimental studies (not used for calibration).

Input: experimental data, parameter values
Settings: number of fitted parameters (for statistics)

The verification form has the same structure as the calibration form; the only part that is different is the TK-TD tab. We can manually enter the parameter we want to validate or choose a parameter set from the TK-TD database. By clicking at "Start Verification", the programs calculates the model prediction using the entered TK-TD parameters and compares them to the given experimental data set (Figure 17).

The statistics is similar to the calibration statistics in Table 2.

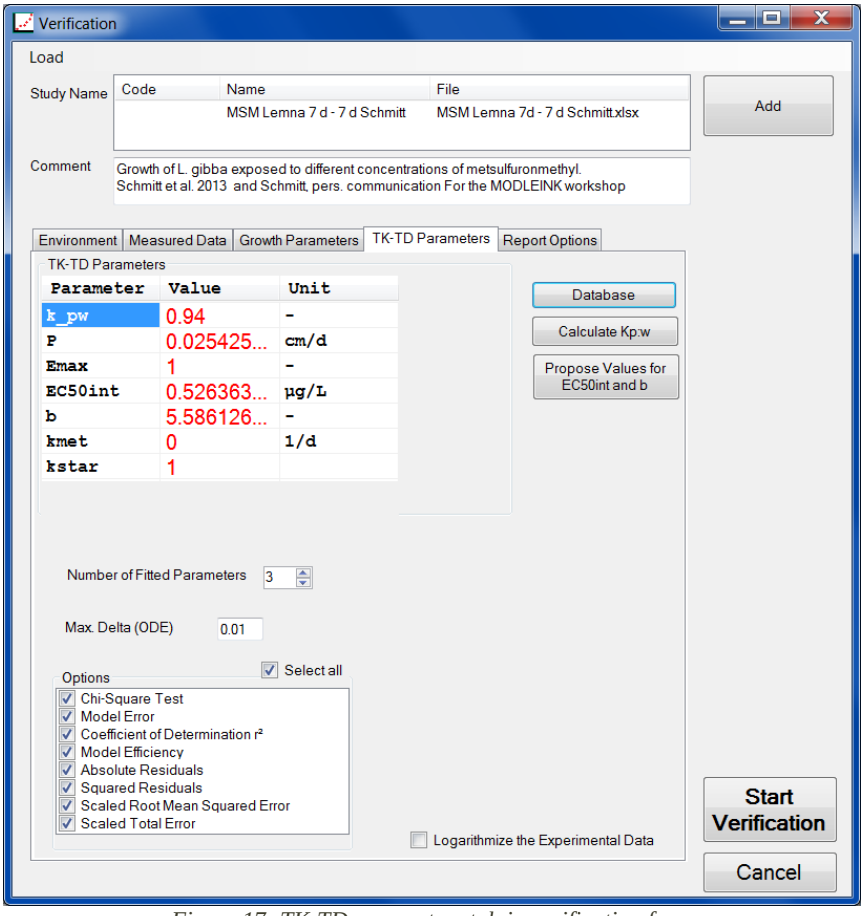


Figure 17: TK-TD parameters tab in verification form

As result, we obtain the time series of exposure, internal concentration, growth (biomass, frond number, or frond area over time) and effect (% of reduction compared to control). It can also be selected which study (if more than one was selected before) should be shown, if all or only specific concentrations should be plotted and if the experimental data should be shown as symbols (Figure 18).

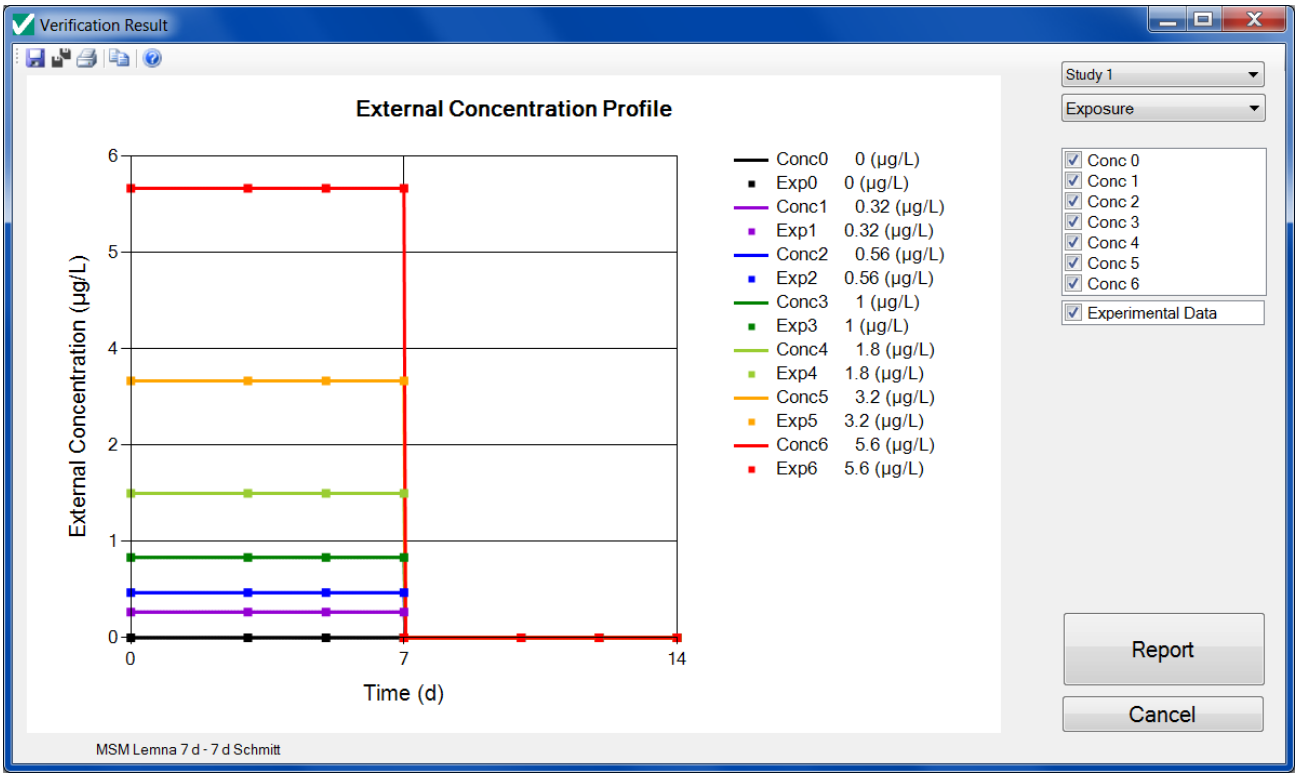


Figure 18: Exposure profile of study in verification result form

In Figure 19, the verification result with respect to the number of fronds can be seen. Experimental data (points) as well as model predictions (line) is presented in arithmetic scale.

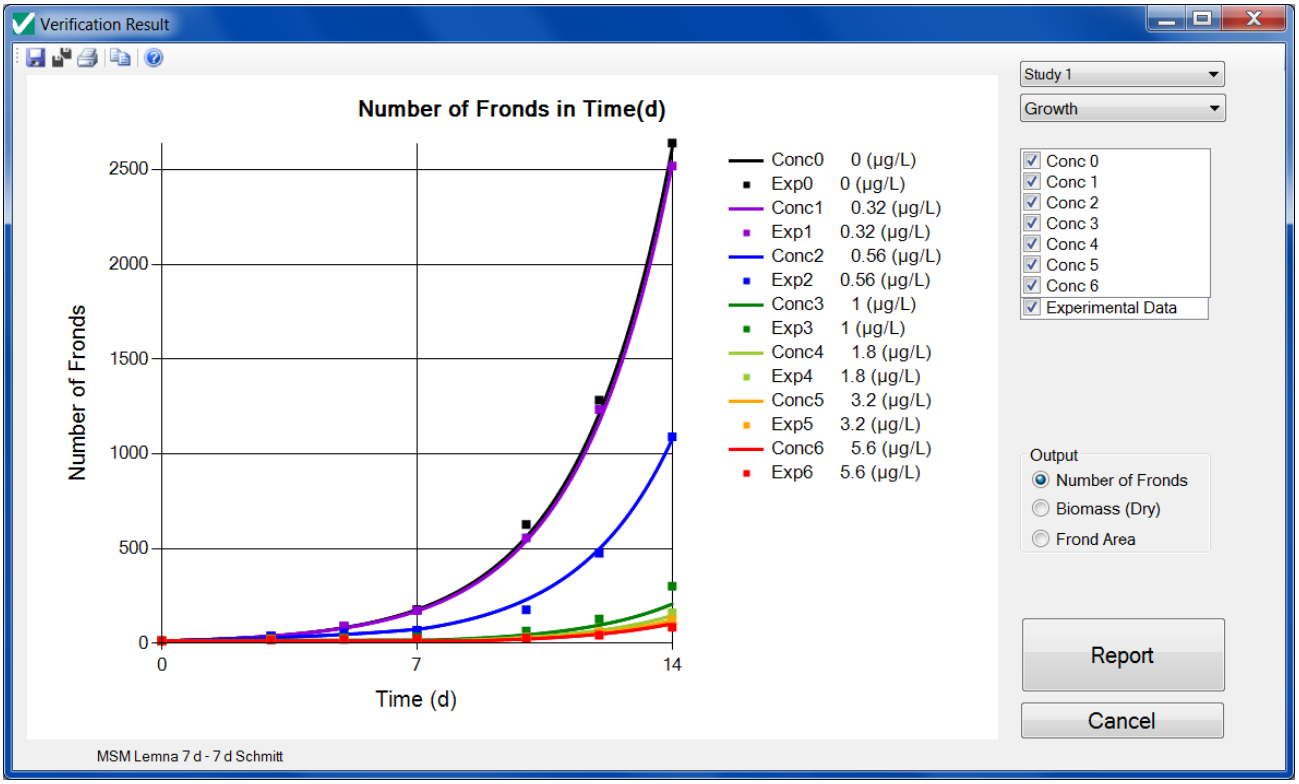


Figure 19: Number of fronds of study in verification result form

It is possible to copy the results separately or all simultaneously. Furthermore, one can save or print the results. Again, the file format is jpeg for the plots, and txt for statistics and the text report.

The results contain a similar statistical assessment and report as the calibration statistics.

3.3 Application

Having found suitable TK-TD parameters in calibration and validation, in “Application” the user can simulate growth of *Lemna* populations in dependence of different environmental and exposure scenarios.
Therefore, before starting the calculation procedures, the user has to choose several input parameters such as the exposure of substance, the environmental conditions, the TK-TD parameters and the output variables. Usually the user has not to change the growth parameters because they depend on the species not the substance but the parameters can be edited if needed.

In the environment tab, exposure, temperature, light and nutrient conditions have to be specified (Figure 21). They can be defined to be constant or they can be read in from txt files.
By clicking at multiplication factor, the program multiplies the exposure pattern by factors to be entered by the user. For this, a range of multiplication factors can be entered. Furthermore, the user can decide whether the external concentration shall be interpolated exponentially or linearly.

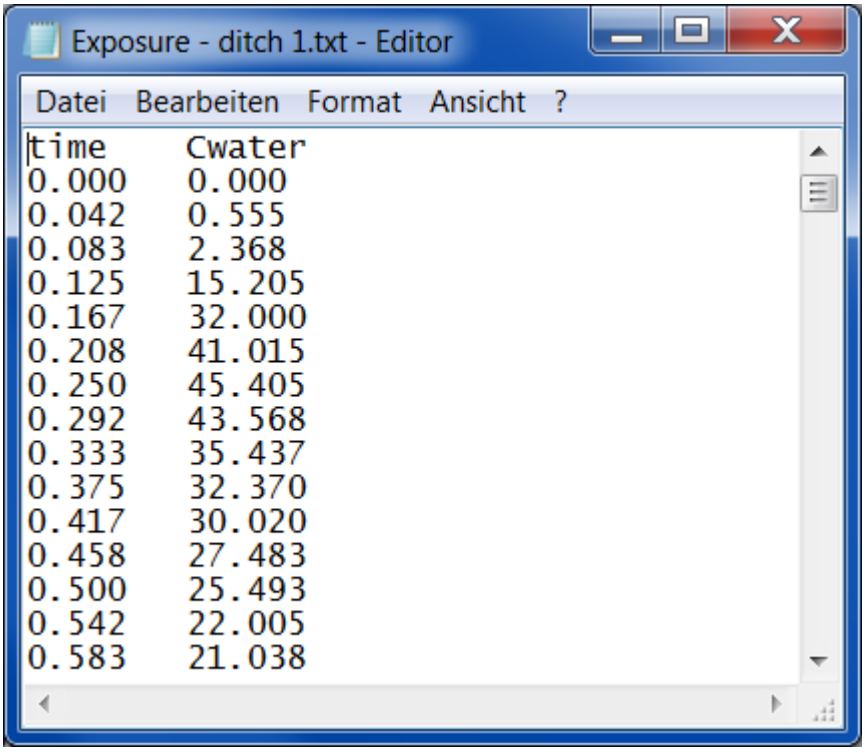


Figure 20: Exemplary concentration input text file

For each environmental factor the user has to create a proper text file containing time and environmental data values separated by tabs (Figure 20).

If laboratory studies should be simulated without explicit simulation of the growth depending on temperature, light and nutrients, the user can use the check boxes below. The check box “Optimal Environmental Conditions” sets the environmental conditions to constants leading to maximum photosynthesis rate. The “Fix Photosynthesis factor” option does not consider environmental influences on photosynthesis. The same option exists for respiration, “Fix Respiration Factor”.

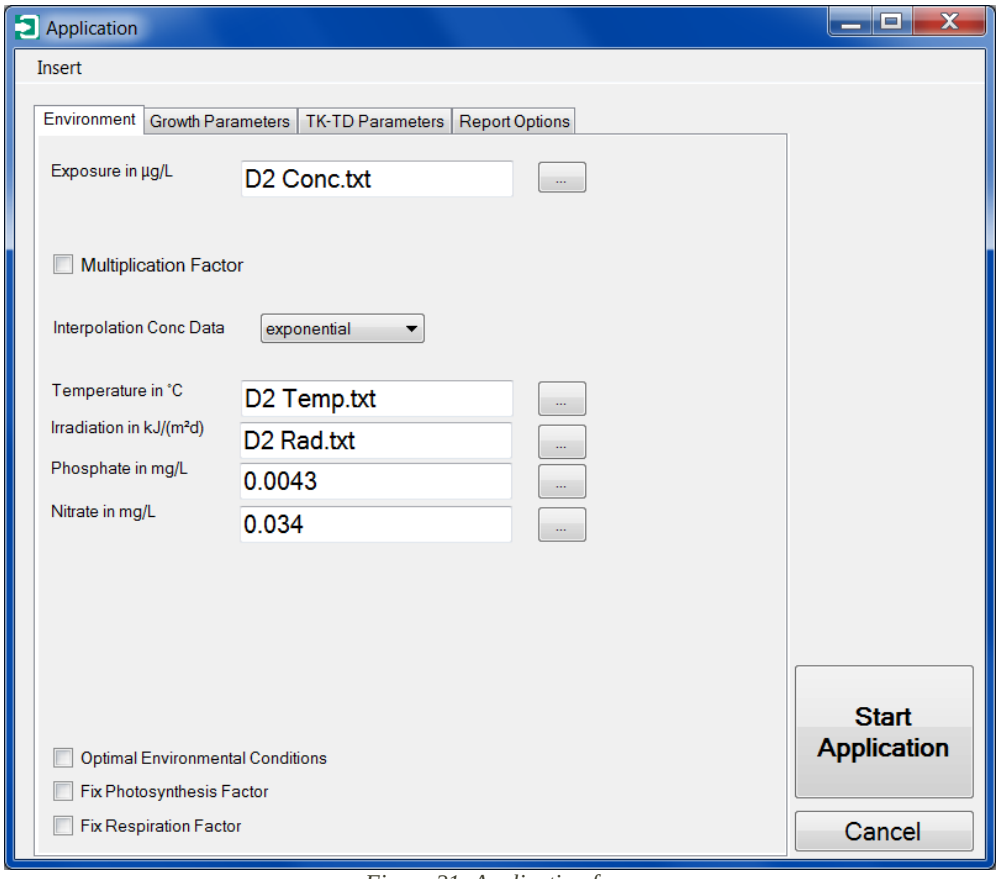


Figure 21: Application form

In the next tab “Growth parameters”, the user can decide between exponential and logistic growth as well as the specific growth parameters (Figure 22).

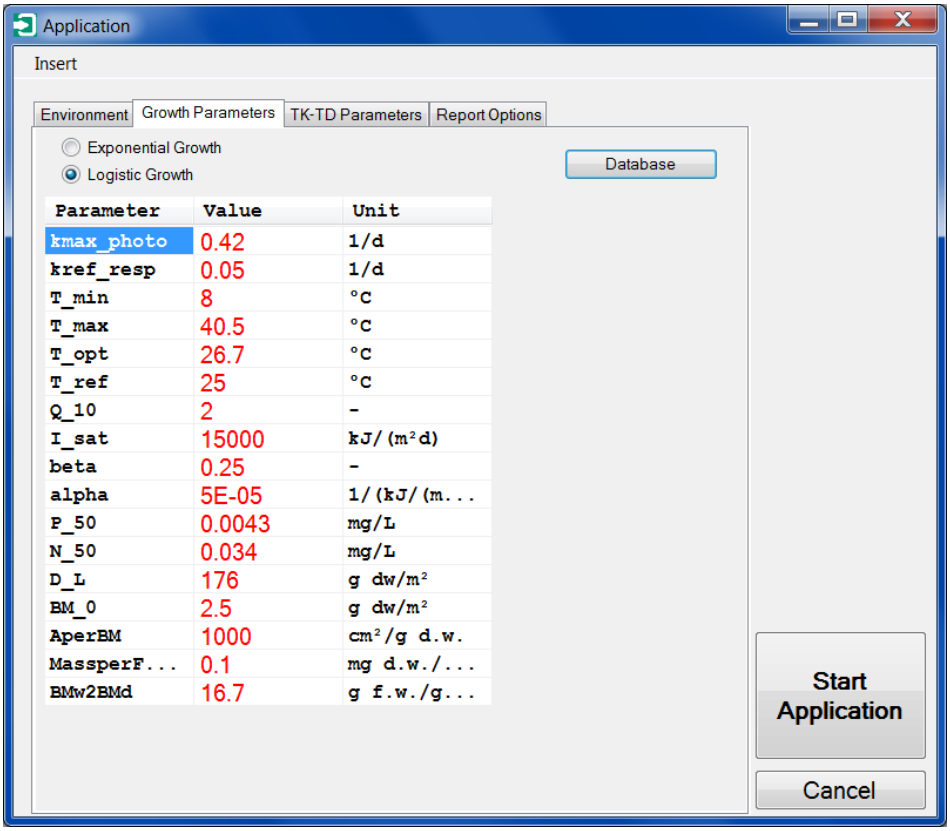


Figure 22: Growth parameters tab in the application form

Similarly the TK-TD parameters can be chosen (Figure 23). Additionally the precision of solving the ordinary differential equations can be changed. This precision value is valid for calculation of both internal concentration and biomass, but a relative value. The smaller the value, the more exact is the solution of differential equations but the longer the calculation of prediction takes.

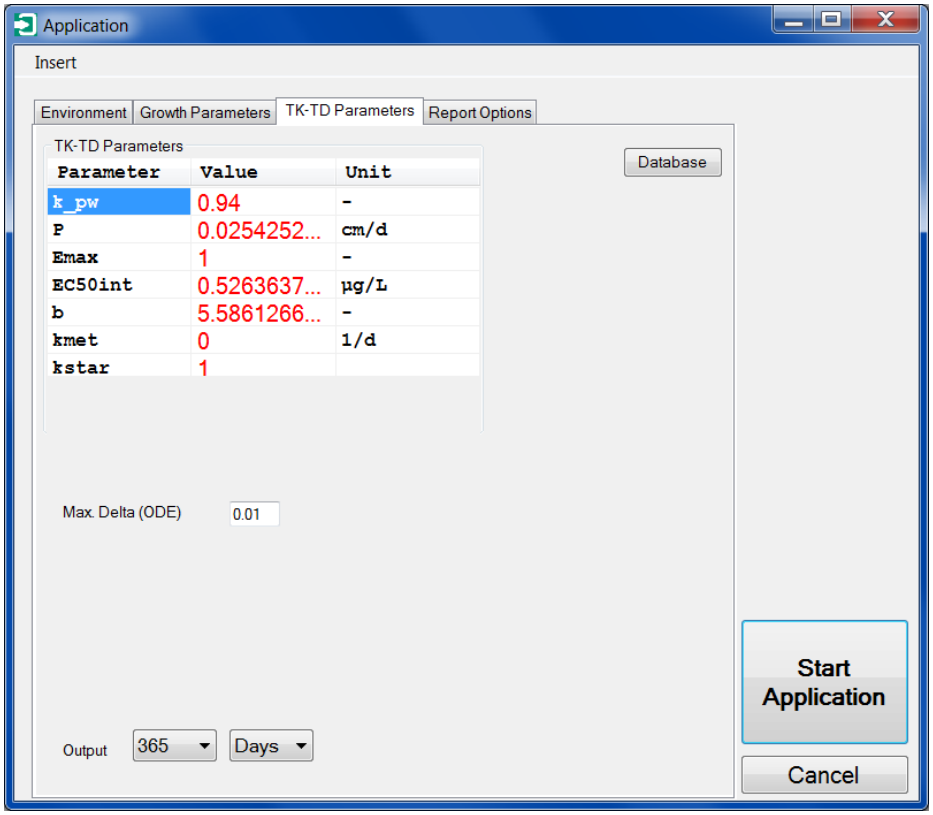


Figure 23: TK-TD parameter tab in the application form

In particular using a long time period, e.g. longer than 485 days, leads to a longer calculation time. The maximum time period is 30 years.

The last tab “Report Options”, Figure 24, is about the setting of the test report.

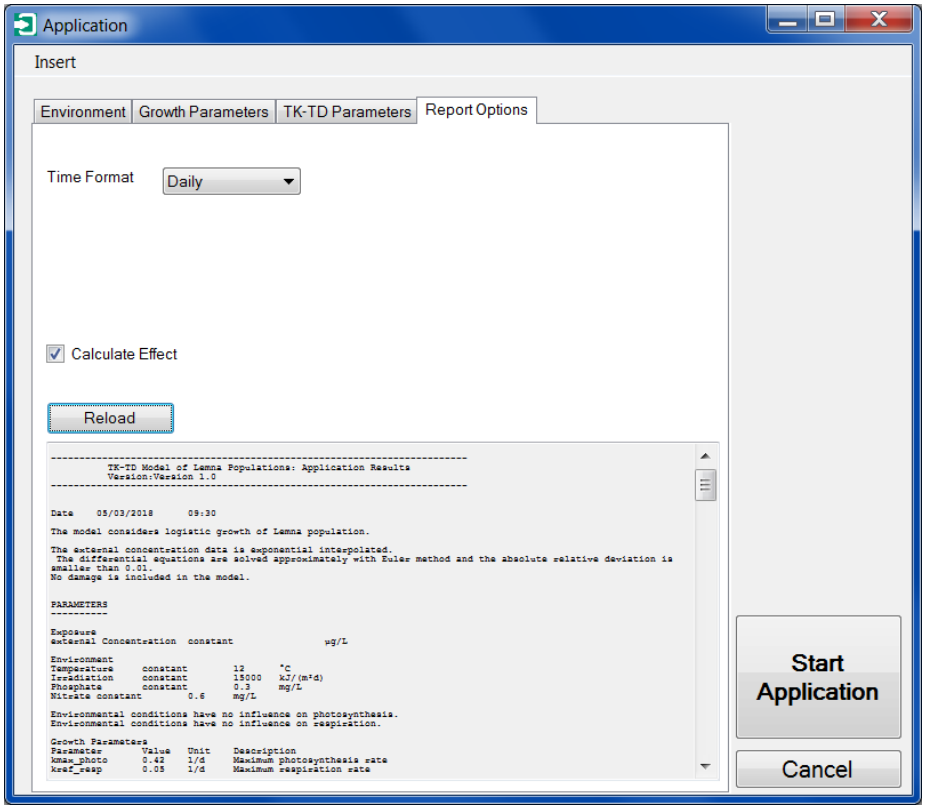


Figure 24: Report options of the application form

After the selections have been made, the "Start Application" button can be clicked to start the simulations. When the application procedure starts, a bar appears to show the progress of the calculation.

The Application Result Form gives an overview of the entered information and the result of the application procedure. Therefore, the program provides different diagrams: the external concentration profile (input, Figure 25), the predicted internal concentration over time (Figure 26), the predicted growth (biomass, frond number, or frond area over time, Figure 27) and the effect (% reduction compared to control) over time.
Furthermore, the user can save, print or copy the diagrams. Additionally the user can create an output file (txt format), which can e.g. be imported in Excel for further evaluation of the results.

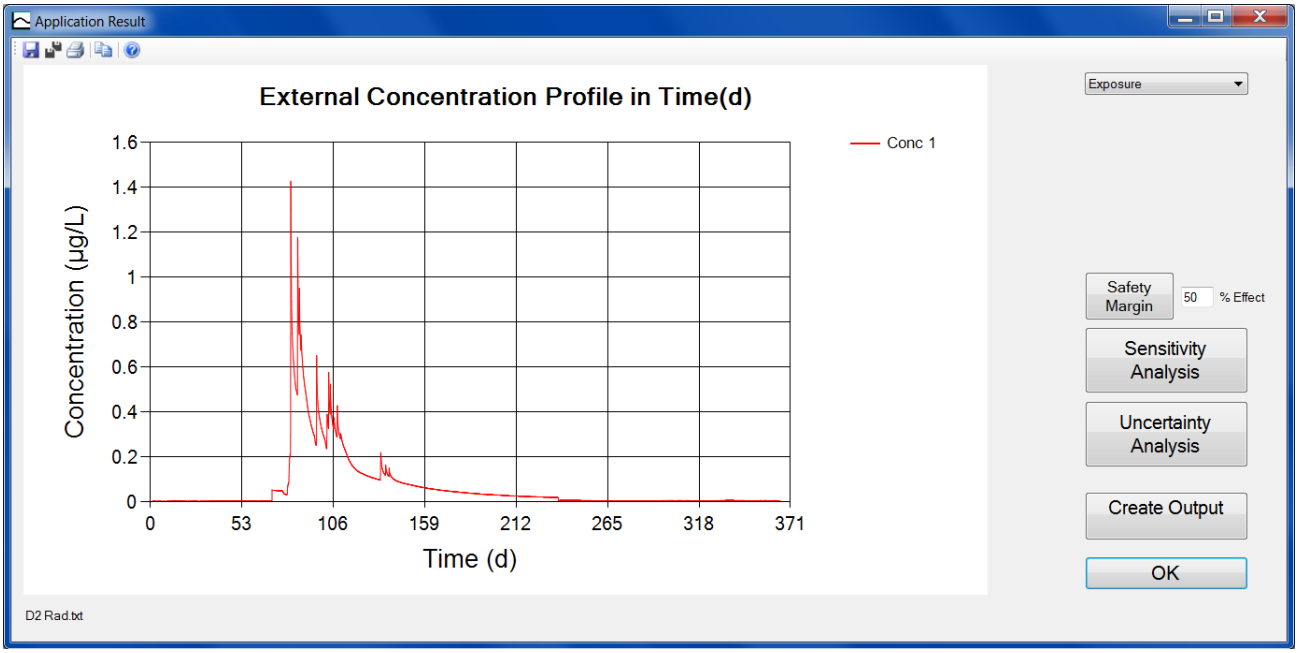


Figure 25: External concentration profile in the application result form

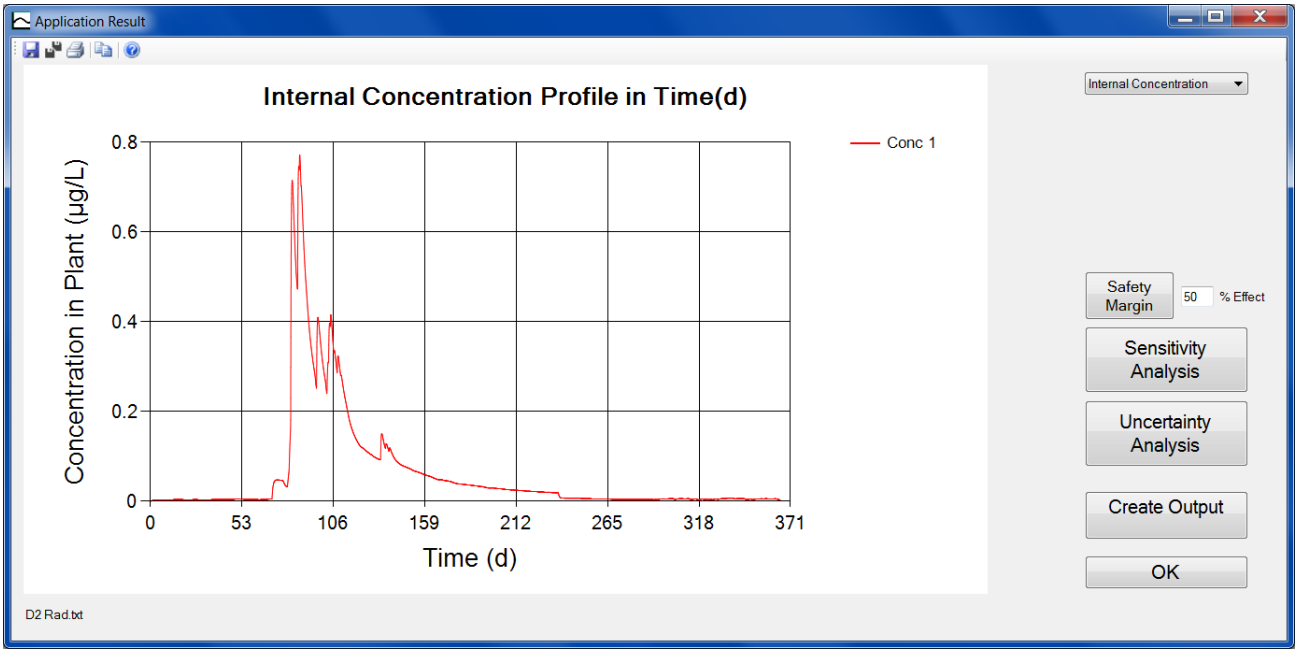


Figure 26: Internal concentration profile in the application result form

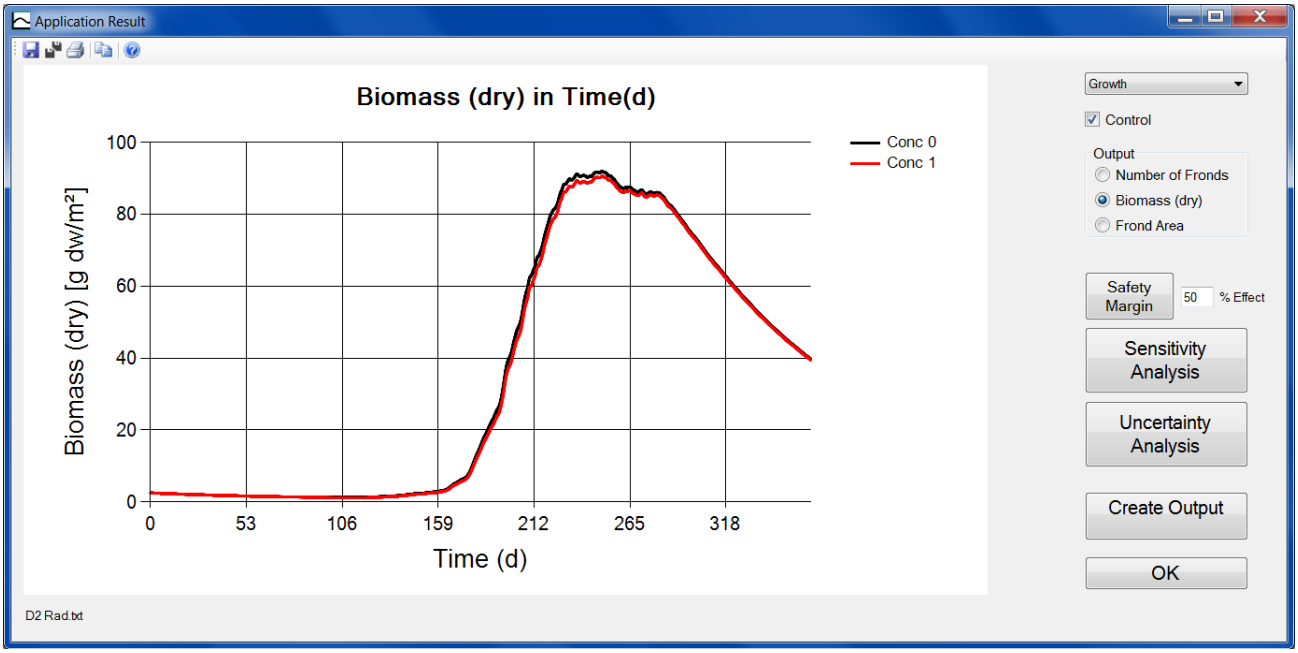


Figure 27: Dry biomass in g/m² in prediction result form

The button "Safety Margin" calculates the multiplication factor that yields x percentage effect, answering the question by which factor do we have to multiply the exposure profile to obtain x percentage effect on the growth rate.

Let $BM(t, j) \in R_+ / \{0\}$ be the dry biomass value of concentration $j = 1,...,m$ at time $i = 0,1,...,n$. The growth rate from time point $t = 0$ to the last time point $t = n$ is calculated by

$$r(j) = \frac{\log(BM(n, j)) - \log(BM(0, j))}{n}$$

The effect on the growth rate as endpoint can be calculated by relating the control growth rate to the growth rate of the treatment $j = 1,...,m$. We define the effect on growth rate as inhibition of growth rate.

$$E_r(j) = 100 - 100 \cdot \frac{r(j)}{r(0)}$$

The calculation of safety margin is done by using the Intermediate value theorem. The program searches in an interval $[0,100 \cdot EC50_{int}]$. The result is only given in a label on the right side next to the safety margin button and the diagram shows the graphical result. In this particular case, we obtain a safety margin of 11.379 (Figure 28).

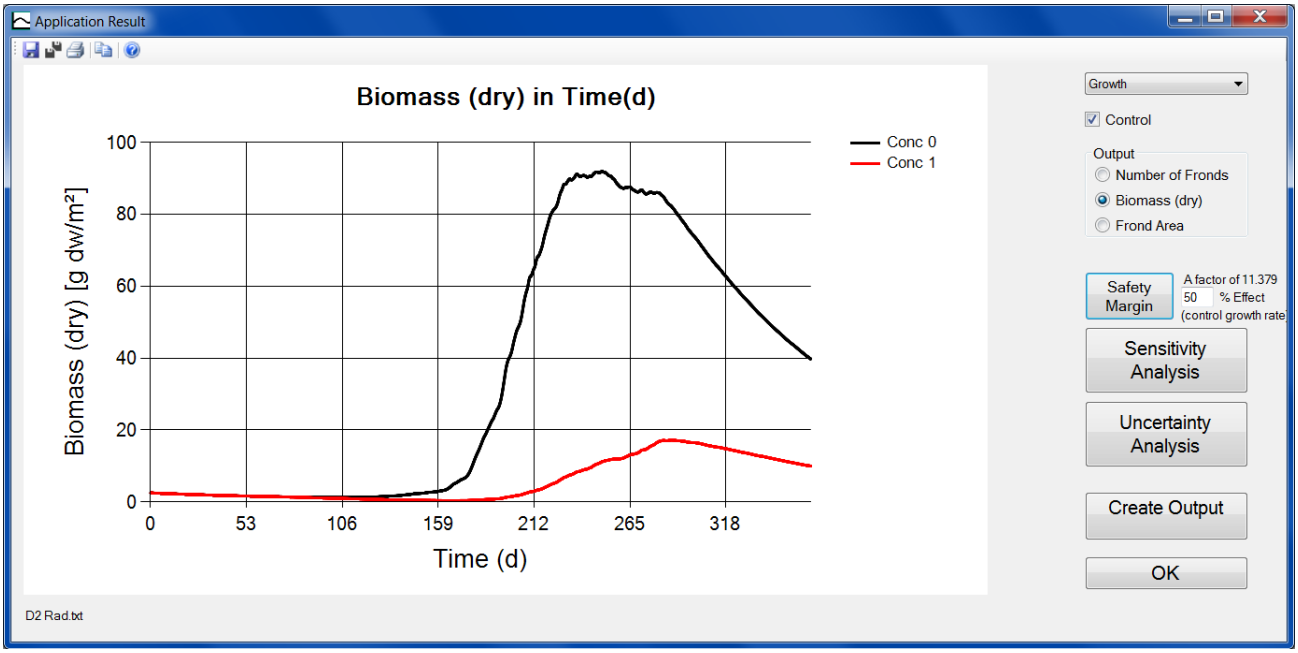


Figure 28: Application result form after clicking at the button "Safety Margin"

Sensitivity analysis is a systematically analysis of the effect of relative changes in parameter value on the model output. For this, only one parameter is changed in systematic manner whereas the other parameters are fixed to their fitted values. It is possible to calculate sensitivity coefficients by dividing the scaled parameter change by the scaled model output (EFSA PPR Panel 2014).
Local sensitivity analysis is when the value of a parameter is varied in a small area around the proper parameter value. Global sensitivity analysis is the consideration of the total range of the parameter (Pianosi et al. 2016). In Grimm et al. 2014 "global sensitivity analysis" is when several or all parameters are varied over their whole ranges.

The TRACE report presents examples for uncertainty and sensitivity analysis (selection of scenario, input, parameter ranges, distribution, and number of simulations).

In principle both forms, sensitivity and uncertainty analysis, are the same. They differ in their "Standard Analysis", namely for the sensitivity analysis we propose to choose the chosen parameters to vary one at a time in equidistant steps. For uncertainty analysis, we vary all parameters at one time using a certain distribution.

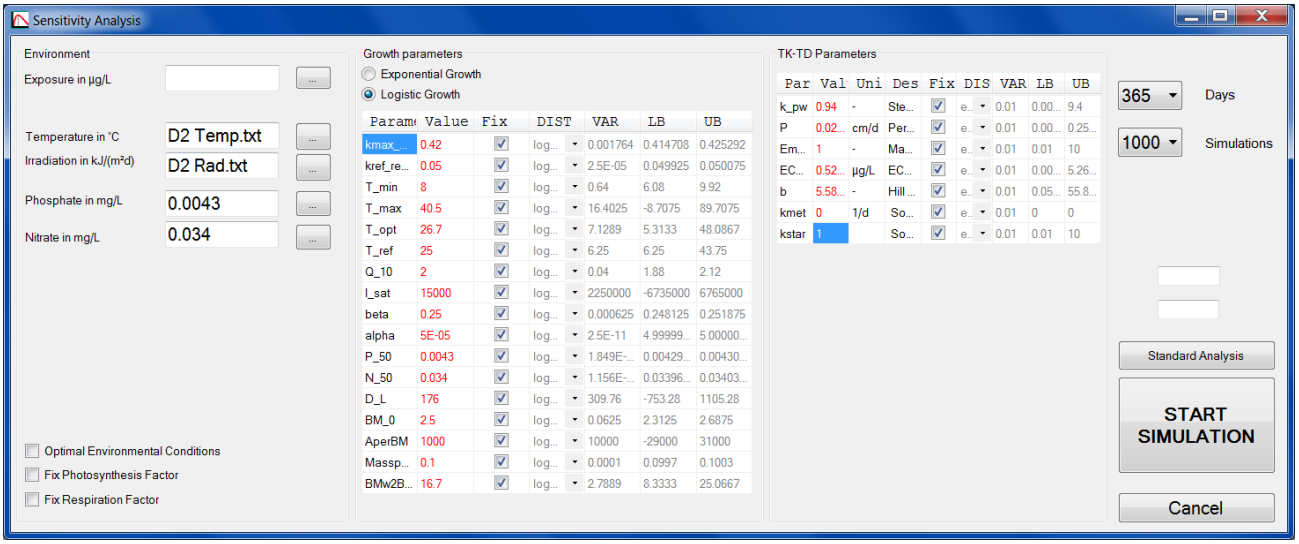


Figure 29: Sensitivity analysis form

The following possibilities are included. One can set a parameter value, growth as well as TK-TD parameter fixed to a constant value or variable in each simulation.
If a parameter is variable, the user can choose between lognormal, normal, uniform, equidistant, triangular and Marsaglia.

Distribution	Algorithm
Marsaglia	Let $u_1, u_2 \approx U(0,1)$ be stochastically independently uniformly distributed random variables. Set $v_i := 2 \cdot u_i - 1$ for $i = 1,2$ as long as it yields that $w := v_1^2 + v_2^2 < 1$. Then the random numbers $z_1 := v_1\sqrt{-2\log(w)/w}$ and $z_2 := v_2\sqrt{-2\log(w)/w}$ are normally distributed.
Triangular	Let $a, b \in \mathbb{R}_+$ with $a < b$ the range of the distributed values. Furthermore, let $c \in (a,b)$ be the peak value. Let $u \in U(0,1)$ be a uniformly distributed random variable. If $u < \frac{c-a}{b-a}$ then $z := a + \sqrt{(b-a)\cdot(c-a)\cdot u}$ else $z := b + \sqrt{(b-a)\cdot(c-a)\cdot(1-u)}$
Uniform	Let $a, b \in \mathbb{R}_+$ with $a < b$ the range of the distributed values. Let $u \in U(0,1)$ be a uniformly distributed random variable. Set $z := (b-a)\cdot u + a$ to obtain a uniformly distributed random variable $z \in [a,b]$.
Normal	Let $x, y \in \mathbb{R}$ and $b \in \mathbb{R}_+$ the border. Let $\mu \in \mathbb{R}_+$ be the expected value and σ^2 the variance. Let $u_1, u_2 \approx U(0,1)$ be uniformly distributed random variables. Do $u_1, u_2 \approx U(0,1)$ $x = 2 \cdot b \cdot u_1 + (\mu - b)$ and

	$y = \frac{1}{\sqrt{2 \cdot \pi \cdot \sigma^2}} \cdot \exp\left(-\frac{(x-\mu)^2}{2 \cdot \sigma^2} \right)$ until $u_2 < y$. Then is $x \in \mathrm{N}\left(\mu, \sigma^2\right)$ a normally distributed random variable (with corresponding probability density function value $y \in \mathbb{R}$).
Equidistant	Let $a, b \in \mathbb{R}_+$ with $a < b$ the range of the distributed values. Furthermore, let $N \in \mathbb{N}$ be the total number of simulations. For each simulation $i = 1, \cdots, N$, we calculate the number $z_i \in [a, b]$ with $z_i = (a + (b-a)) \cdot \frac{i}{N-1}$.
Lognormal	Let $\mu \in \mathbb{R}_+$ be the expected value and σ^2 the variance. Furthermore, let $x \in \mathrm{N}(0,1)$ be a normally distributed random variable. Set $z := \sigma^2 \cdot x + log(\mu)$ to obtain a log normally distributed random variable $z \in \mathbb{R}$.

3.3.1 Insert a Blend

In case that we have already found the TK-TD parameters for two different active substances, it is possible to calculate a blend based on both active substances. This can be done by clicking at “Insert” and then “Blend” in the menu item list in the application form (Figure 30).

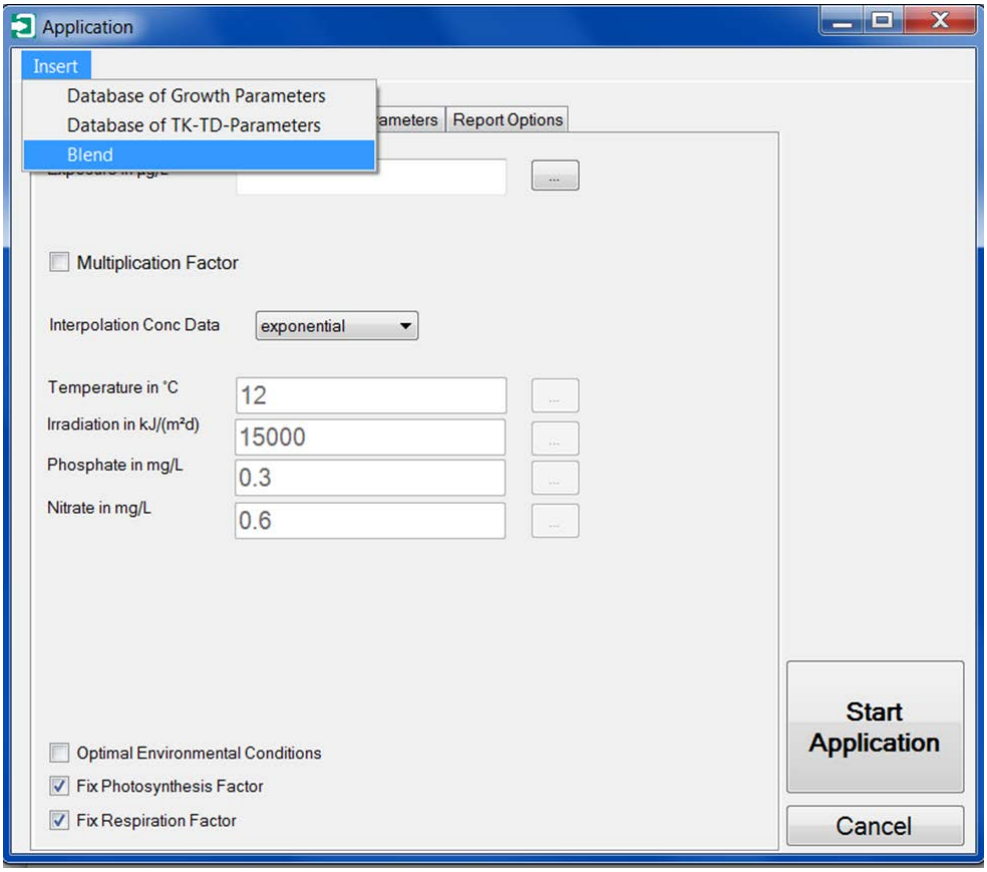


Figure 30: Application form showing the insert blend button

After clicking at “Blend” an additional form opens. Here, the user can enter the TK-TD parameters for each active substance and the corresponding exposure profile (Figure 31).

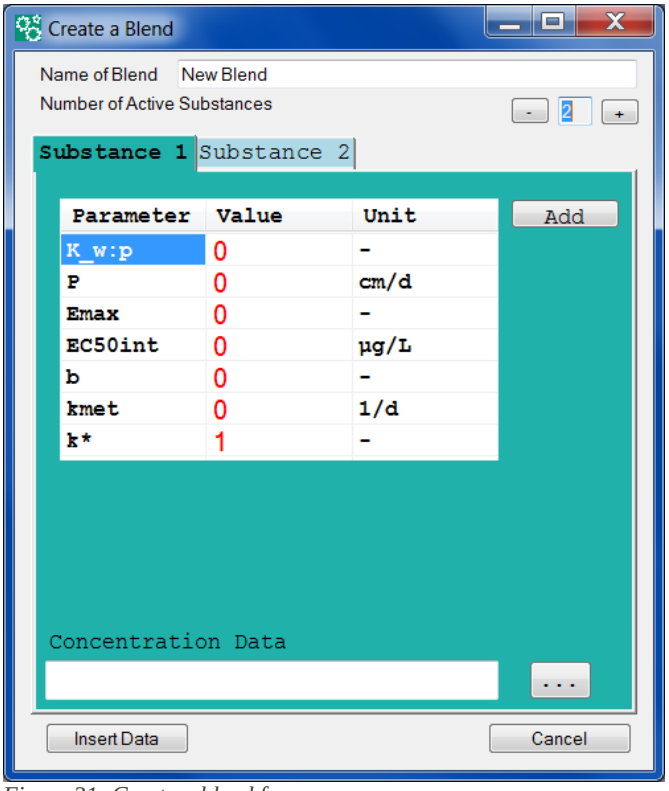


Figure 31: Create a blend form

The user can manually enter the values of TK-TD parameters or we can choose a data set by clicking at “Add” from the internal data base. By clicking at “Insert Data” the form disappears and the information is entered into the application form (Figure 32). The calculation of the effect of mixture is based on Plummer & Short (1990). It is described in more detail in the TRACE document. The user can add more than two active substances, however, we did not test this approach for more than two active substances.

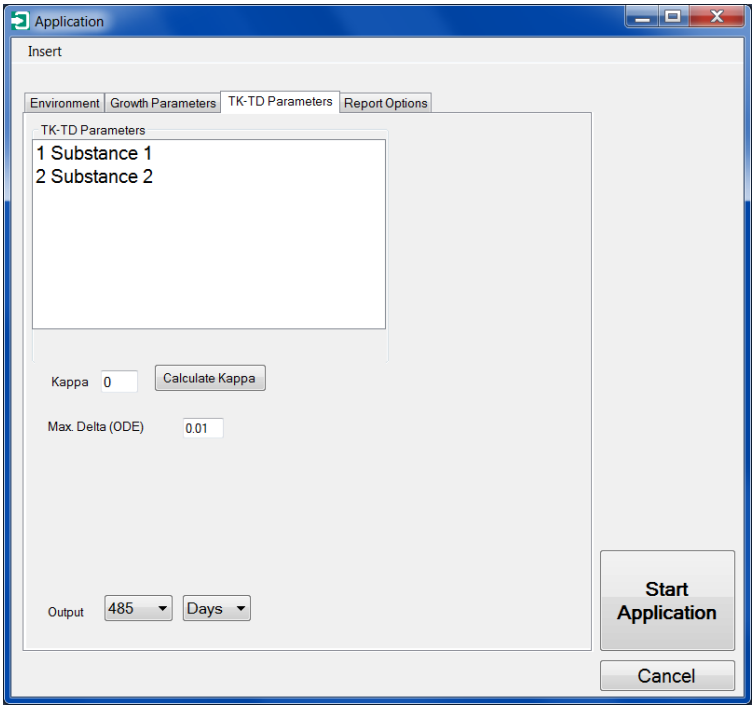


Figure 32: Two active substances inserted as blend in application form

In addition to the respective TK-TD parameter sets, the user has to choose a value for Kappa. This is the parameter describing the relation of the two active substances. A negative value shows antagonism, a positive value synergism and a value equal to zero represents additivity. The user can enter a kappa value, assuming a value or finding a kappa value by calibration using experimental data describing the effect of the mixture (“Calculate Kappa”).

4. References

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Plummer, John L. and Tim G. Short (1990). “Statistical modeling of the effects of drug combinations”. In: Journal of Pharmacological Methods 23.4, pp. 297–309. doi: 10.1016/0160-5402(90)90058-S

Schmitt W., Bruns E., Dollinger M., and Sowig P. (2013). Mechanistic TK-TD-model simulating the effect of growth inhibitors on *Lemna* populations. Ecol Model 255:1– 10.

5. Appendix

5.1 Growth parameter set of Schmitt et al. 2013

Table 3: Growth parameters by Schmitt et al. 2013

Parameter	Value	Unit	Description
kmax_photo	0.42	1/d	Maximum photosynthesis rate
kref_resp	0.05	1/d	Maximum respiration rate
T_min	8	°C	Minimum growth °C temperature
T_max	40.5	°C	Maximum growth °C temperature
T_opt	26.7	°C	Optimum growth °C temperature
T_ref	25	°C	Reference temperature for respiration rate
Q_10	2	-	Q10 for respiration rate
I_sat	15000	kJ/(m²d)	Saturating global radiation
beta	0.25	-	Intercept of radiation dependence
alpha	0.00005	1/(kJ/(m²d))	Slope of radiation dependence
P_50	0.0043	mg/L	P concentration where growth rate is halved
N_50	0.034	mg/L	N concentration where growth rate is halved
D_L	176	g dw/m²	Limit density
BM_0	2.5	g dw/m²	Initial biomass
AperBM	1000	cm²/g d.w.	Frond area per weight
MassperFrond	0.1	mg d.w./frond	Dry weight per frond
BMw2BMd	16.7	g f.w./g d.w.	Fresh weight per dry weight

5.2 Structure of an Experimental Data File

This is an Excel file summarizing the data from one experimental test, shown in Figure 4 and Figure 17. The structure is fixed.

In B1, the name of the study can be entered. In B2, the code of the study can be entered and in B3-B5 possible comments can be inserted by the user. All this information is obligatory.

B6 (number of exposure levels including control) and B7 (number of entered data rows) are necessary inputs. In this case, Figure 33, the number of entered data is from row 12 to 19 and thus, altogether 8 rows.

In the first column the user can enter the time data in days. In this case, we have a test that is in total 14 days long with an exposure period of 7 days (orange) and subsequent 7 days recovery (green). The entered time points indicate the time point when data of frond number was measured. The day seven is entered twice to state the time, when exposure is changed (compare for example J15, J16).

The first five columns (B-F) are reserved for the environmental data. In this case, we have no information on the environmental data of the study in Schmitt et al. 2013.

After these columns, the experimental data concerning the treatments is added. Here, we start with the control data (G-I). The first column (G) is the measured concentration. Additionally in H9 the nominal concentration is given. The second column (H) contains the counted number of fronds, and the third column (I) contains measurements on dry biomass, if available. This structure is the same for all treatments.

For the time points for which no external concentration data is available, the program interpolates linearly or exponentially.

Below or to the right from the table with the input data, summary statistics, diagrams or additional data can be inserted but will not be used by MoLePo.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Name	MSM Lemna 7 d - 7 d Schmitt												
2	Code													
3	Comment	Growth of L. gibba exposed to different concentrations of metsulfuronmethyl.												
4		Schmitt et al. 2013 and Schmitt, pers. communication For the MODLEINK workshop												
5		No further info on test conditions												
6	Levels	7												
7	Data rows	8												
8														
9					Nominal µg a.s./L			0.00			0.32			0.56
10	Time d	Temp	Light	PP	N	P	Exp1	FN1	BM1	Exp2	FN2	BM2	Exp3	FN3
11		°C	Lux	h	mg N/L	mg P/L	µg a.s./L	#	mg	µg a.s./L	#	mg dw	µg a.s./L	#
12	0						0.00	12		0.32	12		0.56	12
13	3							38			39			38
14	5							92			92			54
15	7						0.00	176		0.32	173		0.56	68
16	7						0.00	176		0.00	173		0.00	68
17	10							627			556			177
18	12							1283			1233			475
19	14						0	2640		0	2518		0	1090

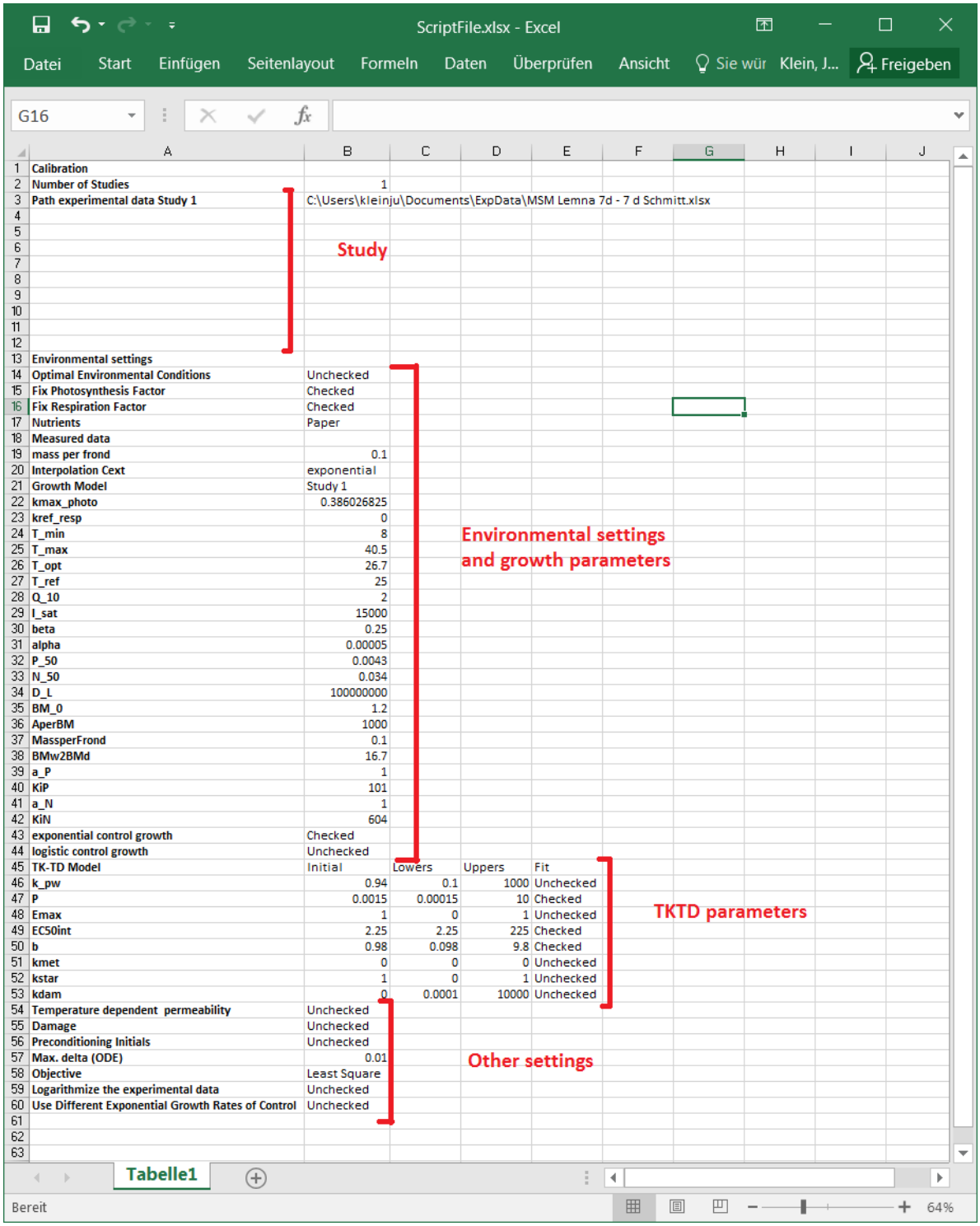
Figure 33: Example Input (Excel file)

5.3 Structure of a Script File

After calibration or verification, the program saves by clicking at “save all”, all reports and graphics including a script file containing all information on the procedure (experimental data file, growth and TK-TD parameter values, initial values, objective …).

In Figure 34, an exemplary script file is presented. This script file contains the setting of a calibration run (A1). In B2, the number of studies used for calibration is given; here, only one study is used. In the next row in the same column, B3, the corresponding file name with path is given. If more than one study is added, the subsequent cells in the column (until B12) are used.

After that the environmental settings are stored (information on the first tab of the program’s surface). After that the growth parameters, and TK-TD parameter information are presented.



	A	B	C	D	E
1	Calibration				
2	Number of Studies	1			
3	Path experimental data Study 1	C:\Users\kleinju\Documents\ExpData\MSM Lemna 7d - 7 d Schmitt.xlsx			
13	Environmental settings				
14	Optimal Environmental Conditions	Unchecked			
15	Fix Photosynthesis Factor	Checked			
16	Fix Respiration Factor	Checked			
17	Nutrients	Paper			
18	Measured data				
19	mass per frond	0.1			
20	Interpolation Cext	exponential			
21	Growth Model	Study 1			
22	kmax_photo	0.386026825			
23	kref_resp	0			
24	T_min	8			
25	T_max	40.5			
26	T_opt	26.7			
27	T_ref	25			
28	Q_10	2			
29	I_sat	15000			
30	beta	0.25			
31	alpha	0.00005			
32	P_50	0.0043			
33	N_50	0.034			
34	D_L	100000000			
35	BM_0	1.2			
36	AperBM	1000			
37	MassperFrond	0.1			
38	BMw2BMd	16.7			
39	a_P	1			
40	KiP	101			
41	a_N	1			
42	KiN	604			
43	exponential control growth	Checked			
44	logistic control growth	Unchecked			
45	TK-TD Model	Initial	Lowers	Uppers	Fit
46	k_pw	0.94	0.1	1000	Unchecked
47	P	0.0015	0.00015	10	Checked
48	Emax	1	0	1	Unchecked
49	EC50int	2.25	2.25	225	Checked
50	b	0.98	0.098	9.8	Checked
51	kmet	0	0	0	Unchecked
52	kstar	1	0	1	Unchecked
53	kdam	0	0.0001	10000	Unchecked
54	Temperature dependent permeability	Unchecked			
55	Damage	Unchecked			
56	Preconditioning Initials	Unchecked			
57	Max. delta (ODE)	0.01			
58	Objective	Least Square			
59	Logarithmize the experimental data	Unchecked			
60	Use Different Exponential Growth Rates of Control	Unchecked			

Figure 34: Script file

Abbreviations

AR	Absolute Residuals
Conc	Concentration
EF	Model Efficiency
Exp	Experimental Data
FN	Frond Number
BM	Biomass
MoLePo	TK-TD Model for *Lemna* Populations
N	Nitrate in mg/L
ODE	Ordinary Differential Equation
P	Phosphate in mg/L
PP	Photoperiod
SR	Squared residuals
SRMSE	Squared Root Mean Squared Error
STE	Scaled Total Error
TD	Toxicodynamic
Temp	Temperature in °C
TK	Toxicokinetic
TRACE	TRansparent And Comprehensive model Evaludation