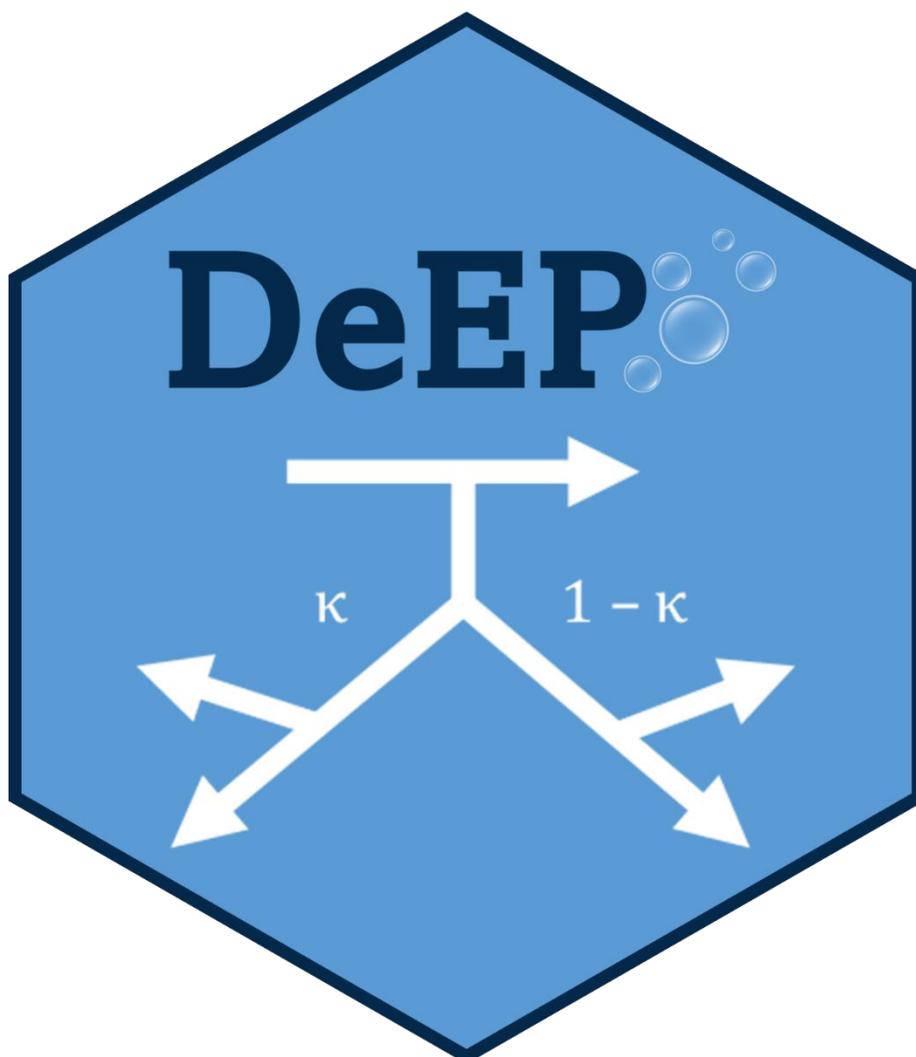# User Manual: DEB-TKTD EPx Predictor (DeEP)

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Contents

1. Introduction and Background

This user manual describes how to use the DEB-TKTD EP_x Predictor (*DeEP*), but it does not go into great detail regarding the underlying theory. *DeEP* makes forward predictions using the DEB-TKTD model 'DEBtox2019' (for full details of the model theory see Jager, 2020). The software is specifically designed to make forward predictions only, not to perform model calibration and validation. Therefore, validated model parameters for a given species and chemical need to be provided. You may derive and validate your own model parameters using other software (e.g. https://www.debtox.info/byom.html) or use a parameter set that has been tested and documented by others.

Specifically, *DeEP* predicts the EP_x multiplier, an endpoint relevant to ecological risk assessment (ERA) of plant protection products (for details see EFSA, 2018). This is the factor by which a given (realistic) exposure profile would need to be multiplied in order to elicit an X% reduction in an endpoint, i.e. either growth or reproduction. The higher the EP_x multiplier, the lower the risk that the environmentally relevant exposure would lead to unacceptable impacts.

The software uses a 'moving time window' approach in which long exposure profiles are broken down into multiple overlapping 'virtual toxicity tests' (VTTs). Further details on this approach are provided below. The lowest EP_x multiplier derived for a compound is then compared to a (user defined) critical value, if that value is exceeded then the test criterion is passed.

2. Methods

This section provides an overview of the underlying methods required to understand how the software works and to interpret its outputs.

DEB-TKTD

The underlying toxicokinetic-toxicodynamic (TKTD model) is based on a simplified version of the dynamic energy budget theory called DEBkiss (Jager et al., 2013). The DEBkiss model simulates the normal life history for the species (growth, maturation, and reproduction rates over time) while the TKTD module simulates toxic effects on biological processes. Toxic effects will lead to reductions in growth and/or reproduction. Only sublethal effects are modelled with this software but user friendly software also exists to model survival with the GUTS framework (Jager et al., 2011; Jager and Ashauer, 2018), which is available e.g. at https://openguts.info/.

Specifically, the tool uses the 'DEBtox2019' implementation (Jager, 2020) which presents the model with the fewest parameters possible, allowing for a streamlined user-interface. Further information about the theoretical models is not provided here but can be found in the references provided.

Moving Time Window

DeEP evaluates each exposure profile using the 'moving time window' approach (Sherborne et al., 2020). This means that the whole profile is broken down into overlapping windows of a fixed length and predictions are made for each window separately. Each simulation can be thought of as a 'virtual toxicity test' or VTT. A simple example would be a 6-day exposure profile with a 3-day time window moving forward in 1-day timesteps (Figure 1). This would break down into four 3-day windows, with consecutive windows having two days of overlap.

The length of the time window should be decided based on the specific question being approached. For example, this may be the duration of laboratory experiments used for model calibration, the length of the species' most vulnerable life stage, or the entire lifespan of the species.

The software also pads the exposure profile with zeros, such that the first window only includes exposure in the last time step. The same number of zeros are added to the end of the profile. This ensures that all possible combinations of age and concentration are considered. In the example above, this would mean that the number of time windows would increase from four to eight. The first window would cover days -2 to 1 and the last would cover days 5 to 8.
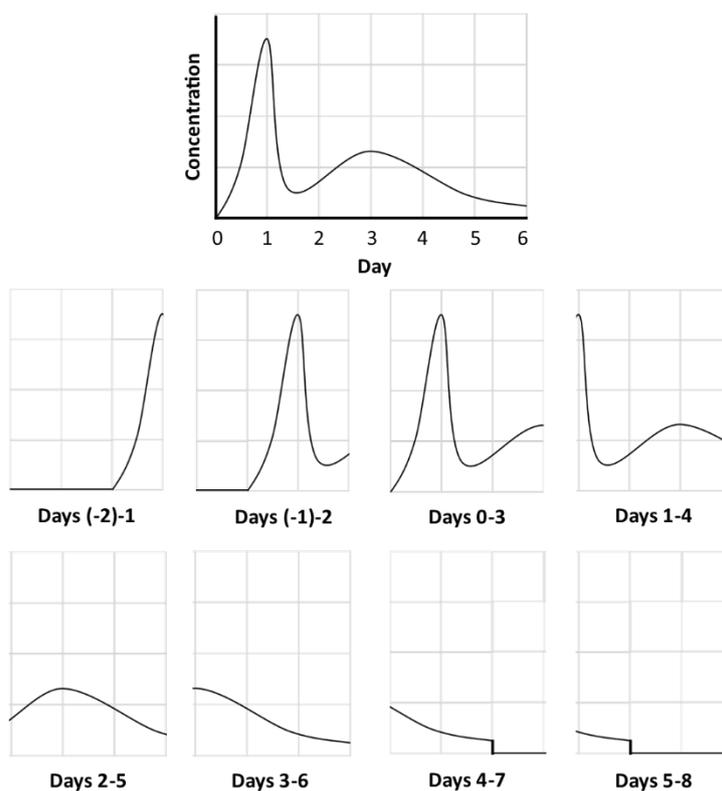
Figure 1: Diagram showing how a 6-day exposure profile would be padded with zeros and broken into eight overlapping windows of three days each.

EP$_x$ Multiplier

The EP$_x$ multiplier is the factor by which the concentrations within a given window would need to be multiplied in order to cause an X% reduction in growth or reproduction (relative to the control treatment). The higher the predicted EP$_x$ multiplier, the lower the ecological risk posed by an active substance and vice versa. The effect size of interest and threshold value for acceptable risk can be selected by the user. A small effect size and a high threshold value would represent a more conservative approach.

3. Navigating the dashboard

Save project **Load project**

DeEP - DEB-TKTD EPx Predictor

DeEP is an easy-to-use platform using DEB-TKTD modelling to predict the EP_x multiplier for a given chemical product and species. The EP_x multiplier is an endpoint relevant to ecological risk assessment (ERA) of plant protection products. It is the factor by which a given (realistic) exposure profile would need to be multiplied in order to elicit an X% reduction in either survival, growth or reproduction.

You can load your parameter values, settings and exposure profiles using the dashboard below and click 'Run Exposure Assessment' in the 'Results' box to quickly generate results. Detailed instructions and some background on the underlying model theory can be found in the manual.

NOTE: Predictions rely on validated parameter values provided by the user, these can be loaded from a file or entered/adjusted manually. Untested parameter values may be used for illustrative or educational purposes, but the predictions will not be valid.

Parameters and settings   +

Exposure profiles   +

Results ⓘ   +

The dashboard of the *DeEP* App is divided into three main sections:

- Parameters and Settings
- Exposure Profiles
- Results

This manual will cover each in turn. In the App click the plus or minus icon in the top right to expand or collapse any section or subsection.

4. Parameters and Settings

In this section the parameter values of the DEB-TKTD model for a specific species and compound as well as the settings of the output analysis are specified.

Project Details
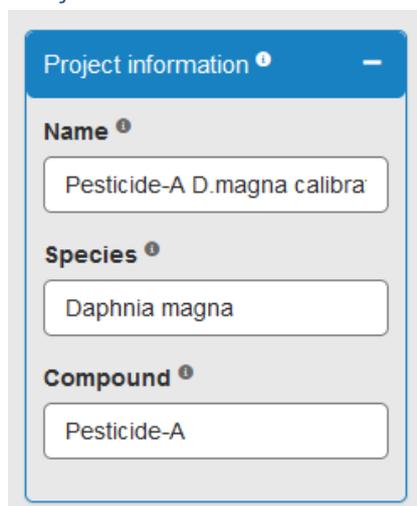
Any parameter set is specific to a species and a chemical compound or product. Here those details can be entered, and a name assigned to the project so that these details are easily identified if the project is saved and loaded again in the future.

Parameter values

Parameter values are grouped according to their function. For most uses, only the parameters in the first three sections will be necessary.

The parameters are divided into three main sections *Physiological parameters*, *Kinetics*, and *Sublethal dynamics*. A brief explanation for each parameter will be provided here. This information can also be accessed by clicking on the information icon next to each parameter in the app.

NOTE: *The units of some parameters depend on those of model inputs (user defined), so only dimensions are specified. For example, parameters which represent concentrations must have the dimensions mass x volume^{-1}, but units may be specified as mg x L^{-1} or µg x L^{-1}. Since the units of some parameters are user-defined, consistency is important. Advice on adjusting units of specific parameters is provided where appropriate below.*

The parameter set for a species and compound can be imported from a file via the Import button or entered manually and exported for future use. To load a parameter set from file, it must be saved as a plain comma-separated .csv or .txt file with two columns without headers. The first column indicates the name of the parameter with its value in the second column.

To save a parameter set for future use, enter the parameter values in the app and click *Save* (recommended). Alternatively, to produce a template file with the default parameter values, open the software, and click the *Save* button. This ensures that the parameter names are entered correctly. The parameter values can then be edited to create a new parameter set.

NOTE: *The parameter names in the file do not always match with how they appear on the user-interface. Instead, the variable names for the underlying computer code are used. A table explaining all the variable names can be found in Appendix 1.*

Physiological parameters

These parameters describe the rates of growth (cm/day) and reproduction (number of offspring/day) in the control treatment.

f: Food intake expressed as the scaled functional response to food availability (dimensionless). This ranges from 0, if no food is available, to 1 if food is unlimited (*ad libitum*).

rB: The Von Bertalanffy growth constant (day^{-1}). This determines the speed of growth up to a maximum length.

L0: The length (cm) of the organism at the start of the VTT.

Lp: The length (cm) of the organism at the onset of puberty.

Lm: The maximum length (cm) that an organism can reach.

Rm: The maximum reproduction rate (no. offspring $\times$ day^{-1}), this occurs when an organism at maximum size and in ideal conditions (*ad libitum* food availability).

Kinetics

Parameters describing the accrual and repair of 'scaled damage'. Scaled damage is an abstract variable (with the same units as the external concentration) that links chemical exposure to stress on the physiological model.

NOTE: *Check that the time units in the exposure profile match the data used in calibration. If not, the value of kd will need to be adjusted accordingly (e.g. to convert from hour^{-1} to day^{-1}, kd must be multiplied by 24).*

kd: Dominant rate constant (day^{-1}). This value determines the rate of 'scaled damage' accrual and repair under exposure to a chemical.

Feedbacks

In this section, the appropriate feedbacks for the TK model should be selected. In principle, this should be the same as used for calibration. The options are (any combination of):

Xu: The rate of damage accrual scales with surface area to volume ratio

Xe: The rate of damage repair scales with surface area to volume ratio

XG: Dilution by growth occurs (as the animal grows, scaled damage is reduced)

XR: Dilution by reproduction occurs (some toxicant or its damage is transferred to offspring)

Sublethal dynamics

These parameters describe the relationship between scaled damage (same units as external concentration) and stress (dimensionless) on the physiological model.

NOTE: *Check that the concentration units in the exposure profile match the data used in calibration. Otherwise, the value of zb and bb will need to be adjusted accordingly (e.g. if the calibration data were in mg/L (or g/m³) and the exposure profile is in µg/L, then zb must be multiplied by 1000 and bb must be divided by 1000).*

zb: The threshold level (same units as damage and external concentration) above which scaled damage places stress on growth and/or reproduction.

bb: Effect strength (damage^{-1}) or the rate at which stress increases with damage above the threshold level.

Mode of Action (pMoA)

In this section the mode of action for the chemical should be selected. This should be known from model calibration. The options are:

SA: Stress on assimilation (reduced assimilation rate from food)

SM: Stress on maintenance (increased costs of maintaining existing body mass)

SG: Stress on growth (increased costs per unit of new body mass)

SR: Stress on reproduction (increased costs per offspring produced)

SH: Stress on reproduction (increased hazard to embryo survival)

Advanced parameters

Some advanced DEB parameter values could be altered. However, these values are difficult to estimate and should often be left at their default values.

Advanced parameters

These parameters are not directly measurable and are challenging to estimate (with the exception of *FBV*, which is measurable but is often not required) so it is recommended they are kept at their default values unless they have been estimated by the user. *FBV* and *KRV* are only used if *XR* is selected as a feedback. The *kap* and *yp* values are only used when the animal experiences starvation (i.e., that stress is so high that maintenance costs cannot be paid anymore from the flux allocated to the soma).

FBV: Dry weight of one egg as a fraction of the mother's dry weight (dimensionless).

KRV: The ratio of scaled damage in egg material and in the body of the mother (dimensionless).

kap: The fraction of assimilates (from food) allocated to the processes of maintaining existing structural body mass and growth of new structure (dimensionless).

yp: The product of two yield parameters (dimensionless). The yield of new structure on assimilates (i.e. the efficiency of growth) and the yield of assimilates on structure during weight loss from starvation. The product describes the proportion of assimilates used in growth that can be recovered during weight loss.

Simulation Settings

The simulation settings section allows the user to set initial conditions for the state variables and to set various assessment criteria. It may be useful to refer to Methods if the terminology is unclear.

% Effect

This is the effect size of interest and so is determined by the risk assessment criteria. If, for example, effects greater than 10% are deemed unacceptable, then **% Effect** should be set to 10. The software will then predict the EP_{10} multiplier for the compound and species given the model parameters specified above.

Window Length and Timestep

Window Length defines the duration of each 'Virtual Toxicity Test' (VTT) while **Timestep** is the amount of time between the start of each VTT. As mentioned, **Window Length** should be decided based on the specific question being approached. For example, this may be the duration of laboratory experiments used for model calibration or the lifespan of the species. However, **Window Length** must be greater than the **Timestep** between the start of each VTT. Otherwise, there would be gaps in the exposure assessment.

Thinning on/off

Check this box to speed up analysis by applying a filter to all the windows. Only windows that might represent the worst-case scenario will be included in analysis. Windows in which the maximum concentration is lower than the minimum concentration of at least one other window in the profile are excluded from the analysis. For more details see Sherborne *at al.* (2020).

Pass/Fail

This value describes the EP_x multiplier below which risk assessment is failed, this is decided by the guidance being followed.

Cutoff

This is the point at which the precise value of the EP_x multiplier is no longer required. For example, if the **Pass/Fail** = 10, **Cutoff** might be set to 1000. If the EP_x multiplier for a time window is above **Cutoff** then the software will return '>1000' rather than a precise value. Since, in this example, the EP_x multiplier is at least 100 times the value required to pass risk assessment, extra precision may not be necessary or desirable. Setting a lower **Cutoff** value also improves the readability of the output plots by limiting the scale on the y-axis.

NOTE: Cutoff must be greater than or equal to **Pass/Fail** otherwise the outputs will not be meaningful. If the **Cutoff** = **Pass/Fail** then exact EP_x multipliers will only be calculated if below the **Pass/Fail** threshold.

Detail on/off

This box may be unchecked if the results are desired only as pass or fail. This has a similar effect to setting **Cutoff** = **Pass/Fail** but in this case, no exact EP_x multipliers will be returned, whether above or below the **Pass/Fail** value.

5. Exposure Profiles

In this section the exposure profiles are selected for which the EP_x calculation will be conducted.

Exposure profiles must be provided as a .csv file (comma separated) or .txt file (tab separated). The profile should be separated into two columns with no headers. Column 1 should contain the time in days while column 2 should contain the chemical concentration at each timepoint.

NOTE: Check that the units for time and concentration match with the data used in model calibration. Otherwise, some parameters need to be adjusted (see Parameters and Settings).

The exposure profiles can be loaded by selecting the "upload your own" radio button, clicking the 'Upload exposure profiles' button and selecting one or more desired file(s). A plot for each profile can be viewed by selecting the desired profile name from the drop-down list. Remove a profile by selecting it from the drop-down list and then clicking 'Remove selected'.

TOXSWA exposure profiles

Whenever *DeEP* is running locally (i.e. either installed locally from the R-package or as the standalone version) the direct import of TOXSWA exposure profiles is supported. After clicking the "Load profile(s)" button a radio button with "TOXSWA profiles" is available. Here TOXSWA profiles can be uploaded from TOXSWA-out files by using the "Upload exposure profiles" button for profile selection followed by the import with "Import files". Please note that the unit in which TOXSWA measures the concentration is in g/m^3 and that therefore all parameter units should be adjusted accordingly (an example is given in the 'Parameters and Settings' section). The exposure profiles can be viewed and removed in the same way as described above.

Select the source of the exposure profiles

○ example profiles ○ upload your own ◉ TOXSWA profiles

⬆ Upload exposure profiles

Profiles that can be directly loaded from the TOXSWA out-files. The unit in which TOXSWA measures the concentration is g/m^3. All parameters should be used accordingly.

Import files

6. Results

Once the parameters, settings and exposure profiles have been loaded the model can be run. Click *Run exposure assessment* (or *Save and run exposure assessment* if you wish to save the results) to generate results for each exposure profiles loaded.

Results ⓘ   —

Click 'Run exposure assessment' to calculate the EP_x multiplier for all exposure profiles loaded above, the results will be summarised in a table. Click on any row of the results table to view the results for a single profile on a plot. For more information on how to interpret the results table and plots, click the info button.
To export all results into a word document, click 'Generate report'. This report gives a summary of the results and explains what they mean in a risk assessment context.

| Save and run exposure assessment | Run exposure assessment | ⬇ Generate report |

Summary Results Table

Profile names	Pass/Fail	EPx	Critical endpoint	Total no. of VTTs	No. of VTTs thinned	No. of VTTs tested
apple_R1_pond_01	Pass	608.908	Reproduction	384	341	43
apple_R2_stream_01	Pass	1000		384	119	265
cereal_D1_ditch_01	Pass	114.948	Reproduction	504	461	43
cereal_D1_stream_01	Pass	608.908	Reproduction	384	341	43

The software returns a summary of results in a table format. Each row represents one of the exposure profiles tested. The columns are as follows:

- **Profile names** – The names assigned to each exposure profile
- **Pass/Fail** – The result for the whole exposure profile (True/False)
- **EPx** – The lowest EP_x multiplier predicted for any time window within the exposure profile
- **Critical Endpoint –** The endpoint for which the lowest EP_x was derived (growth, or reproduction)
- **Total no. VTTs** – The total number of virtual toxicity tests (VTTs, i.e. the total number of time windows)
- **No. VTTs thinned –** The number of VTTs excluded from analysis by thinning
- **No. VTTs tested –** The number of VTTs conducted after thinning

Results Plot

The results for each individual profile can be visualised in a plot like the one below by selecting that row of the results table. The black line shows the lowest EP_x multiplier predicted for any endpoint in each time window vs the start time of that time window. The user-specified pass/fail threshold is shown as a horizontal red line. If the black line drops below the threshold at any point, this represents a failure to meet the risk assessment criterion. Values above the **Cutoff** are not plotted.
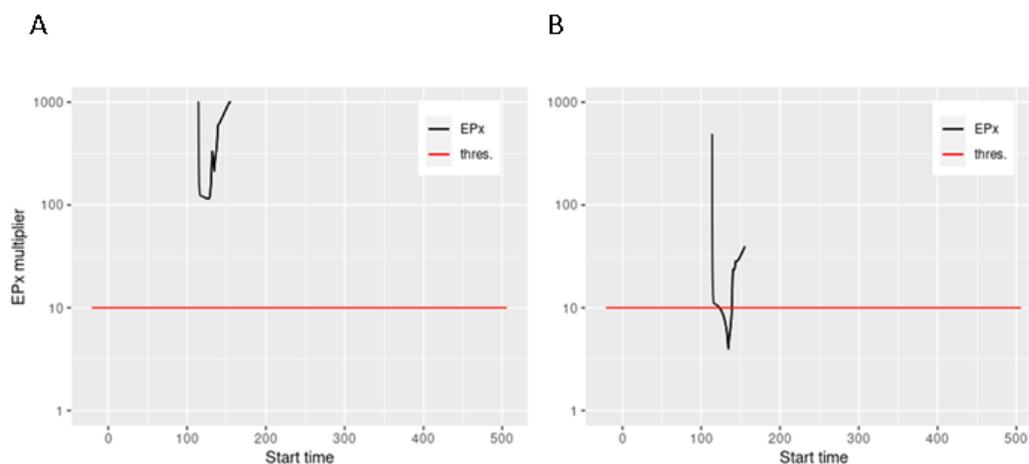

Figure 2: Example results plots. In plot A, the lowest predicted EP_x multiplier is above the Pass/Fail threshold (red horizontal line) so the risk assessment criterion is met. In plot B, the lowest predicted EP_x multiplier is below the Pass/Fail threshold so the risk assessment criterion is not met.

Automatically Generated Report

Results summary

After running the exposure assessment an output report can be created by clicking 'Generate Report'. This produces a word document summarising the results.

The report first describes the overall result for all of the exposure profiles provided. If the predicted EP_x multiplier was above the pass/fail value for all profiles, then the overall result is that the risk assessment criterion is met. If the predicted EP_x multiplier was below the pass/fail value for one or more profiles, then the overall result is that the risk assessment criterion is not met.

Table 1 in the report is the same summary table as shown in the software app, containing the results for each profile.

Results for each profile

The results for each individual profile are then described in more detail alongside the plot which could also be viewed in the software (Figure 2) and a plot of the related exposure profile.

Parameters and settings

Tables 2 & 3 of the report respectively contain all the parameter values and the model settings used in the simulations.

7. Save or load a project

To save your work and return to it later, scroll to the top and click *Save project*. This will save your work (parameter values, exposure profiles and any results) as a .rds file. To continue working later, open the software, click *Load project* and select the desired project file.

8. References

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9. Appendix 1

Table containing an explanation of variable names as they appear in the automatically generated csv file and on the software interface.

Variable name in csv file	Corresponding variable name on software interface
XR	Dilution by reproduction (XR)
Lp	Lp
b_b	bb
kd	kd
Lm_TK	LmTK
yP	yP
K_RV	KRV
F_BV	FBV
y0_1	L0
ECpct	% Effect
SM	Stress to maintenance (SM)
interval	Timestep
Rm	Rm
thinning	Thinning
detail	Detailed EPx output
kap	kap
SR1	Stress to reproduction - reproduction costs (SR)
passFail	Pass/Fail
Xe	Scaled elimination (Xe)
Compound	Compound
z_b	zb
XG	Dilution by growth (XG)
SA	Stress to assimilation (SA)
f	f
rB	rB
Lm	LmTK
Name	Name
Xu	Scaled uptake (Xu)
Species	Species
SR2	Stress to reproduction - embryonic hazard (SH)
windowLength	Window Length
cutOff	Cutoff
Model	N/A (not used in current version)
SG	Stress to growth (SG)
padDt	N/A (Automatically the same as Timestep)