Report of the working group *Lemna* of the SETAC Europe Interest group Effect Modeling

**Refined description of the *Lemna* TKTD growth model based on Schmitt *et al.* (2013) – equation system, default parameters, implementation in R**

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Changes to version 1.0 (22. Sept 2021):

- Inclusion of links to R-implementation, code verification and documentation by Nils Kehrein (in Introduction and Annex 5.3)
- Clarifications / refinements
  - Clarification on the recommended settings for the loss rate in simulations of laboratory tests in text and box 1, removing old appendix 5.4
  - Change in the handling of very low biomass values due to the implementation in R (setting \( \frac{dBM}{dt} \) to zero if \( BM < BM_{\text{min}} \) and \( \frac{dBM}{dt} < 0 \)
  - Correction of typos

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1 Introduction

Toxicokinetic-toxicodynamic (TKTD) models aim to describe the course of effects of a toxicant on an organism over time via the link between external concentration and effect. The TK part describes uptake, transportation within the organisms, biotransformation and elimination processes. The TD part relates the internal concentration to damage and the final adverse effect on growth, reproduction or survival. A well-known and relatively simple TK-TD model is GUTS, describing lethal effects on aquatic organisms where organisms are considered as one compartment (Jager et al. 2011, Jager & Ashauer 2018). More complex models differentiate organisms into several organs and consider transport between and biotransformation in the different compartments (so called pharmacokinetic models, e.g. Krishnan & Peyret (2009)).

In the context of the environmental risk assessment of plant protection products, TK-TD models offer tools to deal with the diversity of dynamic exposure profiles predicted for different uses, landscapes and habitats. Recently the EFSA PPR panel (2018) reviewed available TK-TD models for aquatic organisms.

One of the models reviewed by the PPR panel is a model for the standard test macrophyte Lemna spec. (duckweed), developed by Schmitt et al. (2013). The model was considered ‘ready to be used in risk assessment’ (abstract, EFSA PPR Panel (2018)). However, for the review, the model was documented only as a peer-reviewed publication including R-code in the supplementary information. For its broader use in regulatory risk assessment, a more formalized documentation of the model (equation system), its default parameters and its implementation as well as recommendation for its use including calibration, validation and application to ecological scenarios would be useful.

As a first step, a user-friendly implementation (MoLePo) including TRACE documentation and manual was developed by Klein & Hommen (2018). In order to agree by a broader group of modellers and users on a standard Lemna model version and documentation as well as on recommendations for its use, the working group ‘Lemna’ of the SETAC Europe Interest Group Effect Modelling has been established.

This document is the first report of the Lemna working group addressing the model equations, the default parameters and the implementation in R-code. Therefore, the description in the original publication by Schmitt et al. (2013) and the implementation of the model in the supplementary information are compared. Reasoned suggestions for a refined model description and the default parameters are made if needed.

Based on this, an new implementation in R was programmed and is available free of charge as an R package on CRAN1. The lemma package’s source code and documentation is also available as free and open-source software on GitHub2. The implementation was verified by

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1 The Comprehensive R Archive Network, lemma package: https://cran.r-project.org/package=lemma
2 Lemna package repository on GitHub: https://github.com/nkehrein/lemna
means of data sets used in Schmitt et al. (2013) and Hommen et al. (2016) by comparing results obtained with the original R-Code provided by Schmitt et al. (2013) and the refined implementation presented in this report.

2 Comparison of model description in the paper and the implementation

The main state variable of the model is biomass (as dry weight) of a *Lemna* population per area, i.e. g dw m$^{-2}$ for field populations. The growth of individual plants is not explicitly considered. The biomass is increased by photosynthesis and reduced by respiration, mortality and other losses. Production and loss of biomass are influenced by several environmental factors as indicated in Figure 1.

![Figure 1: Conceptual diagram of the TK-TD population model for *Lemna* sp. by Schmitt et al. (2013).](image)

In the description of the model, we follow the structure in Schmitt et al. (2013):

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1 The verification is described here: [https://cran.r-project.org/web/packages/lemna/vignettes/lemna-verification.html](https://cran.r-project.org/web/packages/lemna/vignettes/lemna-verification.html)

2 For simulation of laboratory populations, e.g. for calibration and validation of the TK-TD parameters, the relation to area is ignored and the biomass or abundance is given as mass or frond number per vessel.
1. Growth Model describing the population growth of *Lemna* sp. depending on different environmental factors
   a. Temperature
   b. Irradiation
   c. Nutrient concentrations
   d. Population density
   e. Toxicant concentrations (TD model)

2. TK model (calculation of the internal concentration of the toxicant)

In each section, first the model equations as given in the paper (Schmitt et al. 2013) and the implementation in R in the supplementary information of the paper are compared. Equations and R-code copied from Schmitt et al. (2013) are indicated by blue framed boxes. Then the parameter values and their units as proposed by Schmitt et al. (2013) as given in the paper and in the R-code are discussed. Finally, our suggestions for a refined description of the model equation and the default model parameter values are given.

Thus, the reader who is interested only in the final model description and its parameters can focus on the proposed equations and parameters in the red framed boxes.

2.1 Population growth model

The basic differential equation for the population dynamics is given in Equation 1 of the paper. $BM$ is the state variable biomass [g dw m⁻²], $t$ is time [d], $\theta$ is the set of actual environmental factors (light, temperature, nutrient and toxicant concentrations in the water and the actual biomass), $f_{photo}$ and $f_{resp}$ are dimensionless functions describing the effect of environmental factors on photosynthesis and respiration, characterized by maximum photosynthesis rate $k_{photo\_max}$ [d⁻¹] and a reference rate respiration rate $k_{resp\_ref}$ [d⁻¹], i.e. the respiration rate at 25 °C, respectively. Respiration is used in a broad sense here, including the loss of biomass via natural mortality:

**Equation 1 in Schmitt et al. (2013): Basic growth equation**

\[
\frac{dBM}{dt} = f_{photo}(\theta)k_{photo\_max}BM - f_{resp}(\theta)k_{resp\_ref}BM
\]

Corrected

\[
\frac{dBM}{dt} = f_{photo}(\theta)k_{photo\_max}BM - f_{resp}(\theta)k_{resp\_ref}BM
\]

There is a small typo in equation 1: $BM$ is accidently written as an exponent of $k_{photo\_max}$ but it should be a factor (see correction of Equation 1 above).

The R-code for the growth differential equation is provided in the following box, explanations are given below the box.
Implementation of the growth equation in mmc3.R (Schmitt et al. 2013)

```r
if (!k_phot_max) {
  k_phot_eff <- k_phot_max * f_R(actRad, k_0, a_k)
  k_phot_eff <- k_phot_eff * f_S(actTemp, Topt, Max, Tmin)
  k_phot_eff <- k_phot_eff * f_C(C_p, CP50, e_p, KiP) * f_N(C_N, CN50, a_N, KiN)
  k_phot_eff <- k_phot_eff * f_E(BM, BM50)
  k_resp_eff <- k_resp * f_T_resp(actTemp, t_ref, q10)
}

# Consider toxic effect
f_Eff <- f_E(b, EC50, Emax, C_int_u)
if (TDmod == 'delayed') {f_Eff <- E}  # delayed effects could be considered
k_phot_eff <- k_phot_eff * f_Eff

# Biomass
dB/dt <- BM * (k_phot_eff - k_resp_eff - k_loss)
# let population extinct if less than one frond/BM
if (BM < mass_per_fron) {dB/dt <- 0}
```

If the growth rate should not be fixed (line 202), i.e. a field population with time variable environmental factors should be modelled, the effective photosynthesis rate \( k_{\text{phot eff}} \) and the effective respiration rate \( k_{\text{resp eff}} \) are calculated by functions of response to the different 'natural' environmental factors (lines 203 – 207, explained later). The expression \( k_{\text{phot eff}} \) corresponds to \( f_{\text{photo}}(\theta) \) in equation 1 multiplied with the maximum photosynthesis rate \( k_{\text{phot max}} \). For the calculation of \( k_{\text{photo eff}} \) the responses for each factor are multiplied.

The expression \( k_{\text{resp eff}} \), the effective respiration rate, is the reference respiration rate \( k_{\text{resp}} \) multiplied with the response to the given temperature.

If growth should be affected only by the toxicant, e.g. for simulating a laboratory test under constant conditions, the effective photosynthesis and respiration rate can be set to default values (lines 209-210).

In lines 214-216 the inhibition of photosynthesis by a toxicant is calculated \( f_{\text{eff}} \), which is explained later in the section on the TD model (section 1.e).

Line 219 is the implementation of Equation 1. The R-code differs from the equation in the paper by using an additional loss term, \( k_{\text{loss}} \), to consider e.g. loss by flow, which is not mentioned in the paper. However, \( k_{\text{loss}} \) is set to zero in the code (see below) and thus, it does not affect the model results.

Biomass values cannot be negative and line 221 avoids this in the model. If the actual biomass is smaller than 5 times the mass per frond - in other words, if there are less than five fronds left (not one frond as said in line 220), the change of biomass is set to zero. Thus, if the population falls below the threshold it will stay constant and cannot recover.

In Table 1 of the paper, 0.42 d\(^{-1}\) and 0.05 d\(^{-1}\) are listed for \( k_{\text{max photo}} \) and \( k_{\text{ref resp}} \), respectively. However, in the supplemented R-code, the values of \( k_{\text{phot max}} \) is given as 0.47 d\(^{-1}\) and it is explained as ‘maximum growth rate + kmort’. The value for \( k_{\text{resp}} \) of 0.05 d\(^{-1}\) is the same as \( k_{\text{resp ref}} \) in the paper but note that \( k_{\text{resp}} \) is explained in the implementation as ‘rate of mortality’.
Lasfar et al. (2007) is given as reference for the value of the \(k_{\text{phot}}\). However, neither a value of 0.42 d\(^{-1}\) nor 0.47 d\(^{-1}\) is explicitly given in this paper. Since the growth rate is the difference of the photosynthesis rate and the rate of loss by respiration and mortality, we assume that the maximum photosynthesis rate should be 0.47 d\(^{-1}\) resulting in a growth rate of 0.42 d\(^{-1}\) if photosynthesis and loss rates are fixed. This is supported by Figure 2 in the text ‘Parameterization of the growth model’ in the supplementary data of Schmitt et al. (2013), showing the effects of temperature on photosynthesis and respiration, the value of 0.47 d\(^{-1}\) is the maximum photosynthesis rate while 0.42 d\(^{-1}\) seems to be the maximum growth rate (see Figure 2 in this document below). In addition, in Table 3 of the paper, summarizing the parameters used in Monte-Carlo simulations, values of 0.47 and 0.05 are used (note that the unit in Table 3 must be d\(^{-1}\) and not g dw d\(^{-1}\)). Thus, 0.47 d\(^{-1}\) seems to be the maximum photosynthesis rate used by Schmitt et al. (2013) and will also be used in the standard model here.

The value of 0.05 d\(^{-1}\) for \(k_{\text{resp}}\) is explained in the supporting information by Schmitt et al. (2013) as follows: ‘The respiration rate has been estimated as the inverse of the typical life span of Lemna fronds that is about 20 days at temperatures around 25 °C with little variation between species (Claus, 1972).’ Thus, \(k_{\text{resp}}\) as parameterized by Schmitt et al. (2013) is mainly a mortality rate.

We suggest the following mathematical formulation of the basic differential equation which describes the change of biomass over time. Since the respiration rate as used by Schmitt et al. (2013) covers respiration and mortality and is parametrised based on life span, we suggest to rename it to loss rate as this describes the process and the data that was used to parameterize the process better.

As Schmitt et al. (2013) we propose to define a minimum biomass value \(BM_{\text{min}}\) to define how the population should develop at very low abundances. If the actual biomass falls below a given threshold \(BM_{\text{min}}\) and the differential quotient \(dBM/dt\) is negative then \(dBM/dt\) is set to zero. By default \(BM_{\text{min}}\) is set to 0.0005 g dw m\(^{-2}\) as used by Schmitt et al. (2013). Note that the population can recover if the conditions allow a positive \(dBM/dt\).
Box 1: Basic differential equation for the change of *Lemna* sp. biomass over time

\[ BM: [0, t_n] \to \mathbb{R}_+ \]

\[
\frac{d}{dt}BM(t) = \left( k_{\text{photo, max}} \cdot f_{\text{photo}}(t) - k_{\text{loss, ref}} \cdot f_{\text{loss}}(t) \right) \cdot BM(t), \quad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}
\]

The biomass \( BM \) is given in g dw m\(^{-2} \) for field populations and in g or mg per vessel as a surrogate for surface area when modelling laboratory testings. The photosynthesis dependency function \( f_{\text{photo}}(t) \) is a dimensionless scaling function between zero and one for photosynthesis depending on the current environmental conditions, including the internal toxicant concentration, changing over time.

The dimensionless biomass loss dependency function \( f_{\text{loss}}(t) \) calculates the relative loss rate depending on actual temperature which is then multiplied with a reference loss rate for a specific temperature.

If the actual biomass falls below a given threshold \( BM_{\text{min}} \) the differential quotient is set to zero. The default settings correspond to Schmitt et al. (2013) and allows extinction.

If \( (BM(t) < BM_{\text{min}} \text{ and } dBM/dt < 0) \) then set \( dBM/dt = 0 \)

Default parameter values:

\[
k_{\text{photo, max}} = 0.47 \text{ d}^{-1} \quad k_{\text{loss, ref}} = 0.05 \text{ d}^{-1}
\]

\[
BM_{\text{min}} = 0.0005 \text{ g dw m}^{-2}
\]

**Remark:**

In case of laboratory conditions (switch variable \( k_{\text{photo, fixed}} = \text{TRUE} \)), the dependencies from \( T, I, N, P, \) and \( BM \) can be ignored, and \( f_{\text{photo}}(t) \) only depends on the internal toxicant concentration. If the model should be used only to model refined exposure laboratory tests, \( k_{\text{loss, ref}} \) can also be set to zero. Then \( k_{\text{photo, max}} \) represents the growth rate. For calibration and validation of the TKTD model, this growth rate can be set to the observed growth rate of the control plants (see explanation below).

\[
\frac{d}{dt}BM(t) = \left( k_{\text{photo}} \cdot f_{\text{photo}}^{\text{intunb}}(t) - k_{\text{loss}} \right) \cdot BM(t), \quad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}
\]

Schmitt et al. (2013) say that for calibrating the TKTD parameters by means of data from a laboratory test ‘the model was parameterized as described above with the exception that the respiration respectively loss rate was set to zero’. However, it seems very unlikely that the default maximum photosynthesis rates of 0.47 d\(^{-1} \) was fitted the control growth of the calibration experiment as well as shown in Fig. 2 of the paper. With respect to the validation of the TKTD model by means of another laboratory test the ‘growth rate of the control was set to observed values’ (Schmitt et al. 2013).

The recommendation of the working group concerning the default value of \( k_{\text{loss}} \) during calibration or validation of the TKTD model using data of laboratory tests depends on the intended use of the model:
If a use of the full model is intended (i.e. simulation of populations under dynamic environmental conditions, e.g. in the field, corresponding to \( k_{\text{photo\_fixed}} = \text{FALSE} \)), we suggest that the loss rate should be set to its reference value (0.05 d\(^{-1}\)) and the photosynthesis rate should be fitted to achieve together with the loss rate the growth observed for the control of the experiment to be simulated. This is recommended because in the full model for dynamic environmental conditions, only the photosynthesis rate is affected by the toxicant. Thus, for such a use, also the calibration of the TKTD model should include a loss rate which is not affected by the toxicant.

If the model should be used only as a Tier 2C approach, i.e. to simulate refined exposure test under constant environmental laboratory conditions (corresponding to \( k_{\text{photo\_fixed}} = \text{TRUE} \)), the loss rate should be set to zero and the photosynthesis rate should be set to the growth rate observed in the control. In such a case, the inhibition of growth is modelled.

### 2.2 Response to environmental factors

In the following we differentiate between response and dependency functions to describe the effects of environmental factors on production and loss of *Lemna* biomass. Since the environmental factors can vary over time, the dependency functions also vary over time. In contrast to this, the response functions describe the response as a function of the environmental factor, e.g. a concentration response function.

Environmental factors in the *Lemna* models are water temperature \( T \) [\(^{\circ}\text{C} \)], irradiance [kJ m\(^{-2}\) d\(^{-1}\)], phosphorus P and nitrogen N concentrations [mg L\(^{-1}\)]. In addition, the actual biomass BM [g/m\(^2\)] is needed for density dependence and the inhibition of the photosynthesis depends of the internal unbound concentration of the toxicant, \( C_{\text{int\_unb}} \), which depends on the environmental factor external concentration in the water \( C_{\text{ext}} \) [e.g. µg/L] as described later in the section on toxicokinetics.

A more detailed mathematical descriptions is given in the following box.
Notes on the mathematical concept and wording to describe the effects on environmental factors on production or loss of biomass

The concept of environmental dependence influencing photosynthesis is for all environmental factors \((x)\) the same. The dependency functions map for each time point \(t\) in the interval \([0, t_n]\) a real number between zero and one: \(f^T_{\text{photo}}: [0, t_n] \to [0,1]\).

\[
\frac{d}{dt} BM(t) = \left( k_{\text{photo, max}} f^p_{\text{photo}}(t) - \frac{K_{\text{ass}}}{f^r_{\text{repl}}(t)} \right) \cdot BM(t), \quad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}
\]

We consider ordered time points \((0 = t_0 < t_1 < \cdots \leq t_n)\). Let \(Z\) be the set of ordered time points \(Z = \{t_0, t_1, \cdots, t_n\}\). For each time point \(t_i \in Z, i = 1, \cdots, n\), there is a measured real value e.g. temperature values \((Z \to \mathbb{R})\). The time series of meteorological variables is what we usually call „driving data“ in pesticide fate and exposure modelling.

For example, it is possible to obtain temperature values in the total interval \([0, t_n]\) using interpolation between measured values or from a function (temperature function \(T\)).

\[T: [0, t_n] \to \mathbb{R}\]

The function mapping each temperature to a value between zero and one is called response function. The response function is indicated by an accent "^". Thus, for the temperature response function \(f^T_{\text{photo}}\) we have:

\[f^T_{\text{photo}}: \mathbb{R} \to [0,1]\]

The variable of the temperature response faction is temperature \(T \in \mathbb{R}\).

Combining both, the temperature function and the temperature response function, we get the temperature dependency function \(f^T_{\text{photo}}\), mapping each time point a value between zero and one (using the temperature response function).

\[f^T_{\text{photo}}: [0, t_n] \to \mathbb{R} \xrightarrow{f^T_{\text{photo}}} [0,1]\]

In Schmitt \textit{et al.} (2013), the photosynthesis dependency function is calculated by multiplying the single environmental dependency functions (including the toxicity response):

\[f_{\text{photo}}(t) = f_{\text{photo}}(t) \cdot f^p_{\text{photo}}(t) \cdot f^T_{\text{photo}}(t) \cdot f^b_{\text{photo}}(t) \cdot f^{\text{int,uib}}_{\text{photo}}(t)\]

\[1\] This equation is not given explicitly in the paper but it is said that ‘fx(θ) is the product of functions depending on single parameters which are described below’ (Schmitt \textit{et al.} 2013, see also the r code given in section 2.1 above).
In contrast to Schmitt et al. (2013), we propose to apply Liebig's Law that growth is controlled by the most limiting resource (limiting factor)\(^1\). We consider the environmental factors temperature, light, phosphorus and nitrate for Liebig's law but density dependence and response to the toxicant as independent and thus, as additional factors.

**Box 2: Photosynthesis dependency function (including Liebig's Law)**

\[
f_{\text{photo}} : [0, t_n] \rightarrow [0,1] \\
f_{\text{photo}}(t) = \min\left( f^T_{\text{photo}}(t), f^I_{\text{photo}}(t), f^P_{\text{photo}}(t), f^N_{\text{photo}}(t) \right) \cdot f^{BM}_{\text{photo}}(t) \cdot f^{C_{\text{int unb}}}_{\text{photo}}(t)
\]

\(t = \text{time [d]}, T = \text{temperature [°C]}, I = \text{irradiance [kJ m}^{-2} \text{ d}^{-1}], P = \text{phosphorus concentration [mg L}^{-1}], N = \text{nitrogen concentration [mg L}^{-1}], BM = \text{biomass [g dw m}^{-2}], C_{\text{int unb}} = \text{internal unbound concentration of the toxicant [µg L}^{-1}]

This refinement results in higher growth rates and higher maximum abundances of the simulated field populations, but it is considered to be more realistic than using the product of all single functions. Example simulations have shown that the effects of the population dynamics are only slight (https://cran.r-project.org/web/packages/lemna/vignettes/lemna-verification.html).

Biomass loss by respiration or mortality is assumed to be only affected by temperature as proposed by Schmitt et al. (2013). Note that it is assumed that the toxicant does not affect biomass loss due to lethal effects or increased respiration. This is probably sufficient for many cases since the standard *Lemna* tests addresses inhibition of population growth and not decline of abundance.

**Box 3: Respiration dependency function**

\[
f_{\text{loss}} : [0, t_n] \rightarrow \mathbb{R}_+ \\
f_{\text{loss}}(t) = f^{T}_{\text{loss}}(t)
\]

\(t = \text{time [d]}, T = \text{temperature [°C]}

### 2.2.1 Temperature response of photosynthesis

The response of photosynthesis to temperature is described by an asymmetric bell shaped function described by three parameters, \(T_{\text{min}}, T_{\text{max}}, T_{\text{opt}}\) (all given in °C). Equation 2 in the paper and the implementation in the code are equivalent (except the approximation of \(\ln(10)\) by 2.3 in the code).

\(^1\) For Liebig’s law, see e.g. https://en.wikipedia.org/wiki/Liebig%27s_law_of_the_minimum
Equation 2 in Schmitt et al. (2013): Temperature response function for photosynthesis

\[ f_{\text{photo}}(T) = \exp \left( -\ln(10) \frac{(T - T_{\text{opt}})^2}{(T_{\text{min}}(\text{max}) - T_{\text{opt}})^2} \right) \]  

In this function, \( T_{\text{min}} \) is used when \( T < T_{\text{opt}} \) and \( T_{\text{max}} \) when \( T > T_{\text{opt}} \).

Temperature response function for photosynthesis in mmc3.R (Schmitt et al. 2013)

```r
# Temperature
# effect on k_phot
f_T <- function(actTemp, Topt, Tmax, Tmin)
{
  if (actTemp <= Topt) {Tx <- Tmin}
  return(exp((-2.3*((actTemp-Topt)/(Tx-Topt))^2)))
}
```

The values for the three parameters defining the response were taken from Lasfar et al. (2007). There are no discrepancies between the values listed in Table 1 of the paper and the implementation.

Parameters for the temperature response function for photosynthesis (Table 1 in Schmitt et al. 2013)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{\text{min}} )</td>
<td>8.0 °C</td>
</tr>
<tr>
<td>( T_{\text{max}} )</td>
<td>40.5 °C</td>
</tr>
<tr>
<td>( T_{\text{opt}} )</td>
<td>26.7 °C</td>
</tr>
</tbody>
</table>

Parameters for the temperature response function for photosynthesis in mmc2.r (Schmitt et al. 2013)

```r
# Temperature dependence
Temp = 12, # [°C] Current temperature (may also be a table)
Tmin = 8.0 , # [°C] Minimum growth temperature [Ref. P 0191, data re-evaluated incl. Amort(71)]
Tmax = 40.5 ; # [°C] Maximum growth temperature [Ref. P 0191, data re-evaluated incl. Amort(71)]
Topt = 26.7 ; # [°C] Optimum growth temperature [Ref. P 0191, data re-evaluated incl. Amort(71)]
```

Note that Temp in line 82 is not a model parameter but a variable which is initialised here.

The resulting response function and the data used by Schmitt et al. (2013) to fit the parameters are shown in Figure 2. For a temperature equal to \( T_{\text{min}} \), respectively \( T_{\text{max}} \) the temperature response function value \( f_{\text{photo}}^T \) is equal to 0.1.
Figure 2: Left: $f_{\text{photo}}(T)$ as used by Schmitt et al. (2015) (modified from Klein 2018)
Right: Dependence of observed (●) and calculated (line) net growth rate $k_{\text{growth}}$ of $L.\ minor$ in dependence of temperature. Symbols (■) show the respiration rates $k_{\text{resp}}$ calculated with the respiration temperature response and (▲) the photosynthesis rates $k_{\text{photo}} = k_{\text{growth}} + k_{\text{resp}}$. was fitted to $k_{\text{photo}}$ (copied from Schmitt et al. 2013, supplemental data)

No changes of the temperature response function of photosynthesis as suggested by Schmitt et al. (2013) are suggested, we just use the term $10^{-X}$ instead of the more complicated but equivalent term $\exp(-\ln(10) \cdot x)$.

\textbf{Box 4: Temperature response of photosynthesis}

\[
\hat{f}_{\text{photo}}: \mathbb{R} \rightarrow [0,1]
\]

\[
\hat{f}_{\text{photo}}(T) = \begin{cases} 
\frac{(T-T_{\text{min}})^2}{10 (T_{\text{opt}}-T_{\text{min}})^2} & \text{if } T \leq T_{\text{opt}} \\
\frac{(T-T_{\text{opt}})^2}{10 (T_{\text{max}}-T_{\text{opt}})^2} & \text{if } T > T_{\text{opt}}
\end{cases}
\]

Default parameter values:

\[
T_{\text{min}} = 8 \, ^\circ\text{C}
\]
\[
T_{\text{max}} = 40.5 \, ^\circ\text{C}
\]
\[
T_{\text{opt}} = 26.7 \, ^\circ\text{C}
\]

2.2.2 Temperature response of biomass loss by respiration and mortality

The only environmental factor considered to influence biomass loss by respiration and mortality in the model is temperature and an approximation of the Arrhenius function (van’t Hoff’s rule) was used by Schmitt et al. (2013). The $Q_{10}$ relation describes the proportional change in response to a temperature increase of 10°C. The implementation in the R-code corresponds to equation 3 in the paper.
Equation 3 in Schmitt et al. (2013): Temperature response function for respiration

\[ f_{\text{resp}}(T) = Q_{10}^{(T-T_{\text{ref}})/10} \]

Temperature response function for respiration in mmc3.r (Schmitt et al. 2013)

```r
# effect on k_resp
f_T_resp <- function(actTemp, t_ref, Q10)
{
  Q10^{((actTemp-t_ref)/10)}
}
```

A reference temperature \( T_{\text{ref}} \) = 25 °C and a \( Q_{10} \) of 2 are used in the paper and the R-code based on Claus (1972) and Wangermann and Ashby (1951).

Parameters for the temperature response function for respiration (Table 1 in Schmitt et al. 2013)

\[ T_{\text{ref}} = 25 \degree C \]
\[ Q_{10} = 2 \]

Parameters for the temperature response function for respiration in mmc2.R (Schmitt et al. 2013)

```r
# k_resp
tr_ref = 25,  # temperature at which t_mort is effective
Q10 = 2,    #
```

Note that in contrast to the response functions for photosynthesis, there is no scaled response between 0 and 1. For temperature values greater than \( T_{\text{ref}} \) the response is greater than one and thus the loss rate is higher than the reference rate (Figure 3).

![Figure 3](image)

Figure 3: The respiration response due to temperature for \( T_{\text{ref}} = 25 \degree C \) and \( Q_{10} = 2 \), taken from Klein 2018. (See also Figure 3 for experimental data)
No changes to Schmitt et al. (2013) are suggested, despite that we use the term 'loss' to indicate the combination of mortality and respiration.

**Box 5: Effect of temperature on biomass loss rate**

\[
\dot{\rho}_{\text{loss}}: \mathbb{R} \rightarrow \mathbb{R}^+ \\
\dot{\rho}_{\text{loss}}(T) = Q_{10}^{\frac{T-T_{\text{ref}}}{10}}
\]

Default parameters:

- \( T_{\text{ref}} = 25 \, ^\circ\text{C} \)
- \( Q_{10} = 2 \)

### 2.2.3 Light response of photosynthesis

The effect of the light given as daily global radiation in kJ m\(^2\) d\(^{-1}\), on photosynthesis is assumed to be a linear function up to the light saturation level \( I_{\text{sat}} \) where \( f_{\text{photo}}(I) \) becomes equal to 1 (no inhibition). Thus, no inhibition by high light intensity is assumed.

The implementation corresponds to the description in Equation 4 except that the parameters were differently named (\( \alpha \) corresponds to \( a_k \) and \( \beta \) to \( k_0 \)). In the R-implementation, the saturation constant \( I_{\text{sat}} \) is not used but \( f_{\text{photo}}(I) \) is set to 1 if the linear function is > 1.

**Equation 4 in (Schmitt et al. 2013): Irradiation response function**

\[
f_{\text{photo}}(I) = \begin{cases} 
\alpha \cdot I + \beta & |I| \leq I_{\text{sat}} \\
1 & |I| > I_{\text{sat}}
\end{cases}
\]

**Irradiation response function for photosynthesis implemented in mmc3.r (Schmitt et al. 2013)**

```r
# Light
f_R <- function(actRad, k_0, a_k)
{
  photfac <- a_k*actRad + k_0
  if (photfac > 1) photfac=1
  return(photfac)
}
```

The intercept values \( \beta \) in Table 1 of the paper and \( k_0 \) in the implementation differ. An intercept of 3 is not possible for a function scaled from 0 to 1. Based on the Figure 4 below, showing experimental data and the fitted function, the value in the paper (0.25) is correct. Since \( f_{\text{photo}}(I) \) is just a dimensionless scaling function the intercept has to be dimensionless and the unit of the slope has to be inverse of the unit of the radiance. Thus, the unit given in the paper for \( \alpha \) (kJ\(^{-1}\)m\(^2\) d) is correct while the unit given for \( \beta \) is wrong as well as the units for both parameters in the R-code.
Parameters for the irradiation response function for photosynthesis (Table 1 in Schmitt et al. 2013)

\[ l_{\text{sat}} = 15,000 \quad [\text{kJ/(m}^2\text{d)}] \]
\[ \beta = 0.25 \quad [\text{kJ/(m}^2\text{d)}] \]
\[ \alpha = 5 \times 10^{-5} \quad [1/(\text{kJ/(m}^2\text{d)})] \]

Parameters for the irradiation response function for photosynthesis in mmc2.r (Schmitt et al. 2013)

```
90 # Light dependence (linear dependence on global radiation (see Hedgeson 1969)
91 \text{Rad} = 15000 \quad \# [\text{kJ/m}^2\text{d}] \quad \text{Radiation (may also be given as table)}
92 k_0 = 3 \quad \# [1/d] \quad \text{Intercept of linear part}
93 a_k = 5 \times 10^{-5} \quad \# [(1/d)/(\text{kJ/m}^2\text{d})] \quad \text{Slope of linear part}
```

Note that \text{Rad} in line 91 of the R code is not a parameter but initialises the irradiation variable.

The function predicts growth also for zero irradiation. This is unrealistic but probably not relevant for the simulations since even for very cloudy days in winter the irradiation will be larger than zero.

![Figure 4: Growth rates of L. minor observed at different temperatures under natural daylight. The line shows the dependence on global radiation evaluated by fitting the light response to the data (copied from Fig, 3 in Schmitt et al. 2013, suppl. data).](image)

The parameter \( l_{\text{sat}} \) defines the light value when the function value is equal to one \( (\alpha \cdot I + \beta = 1 \iff I = \frac{1-\beta}{\alpha}) \). The introduction of an additional parameter \( l_{\text{sat}} \) can result in conflicts with the other parameters since \( l_{\text{sat}} \) is already defined by \( \alpha \) and \( \beta \). Thus, we suggest to neglect the unnecessary parameter \( l_{\text{sat}} \).

The following suggestion uses the linear function suggested by Schmitt et al. (2013) but corrects the parameter values to correspond to Figure 4. A Michaelis-Menten function could be considered as an alternative to the hockey stick model used here. However, since data used
for fitting in Figure 4 are scaled to the maximum growth rates for each of the temperatures tested, the relative growth rate at high light intensities are mostly below 1 and fit by a saturation curve would result in function which does not reach 1 under realistic light conditions.

**Box 6: Effect of irradiance on photosynthesis**

\[
\bar{\tilde{T}}_{\text{photo}}(I) = \begin{cases} 
\alpha \cdot I + \beta & \text{if } I \leq \frac{1 - \beta}{\alpha} \\
1 & \text{if } I > \frac{1 - \beta}{\alpha}
\end{cases}
\]

Default parameters:

\[
\alpha = 5 \times 10^{-5} \text{ kJ}^{-1} \text{ m}^2 \text{ d} \\
\beta = 0.025
\]

\(\frac{1 - \beta}{\alpha}\) corresponds the saturation irradiance where \(\bar{\tilde{T}}_{\text{photo}}(I)\) is set to 1.

2.2.4 **Nutrient response of photosynthesis**

The effect of the availability of nutrients on photosynthesis is modelled as a saturation function characterized by a half-saturation constant (Michaelis-Menten or Monod equation). The inhibition is calculated independently for phosphorus and nitrogen given in mg P L\(^{-1}\) or mg N L\(^{-1}\).

In contrast to this, a more complex function is used in the implementation. It takes into account inhibition at high nutrient concentrations by using an additional parameter: a\_P or a\_N, respectively, as hill coefficient (exponent in the equation) and inhibition constants for very high nutrient concentrations KiP and KiN, respectively.
Equation 5 in Schmitt et al. 2013: Nutrient response function for photosynthesis

\[ f_{\text{photo}}(N) = \frac{[N]}{[N] + [N]_{50}} \]

The same function is used for phosphorus P.

Nutrient response functions for photosynthesis in mmc3.r (Schmitt et al. 2013)

```r
# Phosphorus
f_P <- function(C_P, CP50, a_P, KfP)
{
  C_P^a_P/(C_P^a_P + CP50^a_P) * KfP/(KfP + C_P)
}

# Nitrogen
f_N <- function(C_N, CN50, a_N, KfN)
{
  C_N^a_N/(C_N^a_N + CN50^a_N) * KfN/(KfN + C_N)
}
```

The half-saturation parameter values for the response of photosynthesis to nutrient concentrations are the same in the paper (Table 1) and the implementation. However, in the R-code also values for the additional parameters needed for the more complex description of nutrient dependence used in the code are given.

### Parameters for the nutrient response functions (Table 1 in Schmitt et al. 2013)

- \([P]_{50} = 0.0043\) mg/L
- \([N]_{50} = 0.034\) mg/L

### Parameters for the nutrient response function for photosynthesis in mmc2.r (Schmitt et al. 2013)

- \(c_P = 0.1\) mg/L
- \(CP50 = 0.0043\) mg/L
- \(a_P = 1\)
- \(KfP = 101\) mg/L

- \(c_N = 0.4\) mg/L
- \(CN50 = 0.034\) mg/L
- \(a_N = 1\)
- \(KfN = 404\) mg/L

In Table 3 of the paper, parameters used for Monte-Carlo simulations are listed and other values are given for P50 and N50 are given (0.85 and 0.46 µg L\(^{-1}\), respectively for P respectively N). These values represent probably the assumed P and N concentrations in the simulated water bodies rather than the half saturation constants because they are called together with the initial biomass BM0 ‘site specific parameters’ in this text section. However, the half-saturation constants are properties of \(Lemna\) and site specific parameters of water bodies. Thus, these values of 0.85 µg L\(^{-1}\) and 0.46 µg L\(^{-1}\) are probably the constant water concentrations of P and N assumed for the Monte-Carlo simulations in Schmitt et al. (2013) rather than new values for P50 and N50.

Nonetheless, the two types of functions, using one parameter (as in the paper) or three parameters (as in the R code) are similar for smaller nutrient concentrations. At higher
concentrations the 3-parameters function predicts an increasing inhibition of photosynthesis. This is contrary to the experimental data (Figure 4). Thus, the simple Monod function with one parameter as described in the paper is considered sufficient for implementation.

Figure 5: Growth rates of *L. minor* in dependence of nitrogen and phosphorus concentration as described in the paper and implemented in the R-code. Note that in Figure 4 and 5 in the file 1-s2.0-S0304380013000446-mmc1.doc in the supplementary information, the x-axis labels are switched by mistake.

We suggest to use the Michaelis-Menten equations (as given in the paper) instead of the more complex function in the code describing inhibition by high nutrient levels since the experimental data do not indicate inhibition at very high nutrient levels. The half-saturation constants fit the experimental data used by Schmitt *et al.* (2013) very well and thus, they are considered acceptable as default settings.

**Box 7: Effect of nutrient concentration on photosynthesis**

\[
\tilde{f}_\text{photo}^N: \mathbb{R}_+ \to [0,1], \quad \tilde{f}_\text{photo}^P: \mathbb{R}_+ \to [0,1]
\]

\[
\tilde{f}_\text{photo}^N(N) = \frac{N}{N + N_{50}} \quad \text{respectively} \quad \tilde{f}_\text{photo}^P(P) = \frac{P}{P + P_{50}}
\]

Default parameters

\[P_{50} = 0.0043 \text{ mg P L}^{-1}, N_{50} = 0.034 \text{ mg N L}^{-1}\]

**2.2.5 Density dependence**

Density dependence is relevant to consider e.g. effects of competition for nutrients or space at the water surface and self-shading if *Lemna* fronds overgrow each other. In laboratory tests, the aim is usually to provide conditions allowing continuous exponential growth (e.g. by changing medium and reducing the number of fronds if the experiment is prolonged). However, in some tests, e.g. older studies over 14 days without exchange of medium, the controls might also show slower population growth later in the test, resulting in logistic rather than exponential growth.
Density dependence is modelled as a linear function of the biomass resulting in a logistic growth curve. It needs only one parameter describing the carrying capacity of the system. The parameter is called limit density \( D_L \) in Schmitt et al. (2013). The response function is equal to one at an abundance of zero. In case the density limit \( D_L \) is reached, the response function value is equal to zero.

**Equation 6 in Schmitt et al. (2013): Density dependence function**

\[
 f_{\text{photo}}(I) = \begin{cases} 
 \frac{D_L - D}{D_L} & |D| \leq D_L \\
 0 & |D| > D_L 
\end{cases}
\]

Density dependence implemented in mmc3.r

```r
# Biomass (crowding)
#_BM <- function(BM, BM50)
#
#fact <- (BM50 - BM) / BM50
#return(fact)
```

There is a typo in equation 6 since it should be \( f_{\text{photo}}(D) \) instead \( f_{\text{photo}}(I) \). For consistency, it would also be better to use \( BM \) instead \( D \) since \( BM \) is the state variable used in the basic growth equation (Equation 1). The term used is equivalent to \( 1 - \frac{D}{D_L} \).

In the implementation, the limit density \( D_L \) is called BM50 which can be confused with a half saturation constant – which it is not. The density limit is the carrying capacity in logistic growth while a BM50 sounds like the biomass where the growth rate is 50 % of its maximum value. So, the parameter name BM50 should be changed in a refined R code to be consistent with the model description.

Setting the response to zero if the density is higher than the density limit is not implemented. This is okay since by using the logistic density dependence the modelled population cannot grow above \( D_L \) (if the time steps of the integration routine are sufficiently small).

In Table 1 of the paper as well as in the implementation, a default value of 176 g dw m\(^{-2}\) is used with a reference to Monette et al. (2006). However, the limit density given in Monette et al. (2006) is slightly but not significantly different (177 g dw m\(^{-2}\)).

**Parameters for the density dependence functions (Table 1 in Schmitt et al. 2013)**

\[
D_L\quad 176\text{g d.w./m}^2
\]

**Parameters for the nutrient response function in mmc2.R (Schmitt et al. 2013)**

```r
# - Density dependence -
BM50 = 176,  # [g dw/m²] Cut off BM [Ref. P 0131]
```

We suggest to change the writing to be consistent with the use of \( BM \) as state variable to describe the population in Equation 1 and to use the parameter value given in Monette et al. (2006).
Box 8: Density dependence

\[ f_{\text{photo}}^{BM} : \mathbb{R}_+ \setminus \{0\} \rightarrow [0,1] \]

\[ f_{\text{photo}}^{BM}(BM) = 1 - \frac{BM}{BM_L} \]

Default parameter

\[ BM_L = 177 \text{ g dw m}^{-2} \]

It should be noted that this limit density is never reached in the model. Even if the environmental factors are set to constant not limiting values, the maximum biomass reached is lower than the limit density since the density dependence term affects only the photosynthesis but not the population growth rate (as in the classical Verhulst logistic model). Thus, the density dependence does not consider the loss term and the realised growth rate is always lower than needed to approximate the density limit.

It should also be noted that conditions limiting the photosynthesis (e.g. a temperature below the optimum temperature) also reduce the maximum abundance which is reached because the reduction factor based on density dependence is multiplied by the reduction factors for the other environmental conditions. Thus, if based on the current biomass and the density dependence function, the photosynthesis rate could for example still be 90 % of its maximum value, a low temperature would reduce this further and in consequence zero growth is reached at lower biomass than under higher temperature. The same holds for the effect of a toxicant: constant exposure which reduces the photosynthesis rate would also reduce the carrying capacity which the modelled population can reach.

It could be discussed whether it is realistic that factors like temperature or toxicant concentration in the medium which are not consumable resources reduce the maximum abundance. However, since this affects the risk assessment only in a conservative way, it was decided to keep the original way how density dependence was modelled by Schmitt et al. (2013).

2.2.6 Concentration response (Toxicodynamics)

The effect of a toxicant is modelled by Schmitt et al. (2013) as inhibition of the photosynthesis rate depending on the internal unbound concentration of the toxicant in the plants \( C_{\text{int.unb}} \). The concentration response is modelled as a 3-parameter Hill function defined by the maximum inhibition \( E_{\text{max}} \), the \( EC50_{\text{int}} \) as the internal concentration resulting in 50 % effect and a slope parameter \( b \). There is a typo in equation 7 since it should be \( f_{\text{photo}}(C_{\text{int.unb}}) \) and not \( f_{\text{photo}}(E) \).

The implementation of the concentration response corresponds to the description in the paper. Nonetheless, in the implementation it is also possible to model a delayed effect where the inhibition is not directly linked to actual internal unbound concentration. Therefore an additional state variable \( E \) is modelled via a differential equation (line 224). This state variable \( E \) increases with a specific rate \( k_{\_E\_in} \) multiplied with the effect size resulting from the actual \( C_{\text{int.unb}} \), calculated by the function \( f_{\_E} \) shown above and it decreases with a ‘repair’ rate.
k_E_out multiplied with the actual effect size. This seem to be similar to the full GUTS approach with damage and repair.

Equation 7 in Schmitt et al. (2013): Toxicity response function

\[ f_{\text{photo}}(E) = 1 - E_{\text{max}} \frac{C_{\text{int, unb}}^b}{EC_{50, \text{int}}^b + C_{\text{int, unb}}^b} \]

Toxicity response function implemented in mmc3.r

```r
# Effect
f_E <- function(E, EC50, E_{\text{max}}, C_{\text{active}}) {
  1 - E_{\text{max}} * C_{\text{active}} * E / (EC50^b + C_{\text{active}}^b)
}

# Consider toxic effect
f_eff <- f_E(E, EC50, E_{\text{max}}, C_{\text{int, unb}})
if(T1Time == "delayed") {f_eff <- E} # delayed effects could be considered
k\_phot\_eff <- k\_phot\_eff * E

# Effect (this is the delayed TD model)
d\_dt <- k_E_{\text{out}} * f_E(E, EC50, E_{\text{max}}, C_{\text{int, unb}}) - k_E_{\text{out}} * E
```

The parameters of concentration response are substance specific. Hence, they have to be calibrated using substance specific experimental data. Thus, there are no default parameter values.

In the paper, the substance metsulfuron-methyl is used as example. TK-TD parameters were fitted based on the data from a laboratory test with 7 days of constant exposure to several test concentrations followed by 7 days in clean fresh medium. The values used in the implementation correspond to the values given in Table 2 of the paper. The maximum effect \( E_{\text{max}} \) and the slope \( b \) are dimensionless, whereas the \( EC_{50,\text{int}} \) is given in µg/L (or another mass per volume unit).

Example parameters in Schmitt et al. (2013) for the substance specific toxicity response function

\[
\begin{align*}
E_{\text{max}} &= 0.784 \\
EC_{50,\text{int}} &= 0.3 \\
b &= 4.16
\end{align*}
\]

Example parameters for the toxicity response function in mmc2.R (Schmitt et al. 2013)

```
# - Effect -
Conc = 2,  # [any] Concentration of toxicant (may also be a table)
E_{\text{max}} = 0.784,  # maximum Effect
EC_{50} = 0.3,  # [same as Conc. data] Midpoint of effect curve
b = 4.16,  # [-] Slope of effect curve
```
The parameter $E_{\text{max}}$, $EC_{50\text{int}}$ and slope $b$ are substance specific and have to be calibrated and validated case by case. Thus, no default parameters are given in Box 9. However, the values for metsulfuron-methyl (MSM) as obtained by Schmitt et al. (2013) can be included in the implementation as example.

It can be discussed whether an upper limit for the effect is plausible. It is recommended to set $E_{\text{max}}$ to one by default and decide case by case if $E_{\text{max}}$ should be included in the calibration.

### Box 9: Concentration response function on photosynthesis (Toxicodynamics)

$$\hat{j}^{\text{conc}}_{\text{int}}(C_{\text{int}}): \mathbb{R}_+ \to [1 - E_{\text{max}}, 1]$$

$$f_{\text{photo}}^{\text{conc}}(C_{\text{int,unb}}) = 1 - \frac{E_{\text{max}} \cdot C_{\text{int,unb}}^b}{EC_{50\text{int}}^b + C_{\text{int,unb}}^b}$$

The parameters $EC_{50\text{int}}$ [mass / volume], $b$ [-], and $E_{\text{max}}$ [-] are substance specific and have to calibrated. $E_{\text{max}}$ should be set to 1 by default.

The implementation of modelling delayed effects is not recommended for the standard model here. It needs two additional TD parameters which cannot directly be measured and which will probably result in overfitting if only growth inhibition test data are available for calibration.

To clarify, in the model by Schmitt et al. (2013) the toxicant influences only photosynthesis, not loss of biomass by increased respiration or mortality. The default endpoint of ecotoxicological tests used for calibration or validation of the TKTD model is inhibition of growth or yield, which can be a result of e.g. reduced photosynthesis, inhibition of anabolism or increased catabolism. Lethal effects are not explicitly measured.
2.3 Calculation of internal concentration (TK model)

In Schmitt et al. (2013), a one-compartment model is assumed for the mass balance of the total internal mass $M_{\text{int}}$ of the toxicant with mass fluxes in and out of the plants and metabolism of the substance in the plant.

**Equation 8 in Schmitt et al. (2013): Dynamics of internal mass**

$$\frac{dM_{\text{int}}}{dt} = \Phi_{\text{in}} - \Phi_{\text{out}} - k_{\text{met}} M_{\text{int}}$$

$M_{\text{int}}$ = mass of substance in the plants [mass m² or mass per vessel]
$\Phi_{\text{in}}, \Phi_{\text{out}}$ = substance fluxes (mass d⁻¹) into and out of the plant
$k_{\text{met}}$ = metabolic degradation rate [d⁻¹].

The mass flux in and out of the plants is assumed to be driven mainly permeation through the leaf cuticle depending on the concentration gradient of the chemical. The total permeation depends on the total leaf surface area $A$ [cm²] and the permeability $P$ [cm d⁻¹] of the cuticle.

**Equation 9 in Schmitt et al. (2013): In or out mass flux**

$$\Phi = A \cdot P \cdot C$$

$C$ in equation 9 is the concentration of the permeate out- or inside for $\Phi_{\text{in}}$ and $\Phi_{\text{out}}$, respectively. For the net flux, the concentration gradient outside and inside the plant, $\Delta C$, would be needed. This means that also the internal concentration must be given in mass per volume, e.g. µg/L.

Since permeability is given in cm per day, the product to the right of the equation results in correct unit for mass flow: $\text{area} \cdot \text{length/time} \cdot \text{mass/volume} = \text{mass/time}$.

To calculate the dynamics of the internal concentration, Equation 9 is inserted into Equation 8 and divided by Volume $V$ [cm³] of the population to convert the internal mass into internal concentration (Equation 10).

Since for the model of Lemna field populations the biomass is given as mass per m², also the surface area and the volume of the population must correctly be related to m². However, this gets cancelled out in equation 10.

**Equation 10 in Schmitt et al. (2013): Final TK-equation to describe the dynamics of the internal concentration $C_{\text{int}}$ [µg/L]**

$$\frac{dC_{\text{int}}}{dt} = \frac{P \cdot A}{V} \left( C_{\text{ext}} - \frac{C_{\text{int}}}{K_{p:w}} \right) - k_{\text{met}} C_{\text{int}}$$

In addition, only the unbound, dissolved fraction of the internal concentration is considered for the concentration gradient driving the flux in and out of the plant. To calculate the unbound concentration, the total internal concentration is divided by a partitioning coefficient $K_{p:w}$. 

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\[ C_{\text{int,unb}}(t) = \frac{C_{\text{int}}(t)}{K_{p:w}} \]

\( K_{p:w} \) is originally a bioaccumulation factor, i.e. the quotient of the concentration in the plant divided by the concentration in the (external) water. The concentration in the plant is the \( C_{\text{ext}} \) in the notation by Schmitt et al. 2013. In equilibrium the internal unbound concentration is the same as the external concentration. Thus \( K_{p:w} \) can be written in the following ways:

\[ K_{p:w} = \frac{C_{\text{plant}}}{C_{\text{water}}} = \frac{C_{\text{int}}}{C_{\text{int,unb}}} \]

Thus, Schmitt et al. (2013) assume immediate equilibrium in the plant between bound and unbound toxicant and no kinetics.

Since the equation for internal mass is divided by volume, the units of the internal concentrations (total and unbound) are mass per volume, as for the external concentration in the water, e.g. µg/L. Expressing the internal concentration in mass per volume allows comparing the EC50 from e.g. a standard test as concentration in the medium with the \( EC50_{\text{int}} \) fitted by the model.

Note that for metabolism, not only the unbound fraction, but the total internal concentration is considered. This seems to be not consistent to the assumption that only the unbound bioavailable concentration is driving the toxicity. The metabolite itself is not further considered in the model. Thus, metabolism is only considered an additional process to decrease the internal concentration of the toxicant. If metabolites are expected to result in relevant toxic effects, the model must be refined to consider this.

The full TK concept is visualized in Figure 10.

Figure 7: TK model concept of the Lemna model by Schmitt et al. 2013

In the R-code, the differential equation is written for internal total mass of the toxicant in the total population is used in line 229 instead of internal concentration as in equation 10. Therefore, some conversions are needed which are described below.
Toxicokinetics related to internal mass implemented in mmc3.r

In line 196, the fresh weight of the population is calculated from the dry weight biomass as the state variable to describe the  *Lemna* population. A fixed fresh weight to dry weight ratio is used (BMw2BMd).

The internal concentration is calculated as the internal mass divided by the fresh weight in line 197. This is not correct since the internal concentration should be given in mass per volume (see equation 10). The resulting values are only correct if a density of 1 g fw mL$^{-1}$ is assumed as it seems to be done in the Schmitt *et al.* (2013), but the density should be explicitly mentioned to get correct units. Thus, it is a matter of the documentation which does not affect the numerical results.

Line 198 calculates the internal unbound concentration $C_{\text{int}\_u}$ using the partitioning coefficient $K_{\text{bm}}$ (corresponding to $K_{p:w}$). The absolute value is probably used to stabilize the code. However, $K_{p:w}$ is positive by definition as well as $C_{\text{int}}$. $C_{\text{int}}$ might only become negative if the time steps of the integration are too large.

Line 229 includes the basic differential equation for the total internal mass. Thus, it should correspond to equation 10 multiplied with the population’s volume to get mass instead of concentration. However, there are three discrepancies:

1. The code does not include the metabolism as described in equation 10.
2. The additional elimination term $C_{\text{int}} \cdot \text{BM}_\text{fresh} \cdot (k_{\text{resp\_eff}} + k_{\text{loss}})$ is not given in the paper. It is needed to reduce the internal mass ($C_{\text{int}} \cdot \text{BM}_\text{fresh}$) if the biomass is declining. This correction is not necessary if the TK is described as the change in internal concentration as done in Equation 10. However, the internal concentration has to be corrected for ‘dilution by growth’ if the growth rate is higher than the uptake rate. There is also a problem with the units since the term does not result in mass per time.
3. Line 228 offers the option to consider temperature dependent permeability which is calculated in line 237 – 242. The parameter $P_{\text{up\_eff}}$ is the fixed parameter permeability ($P$ in the paper, $P_{\text{up}}$ in the code) if temperature dependence is not considered. The function for temperature dependence includes some constants and the molecular weight of the chemical to calculate the activation energy. However, the temperature dependent permeability is not documented in the paper.
The permeability $P$ and the partitioning coefficient $K_{p:w}$ are substance specific. Additionally, species specific but substance independent parameters needed for conversion between fresh and dry weight, surface area and volume of the *Lemna* population are used. In the paper the values of these parameters are listed in Table 1. The values in R-code (line 68, line 112-113) for the conversion parameters are the same as given in the paper.

### Parameters in Schmitt et al. (2013) for conversion between fresh and dry weight, surface area and number of fronds

<table>
<thead>
<tr>
<th>Conversion parameters implemented in mmc2.r (Schmitt et al. 2013)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
</tr>
<tr>
<td>111</td>
</tr>
<tr>
<td>112</td>
</tr>
<tr>
<td>113</td>
</tr>
</tbody>
</table>

For the ratio of frond area to dry weight, AperBM = 1000 cm² g⁻¹ dw, a reference is made in the paper to Landolt & Kandeler (1987) who estimated 40 mm² per frond from photographs. With the reported dry weight/frond ratio of 0.1 mg dw/frond, the 40 mm² per frond would result in 4 000 cm² g⁻¹ dw. In the implementation, the 1000 cm² g⁻¹ dw are explained with a reference to ‘Harlan -022’.

Schmitt et al (2013) report a fresh to dry weight ratio for *L. gibba* of 16.7. This corresponds to a dry weight to fresh weight ratio of 0.061 and a 94 % water content.

The dry weight per frond ratio is not needed directly in the model. It is only needed for calibration and validation when model results (BM) have to be compared to observations (frond-number).

As shown before, the model assumes a density of 1 g fw cm⁻³ which is not explicitly mentioned.

Note that for practical reasons all these ratios are assumed to be constant. This implicates that there are no seasonal variation nor effects of toxicants considered.

As the TD parameters also the TK parameters $P$ and $K_{p:w}$ are substance specific and have to be calibrated for each toxicant together with the TD parameters by means of laboratory test results. To reduce the number of parameters to be fitted, Schmitt et al.(2013) suggest to use a regression model for the partitioning coefficient $K_{p:w}$. It relates log $K_{ow}$ to the partitioning coefficient. The regression equation is based on de Carvalho et al. (2007). The equation is experimentally based on measured plant-water partition coefficients $K_{p:w}$ for *Lemna minor* for a set of substances with different log $K_{ow}$ values.

**Equation 10 in Schmitt et al. (2013): Final TK-equation to describe the dynamics of the internal concentration $C_{int}$ [µg/L]**

$$\log(K_{p:w} - 0.71) = 0.73 \cdot \log K_{ow} - 1.37$$

This equation can be solved to estimate $K_{p:w}$ from the $\log K_{ow}$.
\[ K_{p,w} = 10^{0.73 \cdot \log K_{ow} - 1.37} + 0.71 \]

Note that a log \( K_{ow} \) below 1 has only very small effects on the \( K_{p,w} \) (see Figure 8).

![Figure 8: Water:Plant partitioning coefficient \( K_{p,w} \) as a function of log \( K_{ow} \) (copied from Figure 3 in de Carvalho et al. 2007).](image)

For the metsulfuron-methyl example by Schmitt et al. (2013), the regression model by de Carvalho et al. (2007) results in a \( K_{p,w} \) of 0.73 for the given \( K_{ow} \) of -0.48. The \( K_{p,w} \) value is not explicitly given in the paper but a value of 0.75 is used in the implementation. The permeability was fitted together with the TD parameters to be 0.0054 cm d\(^{-1}\). Metabolism as included in Equation 10 is not considered in the implementation.

### Example parameters in Schmitt et al. (2013) for the toxicokinetics of metsulfuron-methyl

\[ P = 0.0054 \text{ cm/d} \]

<table>
<thead>
<tr>
<th>Line</th>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td># P_up</td>
<td>0.0054</td>
<td>Toricokinetics</td>
</tr>
<tr>
<td>69</td>
<td>Kbm</td>
<td>0.75</td>
<td>Biomass(fw):water partition coefficient</td>
</tr>
</tbody>
</table>

For the refined model, the working group agreed on the following:

1. The option to consider metabolism of the toxicant within Lemna is kept but we recommend to set the metabolism rate to zero by default to limit the number of parameters to be calibrated. In case the observed recovery of the population is faster than it can be predicted by the basic model, the metabolism rate can be calibrated, too.

2. The effect of temperature on permeability as implemented in the R-code but not explained in the model description is not included in the proposed standard model. Note that temperature dependence of TK (and TD) does not affect the use of the model for simulating lab tests (Tier 2C).

3. The \( K_{p,w} \) parameter to calculate the unbound fraction of the toxicant in the plant is not necessary and a single compartment model can be used instead:
   Since the unbound concentration is always proportional to the total concentration, using a scaled total concentration instead of an internal unbound concentration will result in the
same effect by just changing the location parameter of the concentration response curve \( EC50_{int} \). A TKTD model without \( K_{p:w} \) will result in the same fit as using \( K_{p:w} \) with just different TD parameters. However, \( K_{p:w} \) affects the toxicokinetics: if \( K_{p:w} \) is larger than 1, the concentration gradient driving uptake or elimination (the term in the brackets) becomes larger for uptake situation but smaller for elimination (related to the absolute value of the gradient). Thus, with \( K_{p:w} > 1 \), uptake becomes faster and elimination slower compared to \( K_{p:w} = 1 \). This relates to the total internal concentration. For \( K_{p:w} < 1 \), the effect of \( K_{p:w} \) is less relevant since \( K_{p:w} \) must not become smaller than the water content (i.e. 0.94 in this model). For a more detailed explanation, please see Annex 9.2.

In conclusion, since the use of \( K_{p:w} \) does not result in better fits, it would be possible to use a simple one-compartment model with a scaled (total) internal concentration \( C_{int} \). The TK model is then similar to the reduced GUTS approach (Jager et al. 2011, Jager and Ashauer 2018) using a dominant rate constant \( k_d \). The \( k_d \) in GUTS corresponds to the \( P \ A \ V \) in Equation 10.

The difference between the two TK models for GUTS and \textit{Lemna} is that in GUTS it is assumed that the biomass (in GUTS of the single organism) is constant while the biomass of the \textit{Lemna} population can change. Growth can result in dilution and thus reduced internal concentration if the uptake is slower than the growth and biomass declines have to be considered for the calculation of the internal mass.

To be consistent and compatible with the original \textit{Lemna} model, we still include the \( K_{p:w} \) parameter but prefer to set it to 1 by default. Note that also according to Schmitt \textit{et al.} (2013), \( K_{p:w} \) is usually not calibrated but determined independently from the log Kow by means of a regression (see Figure 8).

For very low lipophilic and hydrophilic substances (as most herbicides are) the partition coefficient is of less importance. However, for more lipophilic substances it must be considered. Generally, \( K_{p:w} \) should be treated as an independently determined compound specific input parameter. In Schmitt \textit{et al.} (2013) the Carvalho approach was used as a citable example of how to estimate it. Other approaches, also experimental ones, are also possible, but must be defended when applied.

4. We suggest to use the same kind of TK equation in the model description and in the R-code. We suggest to use the \textit{internal mass} because in the R-code the differential equation is solved for mass and to have the same units for both state variables (\textit{Lemna} population and toxicant).

5. With respect to the names of variables and parameters we propose to be consistent as possible in the model description and the implementation.

The use of the name \( P_{\text{up}} \) and its explanation in the R-code is misleading since the permeability drives both uptake and elimination depending on the concentration gradient. Thus, for clarity, \( P \) or \( Perm \) should be used.

We suggest to use \( r \) to indicate model parameters for ratios and define \( r_{A/DW} \) as the surface area per dry weight rate, \( r_{FW/DW} \) as the fresh weight to dry weight ratio and \( r_{FW/V} \) as the fresh weight density and can then calculate \( A(t) \) and \( V(t) \) from \( BM(t) \):

\[
A(t) = BM(t) \cdot r_{A/DW}
\]
\[
V(t) = BM(t) \cdot r_{FW/DW} \cdot \frac{1}{r_{FW/V}}
\]
The dry weight per frond ratio, $r_{DW/FN}$, is not needed in the model itself. However, since in laboratory tests, frond number but not dry weight is measured over time, it is needed for calibration and validation using laboratory data.

Thus, we propose the following TK model similar to line 229 of the R-code. The uptake and elimination is driven by the concentration gradient and the permeability. In addition, the total internal mass is reduced by the amount in the biomass which is lost. If metabolism of the toxicant should be considered it is assumed that only the unbound fraction can be metabolized.

**Box 10: Refined toxicokinetic model for *Lemna***

\[
\begin{align*}
M_{\text{int}}(t) & : [0, t_n] \rightarrow \mathbb{R}_+ \\
\frac{d}{dt} M_{\text{int}}(t) & = P \cdot A(t) \cdot \left( C_{\text{ext}}(t) - \frac{C_{\text{int}}(t)}{K_{p:w}} \right) - \frac{M_{\text{int}}(t)}{K_{p:w}} \cdot k_{\text{met}} - M_{\text{int}} \cdot (t) \cdot k_{\text{loss}} \cdot f_{\text{loss}}(t) \\
M_{\text{int}}(0) & = 0 \\
A(t) & = BM(t) \cdot r_{A/DW} \\
V(t) & = BM(t) \cdot r_{FW/DW} \cdot \frac{1}{r_{FW/V}} \\
C_{\text{int}}(t) & = \frac{M_{\text{int}}(t)}{V(t)} = \frac{M_{\text{int}}(t) \cdot r_{FW/V}}{BM(t) \cdot r_{FW/DW}} \\
M_{\text{int}}(t) & = \text{mass of the toxicant in the plant population [mass m}^{-2}\text{] or [mass per vessel]} \\
P & = \text{permeability [cm d}^{-1}\text{]} \\
A(t) & = \text{total surface area of the plant population [cm}^{2}\text{ m}^{-2}\text{] or [cm}^{2}\text{ per vessel]} \\
V(t) & = \text{total volume of the population [cm}^{3}\text{ m}^{-2}\text{] or [cm}^{3}\text{ per vessel]} \\
C_{\text{ext}}(t) & = \text{external concentration in the water [mass/volume]} \\
C_{\text{int}}(t) & = \text{internal concentration [mass/volume]} \\
r_{A/DW} & = \text{area per dry weight ratio [cm}^{2}\text{ g}^{-1}\text{]} \\
r_{FW/DW} & = \text{fresh weight per dry weight ratio [-]} \\
r_{FW/V} & = \text{fresh weight density [g cm}^{-3}\text{]} \\
r_{DW/FN} & = \text{dry weight per frond [g]} \\
K_{p:w} & = \text{partitioning coefficient plant:water [-]} \\
k_{\text{met}} & = \text{metabolisation rate [d}^{-1}\text{]} \\
\end{align*}
\]

Default parameters:

\[
\begin{align*}
r_{A/DW} & = 1000 \text{ cm}^{2}\text{ g}^{-1} \\
r_{FW/DW} & = 16.7 \\
r_{FW/V} & = 1 \text{ g cm}^{-3} \\
r_{DW/FN} & = 0.0001 \text{ g} \\
K_{p:w} & = 1 \\
k_{\text{met}} & = 0 \text{ d}^{-1} \\
\end{align*}
\]

The permeability $P$ is a substance specific parameter which has to be calibrated together with the TD parameters. The partitioning coefficient $K_{p:w}$ can be set to 1 by default and can be calibrated if internal concentrations are measured. Also the metabolism rate $k_{\text{met}}$ should only be calibrated if needed.
3 Summary

3.1 Variables used in the model

Table 1: Variables used in the model

<table>
<thead>
<tr>
<th>Variable</th>
<th>Description</th>
<th>Start value</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>State variables</strong> (calculated by differential equation system)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BM</td>
<td>Biomass (dry weight) of <em>Lemna</em> population</td>
<td>Study specific &gt; 0</td>
<td>g dw m⁻² (field) mg dw (lab)</td>
</tr>
<tr>
<td>Mint</td>
<td>Internal mass of toxicant in the <em>Lemna</em> population</td>
<td>Usually 0</td>
<td>Case specific, e.g. mg or µg m⁻² (field) mg or µg (lab)</td>
</tr>
<tr>
<td><strong>Help variables</strong> (can be calculated from state variables, no differential equations)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Volume of <em>Lemna</em> population</td>
<td>Proportional to BM</td>
<td>cm³ m⁻² (field) cm³ (lab)</td>
</tr>
<tr>
<td>A</td>
<td>Surface area of <em>Lemna</em> population</td>
<td>Proportional to BM</td>
<td>cm² m⁻² (field) cm² (lab)</td>
</tr>
<tr>
<td>C_{int}</td>
<td>Internal concentration</td>
<td>$M_{nt}/V$</td>
<td>Same as $C_{ext}$</td>
</tr>
<tr>
<td>C_{int_unb}</td>
<td>Internal unbound concentration</td>
<td>Proportional to $C_{int}$</td>
<td>Same as $C_{ext}$</td>
</tr>
<tr>
<td>FN</td>
<td>Frond number</td>
<td>Proportional to BM</td>
<td>m² (field) - (lab)</td>
</tr>
<tr>
<td><strong>External variables</strong> (forcing functions, not affected by state variables but model inputs)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$C_{ext}$</td>
<td>Concentration in the medium, e.g. measured concentration or PEC</td>
<td>Study specific, e.g. FOCUSsw profile</td>
<td>Case specific, e.g. mg L⁻¹ or µg·L⁻¹:</td>
</tr>
<tr>
<td>T</td>
<td>Temperature (for <em>Lemna</em> this could be water or air temperature)</td>
<td>Study specific, e.g. from FOCUSsw</td>
<td>°C</td>
</tr>
<tr>
<td>Rad</td>
<td>Global radiation</td>
<td>Study specific, e.g. from FOCUSsw</td>
<td>kJ m⁻² d⁻¹</td>
</tr>
<tr>
<td>N, P</td>
<td>Nitrogen and Phosphorus concentrations</td>
<td>Study specific</td>
<td>mg N L⁻¹ mg P L⁻¹</td>
</tr>
</tbody>
</table>
### 3.2 Growth model for *Lemna* biomass

\[
\frac{d}{dt} BM(t) = \left( k_{\text{photo,max}} \cdot f_{\text{photo}}(t) - k_{\text{loss}} \cdot f_{\text{loss}}(t) \right) \cdot BM(t), \quad BM(0) = BM_0 \in \mathbb{R}^+ \setminus \{0\}
\]

If \((BM(t) < BM_{\text{min}} \text{ and } dBM/dt < 0)\) then \(dBM/dt = 0\)

**Dependencies of photosynthesis**

\[
f_{\text{photo}}(t) = \min \left( f^T_{\text{photo}}(t), f^I_{\text{photo}}(t), f^P_{\text{photo}}(t), f^N_{\text{photo}}(t) \right) \cdot f^{BM}_{\text{photo}}(t) \cdot f^{C_{\text{int, unb}}}_{\text{photo}}(t)
\]

\[
f_{\text{loss}}(t) = \frac{T - T_{\text{opt}}}{(T_{\text{opt}} - T_{\text{opt}})^2} \quad \text{if } T \leq T_{\text{opt}}
\]

\[
f_{\text{loss}}(t) = \frac{T_{\text{opt}} - T}{(T_{\text{opt}} - T_{\text{opt}})^2} \quad \text{if } T > T_{\text{opt}}
\]

\[
f^I_{\text{photo}}(I) = \begin{cases} 
\alpha \cdot I + \beta & \text{if } I \leq I_{\text{sat}} \\
1 & \text{if } I > I_{\text{sat}}
\end{cases}
\]

\[
f^N_{\text{photo}}(N) = \frac{N}{N + N_{50}} \quad \text{respectively} \quad f^P_{\text{photo}}(P) = \frac{P}{P + P_{50}}
\]

\[
f^{BM}_{\text{photo}}(BM) = 1 - \frac{BM}{BM_L}
\]

\[
f^{C_{\text{int, unb}}}_{\text{photo}}(C_{\text{int, unb}}) = 1 - \frac{E_{\text{max}} \cdot (C_{\text{int, unb}}/K_p: w)^b}{E_{50}^b + (C_{\text{int, unb}}/K_p: w)^b}
\]

**Dependencies of biomass loss**

\[
f^T_{\text{loss}}(T) = Q^{T - T_{\text{ref}}}_{10}
\]

**Modelling exponential growth under constant environmental conditions**

For calibration and validation of the TKTD model and for simulation of refined exposure tests in the laboratory (Tier 2C, EFSA PPR panel 2013, 2018), the dependencies from \(T, I, N, P,\) and \(BM\) can be ignored and the model can be simplified to

\[
\frac{d}{dt} BM(t) = \left( k_{\text{photo}} \cdot f^{C_{\text{int, unb}}}_{\text{photo}}(t) - k_{\text{loss}} \right) \cdot BM(t), \quad BM(0) = BM_0 \in \mathbb{R}^+ \setminus \{0\}
\]

The model implementation includes this simplification by providing the switch variable \(k_{\text{photo, fixed}}\) (\(k_{\text{phot, fix}}\) in Schmitt et al., 2013, see Section 2.1). In case of laboratory conditions, \(k_{\text{photo, fixed}}\) is set to \(\text{TRUE}\), and the simplified growth equation is used.
3.3 Toxicokinetic model for internal mass

\[
\frac{d}{dt} M_{\text{int}}(t) = P \cdot A(t) \left( C_{\text{ext}}(t) - \frac{C_{\text{int}}(t)}{K_{p,w}} \right) - \frac{M_{\text{int}}(t)}{K_{p,w}} \cdot k_{\text{met}} - M_{\text{int}} \cdot (t) \cdot k_{\text{loss}} \cdot f_{\text{loss}}(t)
\]

\[
A(t) = BM(t) \cdot r_{A/\text{DW}}
\]

\[
V(t) = BM(t) \cdot r_{\text{FW/\text{DW}}} \cdot \frac{1}{r_{\text{FW}/V}}
\]

\[
C_{\text{int}}(t) = \frac{M_{\text{int}}(t)}{V(t)} = \frac{M_{\text{int}}(t) \cdot r_{\text{FW}/V}}{BM(t) \cdot r_{\text{FW/\text{DW}}}}
\]
### 3.4 Model parameters

**Table 2: Default model parameters**

<table>
<thead>
<tr>
<th>Parameter name in equation system (section 3.2)</th>
<th>Name in R code (Annex 5.3)</th>
<th>Default value</th>
<th>Unit</th>
<th>Description</th>
<th>Reference in Schmitt et al. 2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{\text{photo_max}})</td>
<td>k_photo_max</td>
<td>0.47</td>
<td>d(^{-1})</td>
<td>Maximum photosynthesis rate</td>
<td>Lasfar (2007)</td>
</tr>
<tr>
<td>(k_{\text{photo_fixed}})</td>
<td>k_photo_fixed</td>
<td>FALSE</td>
<td></td>
<td>Set to TRUE for exponential growth conditions</td>
<td>-</td>
</tr>
<tr>
<td>(k_{\text{loss}})</td>
<td>k_loss</td>
<td>0 (Tier 2C)</td>
<td>d(^{-1})</td>
<td>Respiration resp. mortality rate at reference temperature</td>
<td>Claus (1972)</td>
</tr>
<tr>
<td>(T_{\text{min}})</td>
<td>T_min</td>
<td>8</td>
<td>°C</td>
<td>Minimum growth temperature</td>
<td>Lasfar (2007)</td>
</tr>
<tr>
<td>(T_{\text{max}})</td>
<td>T_max</td>
<td>40.5</td>
<td>°C</td>
<td>Maximum growth temperature</td>
<td>Lasfar (2007)</td>
</tr>
<tr>
<td>(T_{\text{opt}})</td>
<td>T_opt</td>
<td>26.7</td>
<td>°C</td>
<td>Optimum growth temperature</td>
<td>Lasfar (2007)</td>
</tr>
<tr>
<td>(T_{\text{ref}})</td>
<td>T_ref</td>
<td>25</td>
<td>°C</td>
<td>Reference temperature for respiration rate</td>
<td>Claus (1972)</td>
</tr>
<tr>
<td>(Q_{10})</td>
<td>Q10</td>
<td>2</td>
<td></td>
<td>Q10 for respiration rate</td>
<td>Wangermann &amp; Ashby (1951)</td>
</tr>
<tr>
<td>(\alpha)</td>
<td>alpha</td>
<td>5.00E-05</td>
<td>m(^2) d kJ(^{-1})</td>
<td>Slope of radiation dependence</td>
<td>Hodgson (1970)</td>
</tr>
<tr>
<td>(\beta)</td>
<td>beta</td>
<td>0.025</td>
<td></td>
<td>Intercept of radiation dependence(^1)</td>
<td>Hodgson (1970)</td>
</tr>
<tr>
<td>(N_{50})</td>
<td>N_50</td>
<td>0.034</td>
<td>mg N L(^{-1})</td>
<td>N-conc. where growth rate is halved</td>
<td>Lüönd (1983)</td>
</tr>
<tr>
<td>(P_{50})</td>
<td>P_50</td>
<td>0.0043</td>
<td>mg P L(^{-1})</td>
<td>N-conc. where growth rate is halved</td>
<td>Lüönd (1983)</td>
</tr>
<tr>
<td>(BM_L)</td>
<td>BM_L</td>
<td>177</td>
<td>g m(^{-2})</td>
<td>Limit density (carrying capacity)</td>
<td>Monette (2006)</td>
</tr>
<tr>
<td>Parameter name in equation system (section 3.2)</td>
<td>Parameter name in R code</td>
<td>Default value</td>
<td>Unit</td>
<td>Description</td>
<td>Reference in Schmitt et al. 2013</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>--------------------------</td>
<td>--------------</td>
<td>------</td>
<td>-------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>$BM_{min}$</td>
<td>BM_min</td>
<td>0.0005</td>
<td>g m$^{-2}$</td>
<td>Threshold density for setting $dBM/dt$ to zero</td>
<td>Schmitt et al. (2013)</td>
</tr>
<tr>
<td>$r_{DW/FN}$</td>
<td>r_DW_FN</td>
<td>0.0001</td>
<td>g$^{-1}$</td>
<td>Dry weight/frond (conversion factor, laboratory)</td>
<td>Determined for L. gibba (Schmitt et al. 2013)</td>
</tr>
<tr>
<td>$r_{FW/DW}$</td>
<td>r_FW_DW</td>
<td>16.7</td>
<td>-</td>
<td>Fresh weight/dry weight</td>
<td>Determined for L. gibba (Schmitt et al. 2013)</td>
</tr>
<tr>
<td>$r_{A/DW}$</td>
<td>r_A_DW</td>
<td>1000</td>
<td>cm$^2$ g$^{-1}$</td>
<td>Surface area per dry weight</td>
<td>Landolt and Kandeler (1987)</td>
</tr>
<tr>
<td>$r_{FW/V}$</td>
<td>r_FW_V</td>
<td>1</td>
<td>g cm$^{-3}$</td>
<td>Fresh weight density</td>
<td>Assumption</td>
</tr>
</tbody>
</table>

Substance specific TKTD parameters used in Schmitt et al. (2013) for Metsulfuron-methyl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Default value</th>
<th>Unit</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P$</td>
<td>0.0054</td>
<td>cm d$^{-1}$</td>
<td>Permeability</td>
<td>Calibrated</td>
</tr>
<tr>
<td>$K_{pw}$</td>
<td>0.75</td>
<td>-</td>
<td>Partitioning coefficient plant:water</td>
<td>Regression Carvalho et al. (2007). (default = 1)</td>
</tr>
<tr>
<td>$k_{met}$</td>
<td>0</td>
<td>d$^{-1}$</td>
<td>Metabolism rate</td>
<td>Set (default)</td>
</tr>
<tr>
<td>$E_{max}$</td>
<td>0.784</td>
<td>-</td>
<td>Maximum effect</td>
<td>Calibrated (default = 1)</td>
</tr>
<tr>
<td>$EC50_{int}$</td>
<td>0.3</td>
<td>Same as $C_{ext}$</td>
<td>Internal EC50</td>
<td>Calibrated</td>
</tr>
<tr>
<td>$b$</td>
<td>4.16</td>
<td>-</td>
<td>Slope</td>
<td>Calibrated</td>
</tr>
</tbody>
</table>
4 References


5 Annex

5.1 Schematic of the R-code provided in Schmitt et al. (2013)

The following figure created by Cecilie Rendal is a basic schematic of the (original) code structure in the mmc2.r and mmc3.r files provided in Schmitt et al. (2013).
5.2 Notes on the use of the plant-water partitioning coefficient in the TK model

08.06.2020 Judith Klein, Stefan Reichenberger, Udo Hommen

**Equation 10 in Schmitt et al. (2013): Final TK-equation to describe the dynamics of the internal concentration $C_{\text{int}}$ [µg/L]**

\[
\frac{dC_{\text{int}}}{dt} = \frac{P \cdot A}{V} \left( \frac{C_{\text{ext}}}{K_{p:w}} - \frac{C_{\text{int}}}{K_{p:w}} \right) - k_{\text{met}}C_{\text{int}}
\]

- $P =$ Permeability, $A =$ surface area of the population, $V =$ Volume of the population, $C_{\text{int}} =$ Total internal concentration, $K_{p:w}$ Partition coefficient plant to water

In the following, we ignore the metabolism term. Note that if the term would be included it would be more logical to relate metabolism to the unbound fraction of the internal concentration only.

General thoughts:

The internal unbound concentration is always proportional to the total concentration: $C_{\text{int,unb}} = \frac{C_{\text{int}}}{K_{p:w}}$.

Instantaneous equilibrium between unbound and bound concentration is assumed – no process rate exists.

If $C_{\text{int,unb}}$ is proportional to $C_{\text{int}}$, it does not matter for the toxicodynamics which internal concentration is used (it just changes the EC50int value).

However, $K_{p:w}$ affects the toxicokinetics: if $K_{p:w}$ is larger than one, the concentration gradient driving uptake or elimination (the term in the brackets) becomes larger for uptake situation but smaller for elimination (related to the absolute value of the gradient). Thus, with $K_{p:w} > 1$, 1 uptake becomes faster and elimination slower compared to $K_{p:w} = 1$. This relates to Cint (total).

For $K_{p:w} < 1$, it is less relevant since $K_{p:w}$ must not become smaller the water content (i.e. 0.94 in this model).

However, following the suggestion of Schmitt, $K_{p:w}$ is not calibrated like P but taken from a regression function. If a calibration with this fitted $K_{p:w}$ is not possible, including $K_{p:w}$ in the calibration might offer a more flexible TK.

To reduce the number of parameters to be fitted, Schmitt et al. (2013) suggest to use a regression model for the partitioning coefficient $K_{p:w}$. It relates log $K_{ow}$ to the partitioning coefficient. The regression equation is based on measured plant-water partition coefficients $K_{p:w}$ for *Lemna minor* for a set of substances with different log $K_{ow}$ values (Carvalho et al. 2007).

**Equation 11 in Schmitt et al. (2013): Final TK-equation to describe the dynamics of the internal concentration $C_{\text{int}}$ [µg/L]**

\[
\log(K_{p:w} - 0.71) = 0.73 \cdot \log K_{ow} - 1.37
\]

This equation can be solved to estimate $K_{p:w}$ from the log $K_{ow}$.
\[ K_{p,w} = 10^{0.73 \times \log K_{ow} - 1.37} + 0.71 \]

Note that a log \( K_{ow} \) below 1 has only very small effects on the \( K_{p,w} \) (see Figure 8).

In the following, the effect is \( K_{p,w} \) on the TK is analyzed more mathematically. The figures illustrate the effect of \( K_{p,w} \) on the internal and internal unbound concentration:

We simplify the differential equation. Let \( k_1, k_2 \in \mathbb{R}^+ \). Set \( k_1 = \frac{P \cdot A}{V} \) and \( k_2 = \frac{k_1}{K_{p,w}} \).

Then, we have

\[ \frac{d}{dt} C_{int}(t) = k_1 \cdot C_{ext}(t) - k_2 \cdot C_{int}(t). \]

If \( K_{p,w} > 1 \) then it yields \( k_2 < k_1 \).

If \( K_{p,w} = 1 \) then it yields \( k_2 = k_1 \).

If \( K_{p,w} < 1 \) then it yields \( k_2 > k_1 \).

Example:

Set \( k_1 = 2 \) and try \( K_{p,w} = 1.5, K_{p,w} = 1 \) and \( K_{p,w} = 0.94 \).

External concentration function is defined by

\[ C_{ext}(t) = \begin{cases} 10, & \text{if } t \leq 7 \\ 0, & \text{if } t > 7 \end{cases} \]

In the following figure, the external concentration profile as well as the internal concentration profiles for different \( K_{p,w} \) are presented. A \( K_{p,w} > 1 \) leads to an internal concentration higher than the external concentration. The plateau is approximately equal to \( C_{ext} \cdot K_{p,w} \).

After transforming to unbound concentration by dividing by the respective \( K_{p,w} \) value, we get the following curves. The plateau value is equal to the external concentration value. A higher \( K_{p,w} \) leads to a slower uptake and to a slower elimination (in contrast to the faster increase of total internal concentration, see figure above).
To test a reasonable range of $K_{p,w}$ values, we assume that pesticides may have maximal a log Kow value of 3.5. This results in a $K_{p,w}$ approximately equal to 16 based on the regression.

In the example, the equilibrium is not reached within the 7 days of exposure. On the other hand, it takes much longer until the plants are ‘clean’ again when exposure is stopped.

In conclusion, for TD $K_{p,w}$ shouldn’t matter since the unbound con is always proportional to the total internal concentration. For TK, $K_{p,w}$ affects the speed of uptake and elimination, but for the
course of Cint_unb it affects both in the same way. Thus, the effect of $K_{p:w}$ on the course of Cint_unb can also be reached just by changing the permeability value. Thus, is seems that without $K_{p:w}$, the same effect over time can be achieved just by changes of EC50 and Perm.

The only reason for keeping $K_{p:w}$ in the model seems to be the effect on the course of Cint-total - and to stick close to the original model.

Additional remark 1: Why $K_{p:w}$ affects increase and decrease of $C_{int\_unb}$ in the same way (in contrast to Cint_total):

$$\frac{d}{dt} C_{int}(t) = k_1 \cdot C_{ext}(t) - k_2 \cdot C_{int}(t)$$

$$\frac{d}{dt} C_{int}(t) = \frac{k_1}{K_{p:w}} \cdot C_{ext}(t) - k_2 \cdot C_{int}(t)$$

$$c_{int\_unb}(t) = \frac{C_{int}(t)}{K_{p:w}} \Rightarrow \frac{d}{dt} c_{int\_unb}(t) = \frac{k_1}{K_{p:w}} \cdot C_{ext}(t) - k_2 \cdot c_{int\_unb}(t)$$

$$\frac{k_2}{K_{p:w}} = \frac{k_1}{K_{p:w}} \Rightarrow \frac{d}{dt} c_{int\_unb}(t) = \frac{k_1}{K_{p:w}} \left( C_{ext}(t) - c_{int\_unb}(t) \right)$$

Additional remark 2: How to choose $P$ to get the same internal unbound concentration as before using $K_{p:w}$

As we wish not to consider the plant water coefficient, we set the new value equal to one ($K_{p:w}^* = 1$).

The adjusted parameter value describing uptake and elimination is $k_1^* = k_2 = \frac{k_1}{K_{p:w}}$ to obtain the same time course of internal unbound concentration. Notice that by star annotated parameters refer to the new values, parameters having no star refer to the old parameter value.

Thus, for example, choosing $k_1 = 2$ and $K_{p:w} = 16$ ($k_2 = \frac{2}{16} = 0.125$) yields the same internal unbound concentration as choosing $k_1^* = k_2^* = k_2 = \frac{k_1}{K_{p:w}^*} = 0.125$ with $K_{p:w}^* = 1$. The value of the permeability is obtained by $k_1^* = \frac{P \cdot A}{V} \Leftrightarrow P = k_1^* \cdot \frac{V}{A}$. 
The internal unbound concentration is the same whereas the internal concentration is different. However, only the internal unbound concentration is used for the description of the effect.

**Additional remark 3: Why $k_{pw}$ must not be smaller than the water content**

The use of estimation of $k_{pw}$ from the regression in Figure 8 also creates problems with the mass balance within the plant. If the $k_{pw}$ is lower than the water content the internal bound mass must be negative as shown below:

\[
\begin{align*}
M_{int} &= M_{int_{amb}} + M_{int_b} \\
&= M_{int_{amb}} + BM_{FW} \cdot 0.94 \cdot \frac{C_{int_{amb}}}{K_{pw}} + M_{int_b} \\
&= M_{int_{amb}} + BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} + M_{int_b}
\end{align*}
\]

(definition of $C_{int_{amb}}$ according to Schmitt et al. [2013])

\[
\begin{align*}
M_{int_b} &= M_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} \\
&= M_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} \\
&= M_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}}
\end{align*}
\]

The left-hand side, the bound internal concentration has to be non-negative due to physical reason. Thus, also the right-hand side has to be non-negative:

\[
M_{int_b} \geq 0 \iff C_{int} \cdot BM_{FW} \cdot \left(1 - \frac{0.94}{K_{pw}}\right) \geq 0
\]

As the internal concentration and the fresh biomass value are non-negative per definition, the internal bound mass is non-negative if the term in the brackets is non-negative. This is only the case, when the plant water coefficient is greater than or equal to 0.94.

\[
1 - \frac{0.94}{K_{pw}} \geq 0 \iff \frac{0.94}{K_{pw}} \leq 1 \iff 0.94 \leq K_{pw}
\]
5.3 Implementation in R, verification and documentation

The model’s reference implementation is available free of charge as an R package on CRAN\(^1\). The `lemna` package’s source code, documentation and manual is also available as free and open-source software on GitHub\(^2\).

The reference implementation was developed using the following R shell and packages:

<table>
<thead>
<tr>
<th>Name</th>
<th>Version</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>R shell</td>
<td>4.1.0</td>
<td>Runtime</td>
</tr>
<tr>
<td>deSolve</td>
<td>1.3.0</td>
<td>Numerical ODE solver</td>
</tr>
<tr>
<td>ggplot2</td>
<td>3.3.5</td>
<td>Data plotting</td>
</tr>
<tr>
<td>gridExtra</td>
<td>2.3</td>
<td>Arranging plots</td>
</tr>
</tbody>
</table>

All model variables and parameters carry the units as described in section 3.1 and 3.4. A manual of all package functions and expected data types as well as a detailed vignette describing package use are available as part of the package. The package also contains a vignette documenting the verification of the model implementation and comparisons with published results. Data sets used in Schmitt et al. (2013) and Hommen et al. (2016) were used to compare results obtained with the original R-Code provided by Schmitt et al. (2013) and the refined implementation presented in this report and are available at GitHub\(^3\).

A Lemna growth scenario can be simulated by providing the necessary scenario elements as arguments to the `lemna()` function. Required scenario elements are initial system state, output times, model parameters as well as (time-series of) environmental variables. The function integrates the model over time and provides facilities to ease the definition of Lemna scenarios such as automatic interpolation of environmental variable data and optional loading of datasets from files. Please refer to the vignette Introduction to the Lemna package or the package manual for details.

Example package use:

```r
# initial state of the model system: 1.0 g dw biomass, 0.0 ug/m^2 internal toxicant
myinit <- c(BM = 1, M_int = 0)
# simulated period and output time points: [0,7], output each day
mytimes <- 0:7 # (days)
# default model parameters + substance specific values
myparam <- param Defaults(c(
  EC50_int = 4.16, # (ug/L) internal EC50
  b = 0.3, # (slope) parameter
  P = 0.0054, # (cm d^-1) permeability
))
# constant environmental conditions, including exposure
myenvir <- list(
  tmp = 18, # (°C) ambient temperature
  irr = 15000, # (kJ m^-2 d^-1) irradiance
  P = 0.3, # (mg L^-1) Phosphorus concentration
  N = 0.6, # (mg L^-1) Nitrogen concentration
  conc = 1 # (ug/L) toxicant concentration
)
# simulate the Lemna TKTD scenario
lemna(init = myinit, times = mytimes, param = myparam, envir = myenvir)
```

---

\(^1\) The Comprehensive R Archive Network, `lemna` package: [https://cran.r-project.org/package=lemna](https://cran.r-project.org/package=lemna)

\(^2\) Lemna package repository on GitHub: [https://github.com/nkehrein/lemna](https://github.com/nkehrein/lemna)

\(^3\) Verification: [https://cran.r-project.org/web/packages/lemna/vignettes/lemna-verification.html](https://cran.r-project.org/web/packages/lemna/vignettes/lemna-verification.html)