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 and default parameters

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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. In the next step, an agreed R-code will be provided.

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[1]. The growth of individual plants is not explicitly considered. The biomass is increased by photosynthesis and reduced by respiration, mortality

[1] 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.

and other losses. Production and loss of biomass are influenced by several environmental factors as indicated in Figure 1.

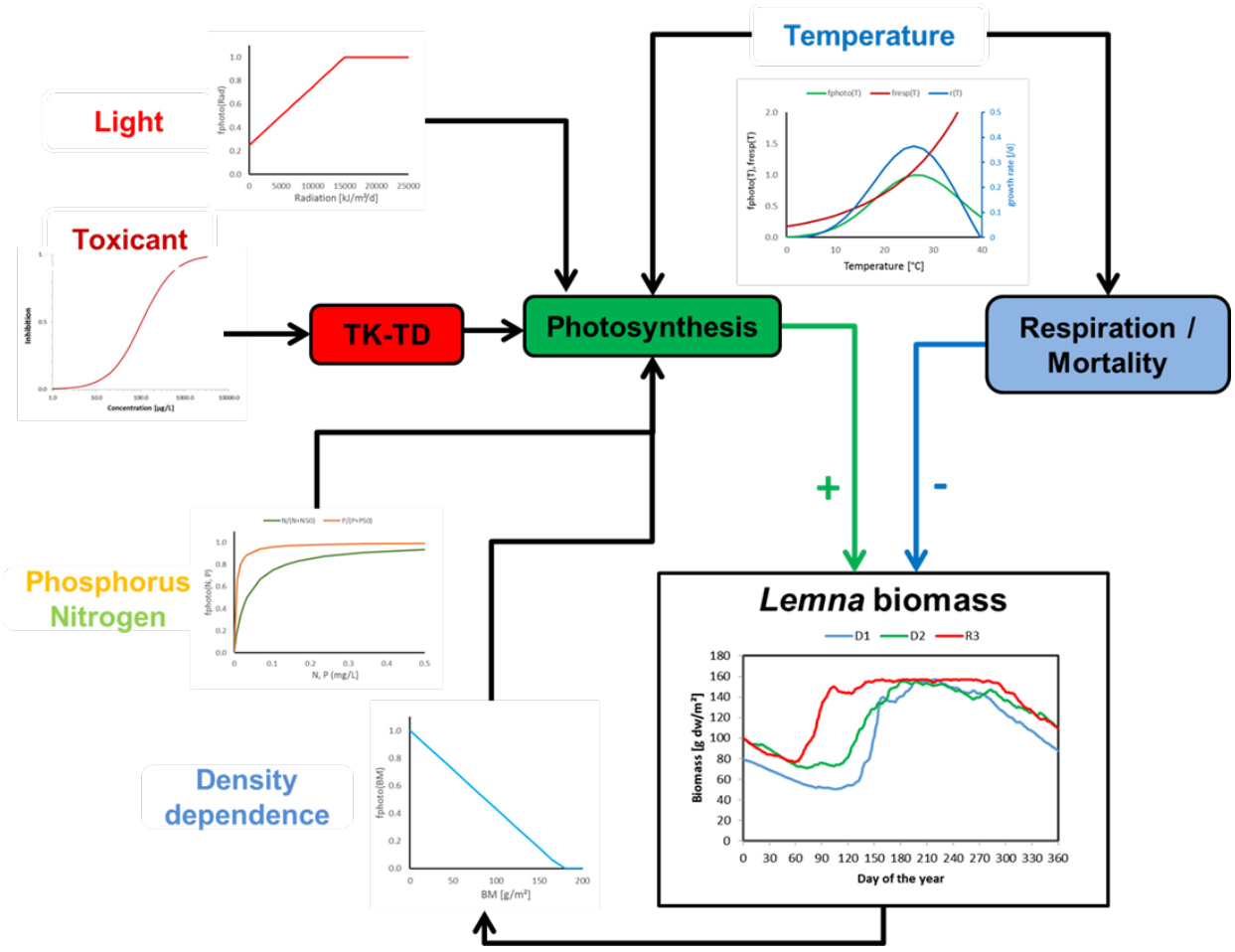


Figure 1: Conceptual diagram of the TK-TD population model for *Lemna sp*. by Schmitt *et al.* (2013).

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

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], θ 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 $kresp\_ref$ [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)

```
201  # Calculate effective growth rate
202  if(!k_phot_fix){
203    k_phot_eff <- k_phot_max * f_R(actRad, k_0, a_k)
204    k_phot_eff <- k_phot_eff * f_T(actTemp,Topt,Tmax,Tmin)
205    k_phot_eff <- k_phot_eff * f_P(C_P, CP50, a_P, KiP) * f_N(C_N, CN50, a_N, KiN)
206    k_phot_eff <- k_phot_eff * f_BM(BM, BM50)
207    k_resp_eff <- k_resp*f_T_resp(actTemp,t_ref,Q10)
208  }else{
209    k_phot_eff <- k_phot_max
210    k_resp_eff <- k_resp
211  }
212
213  # Consider toxic effect
214  f_Eff <- f_E(b,EC50,Emax,C_int_u)
215  if(TDMod=='delayed'){f_Eff <- E}  # delayed effects could be considered
216  k_phot_eff <- k_phot_eff*f_Eff
217
218  # Biomass
219  dBMdt <- BM*(k_phot_eff-k_resp_eff-k_loss)
220  # let population extinct if less than one frond/m²
221  if(BM<5*mass_per_frond){dBMdt <- 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_phot_eff and the effective respiration rate k_resp_eff are calculated by functions of response to the different

'natural' environmental factors (lines 203 – 207, explained) later). The expression k_phot_eff corresponds to $f_{photo}(\theta)$ in equation 1 multiplied with the maximum photosynthesis rate k_phot_max. For the calculation of k_photo_eff the responses for each factor are multiplied. The expression k_resp_eff, the effective respiration rate, is the reference respiration rate k_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_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_loss, to consider e.g. loss by flow, which is not mentioned in the paper. However, k_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_{max_photo} and k_{ref_resp}, respectively. However, in the supplemented R-code, the values of k_phot_max is given as 0.47 d^{-1} and it is explained as 'maximum growth rate + kmort'. The value for k_resp of 0.05 d^{-1} is the same as k_{resp_ref} in the paper but note that k_resp is explained in the implementation as 'rate of mortality'.

Default parameters for the basic growth equation (Table 1 in Schmitt *et al.* 2013)

k_{photo}^{max}	0.42	1/d
k_{resp}^{ref}	0:05	1/d

Default parameters for the basic growth equation implemented in mmc2.r (Schmitt *et al.* 2013)

```
74  #       - Fate of biomass -
75  k_phot_fix  = F,   #  T/F          If True k_G_max is not changed by environmental factors
76  k_phot_max  = 0.47, #  [1/d]       Maximum growth rate of biomass + kmort     [Ref. F 0191, Harlan-011]
77  k_resp   = 0.05, #  [1/d]        Rate of mortality                [Ref. Harlan-011, rough estimate]
78  k_loss   = 0.0, #  [1/d]        Some rate of loss (e.g. Flow rate)
79  #
```

Lasfar *et al.* (2007) is given as reference for the value of the k_phot_max. 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_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_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.

We propose to define a minimum biomass value BM_{min} to define how the population should develop at very low abundances. If the actual biomass falls below a given threshold $BM_{threshold}$ it is set to BM_{min}. The higher BM_{min} the larger the reservoir for recovery. By default BM_{min} is set to zero to allow extinction and $BM_{threshold}$ to 0.0005 g dw m^{-2} as used by Schmitt *et al.* (2013).

Box 1: Basic differential equation for the change of *Lemna* sp. biomass over time

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

$$\frac{d}{dt}BM(t) = \left(k_{photo\_max} \cdot f_{photo}(t) - k_{loss} \cdot f_{loss}(t)\right) \cdot BM(t), \qquad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}$$

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 $f_{photo}(t)$ 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 $f_{loss}(t)$ 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 $BM_{threshold}$ it is set to a defined minimum value. The default settings correspond to Schmitt e al. (2013) and allows extinction.

If $BM(t) < BM_{threshold}$ then set $BM(t)$ to BM_{min}.

Default parameter values:

$$k_{photo\_max} = 0.47\ d^{-1} \qquad k_{loss_{ref}} = 0.05\ d^{-1}$$

$$BM_{threshold} = 0.0005\ g\ dw\ m^{-2} \qquad BM_{min} = 0\ \ g\ dw\ m^{-2}$$

In the paper it is written 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).

Thus, we suggest that for simulating laboratory tests with constant environmental factors, 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 respiration rate the growth observed for the control of the experiment to be simulated. This handling of the loss rate is only relevant if the full model (i.e simulation of populations under dynamic environmental conditions, e.g. in the field) should be used. If the model is only used as a Tier 2C approach to simulate refined exposure tests in the laboratory, the loss rate can also be set to zero and the photosynthesis rate can be set to the growth rate observed in the control. See also 2.11 in the Annex.

2.2 Response to environmental factors

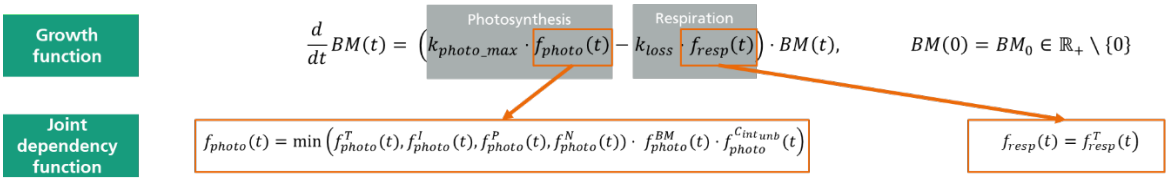
In the following we differentiate between response and dependency functions to describe the effects of environmental factors on production and loss of *Lemna* biomass. Since the environmental factors can vary over time, the dependency functions also vary over time. In contrast to this, the response functions 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 [°C], irradiance [kJ m^{-2} d^{-1}], phosphorus P and nitrogen N concentrations [mg L^{-1}] [1]. In addition, the actual biomass BM [g/m²] is needed for density dependence and the inhibition of the photosynthesis depends of the internal unbound concentration of the toxicant, $C_{int:unb}$, which depends on the environmental factor external concentration in the water C_{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: $f^{x}_{photo}: [0, t_n] \rightarrow [0,1]$.



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 ($Z \rightarrow \mathbb{R}$). 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)**.

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

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** $\hat{f}^{T}_{photo}$ we have:

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

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}_{photo}, mapping each time point a value between zero and one (using the temperature response function).

$$f^{T}_{photo}: [0, t_n] \xrightarrow{T} \mathbb{R} \xrightarrow{\hat{f}^{T}_{photo}} [0,1]$$

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

Photosynthesis dependency function in Schmitt *et al.* (2013)[1]

$$\boldsymbol{f_{photo}(t) = f^{T}_{photo}(t) \cdot f^{I}_{photo}(t) \cdot f^{P}_{photo}(t) \cdot f^{N}_{photo}(t) \cdot f^{BM}_{photo(t)} \cdot f^{C_{int\,unb}}_{photo}(t)}$$

[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 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)

$f_{photo}: [0, t_n] \rightarrow [0,1]$

$$f_{photo}(t) = \min\left(f^{T}_{photo}(t), f^{I}_{photo}(t), f^{P}_{photo}(t), f^{N}_{photo}(t)\right) \cdot f^{BM}_{photo}(t) \cdot f^{C_{int_{unb}}}_{photo}(t)$$

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

This refinement can result in higher growth rates of the simulated field populations and thus, higher recovery rates after stress compared to the original model but it is considered to be more realistic than using the product of all single functions.

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

$f_{loss}: [0, t_n] \rightarrow \mathbb{R}_+$

$$f_{loss}(t) = f^{T}_{loss}(t)$$

t = time [d], T = 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_{min}, T_{max}, T_{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).

Equation 2 in Schmitt *et al.* (2013): Temperature response function for photosynthesis

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

In this function, T_{min} is used when $T < T_{opt}$ and T_{max} when $T > T_{opt}$.

[1] For Liebig's law, see e.g. https://en.wikipedia.org/wiki/Liebig%27s_law_of_the_minimum

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

```
255   # Temperature
256   # effect on k_phot
257   f_T <- function(actTemp,Topt,Tmax,Tmin)
258   {
259   Tx<-Tmax
260   if(actTemp<=Topt){Tx<-Tmin}
261   return(exp(-2.3*((actTemp-Topt)/(Tx-Topt))^2))
262   }
```

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)

T_{min}	8.0	°C
T_{max}	40.5	°C
T_{opt}	26.7	°C

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

```
80   #       - Temperatur dependence -
81   # k_phot
82   Temp      = 12,    #  [°C]        Current temperature (may also be a table)
83   Tmin      = 8.0  , #  [°C]         Minimum growth temperature      [Ref. F 0191, data re-evaluated  incl. kmort(T)]
84   Tmax      = 40.5 , #  [°C]         Maximum growth temperature      [Ref. F 0191, data re-evaluated  incl. kmort(T)]
85   Topt      = 26.7 , #  [°C]         Optimum growth temperature      [Ref. F 0191, data re-evaluated  incl. kmort(T)]
```

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_{min}respectively T_{max} the temperature response function value $\hat{f}^{T}_{photo}$ is equal to 0.1.

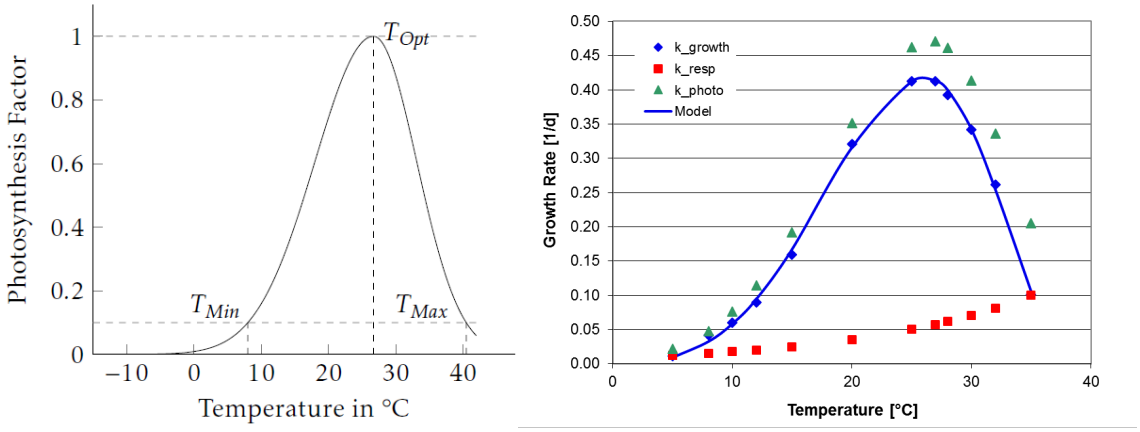


Figure 2: Left: $f_{photo}(T)$ as used by Schmitt *et al.* (2015) (modified from Klein 2018)
Right: Dependence of observed (◆) and calculated (line) net growth rate k_{growth} of *L. mi-*

nor in dependence of temperature. Symbols (■) show the respiration rates k_{resp} calculated with the respiration temperature response and (▲) the photosynthesis rates k_{photo} = k_{growth} + k_{resp}. was fitted to k_{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 $\exp(-\ln(10) \cdot x)$.

Box 4: Temperature response of photosynthesis

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

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

Default parameter values:

$$T_{min} = 8\ °C$$

$$T_{max} = 40.5\ °C$$

$$T_{opt} = 26.7\ °C$$

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

$$f_{resp}(T) = Q_{10}^{(T-T_{ref})/10}$$

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

```
263  # effect on k_resp
264  f_T_resp <- function(actTemp,t_ref,Q10)
265  {
266  Q10^((actTemp-t_ref)/10)
267  }
```

A reference temperature T_{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_{ref} = 25 °C

Q_{10} = 2

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

```
# k_resp
t_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_{ref} the response is greater than one and thus the loss rate is higher than the reference rate (Figure 3).

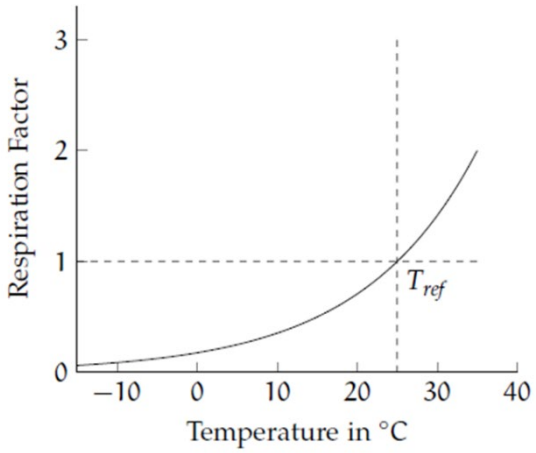


Figure 3: The respiration response due to temperature for $T_{ref} = 25$ °C and $Q_{10} = 2$, taken from Klein 2018. (See also Figure 3 for experimental data)

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

$\hat{f}_{loss}^{T}: \mathbb{R} \rightarrow \mathbb{R}_{+}$

$$\hat{f}_{loss}^{T}(T) = Q_{10}^{\frac{T-T_{ref}}{10}}$$

Default parameters:

T_{ref} = 25 °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_{sat} where $f_{photo}(I)$ 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 (α corresponds to a_k and ß to k_0). In the R-implementation, the saturation constant I_{sat} is not used but $f_{photo}(I)$ is set to 1 if the linear function is > 1.

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

$$f_{photo}(I) = \begin{cases} \alpha \cdot I + \beta & |I \le I_{sat} \\ 1 & |I > I_{sat} \end{cases}$$

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

```
247   # Light
248   f_R <- function(actRad, k_0, a_k)
249   {
250   photfac <- a_k*actRad + k_0
251   if(photfac >1){photfac=1}
252   return(photfac)
253   }
```

The intercept values ß 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 $f_{photo}(I)$ 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 α ($kJ^{-1}m^2$ d) is correct while the unit given for ß 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)

I_{sat}	15,000	kJ/(m^2d)
β	0.25	kJ/(m^2d)
α	5×10^{-5}	1/(kJ/(m^2d))

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

```
90   #      - Light dependence (linear dependence on global radiation (see Hodgeson 1969)
91   Rad         = 15000 , #  [kJ/m²/d]  Radiation  (may also be given as table)
92   k_0         = 3 ,     #  [1/d]      Intercept of linear part
93   a_k         = 5E-5 ,  #  [(1/d)/(kJ/m?/d)]       Slope of linear part
94
```

Note that `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.

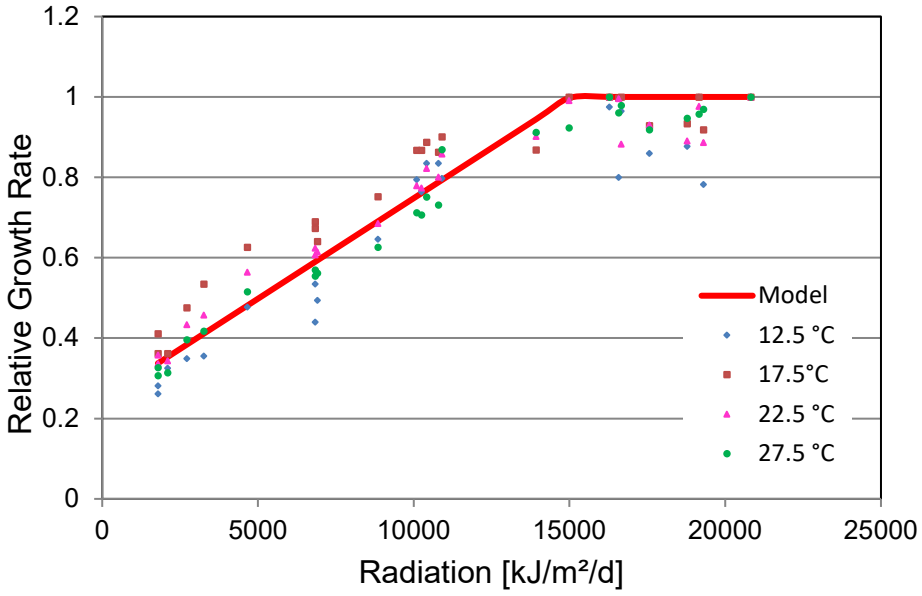


Figure 4: Growth rates of *L. minor* observed at different temperatures under natural daylight. The line shows the dependence on global radiation evaluated by fitting the light response to the data (copied from Fig, 3 in Schmitt *et al.* 2013, suppl. data).

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

$\hat{f}^{I}_{photo}: \mathbb{R}_{+} \rightarrow [0,1]$

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

Default parameters:

α = 5 10^{-5} kJ^{-1} m^2 d

β = 0.025

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

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

The same function is used for phosphorus P.

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

```
269   # Phosphorus
270   f_P <- function(C_P, CP50, a_P, KiP)
271   {
272   C_P^a_P/(C_P^a_P + CP50^a_P) * KiP/(KiP + C_P)
273   }
274
275   # Nitrogen
276   f_N <- function(C_N, CN50, a_N, KiN)
277   {
278   C_N^a_N/(C_N^a_N + CN50^a_N)* KiN/(KiN + C_N)
279   }
```

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)

```
#       - Phosphorus dependence (Hill like dependence) -
C_P     = 0.3,          #   [mg/L]      Phosporus concentration in water
CP50    = 0.0043,       #   [mg/L]      P-conc. where growth rate is halfened   [Data from L??nd, 1983 evaluated with monod model]
a_P     = 1,        #   []          Hill coefficient
KiP     = 101,          #   [mg/L]      P-inhibition constant for very high P-conc.   [Ref. F 0191]

#       - Nitrogen dependence (Hill like dependence) -
C_N     = 0.6,          #   [mg/L]      Nitrogen concentration in water
CN50    = 0.034,        #   [mg/L]      N-conc. where growth rate is halfened   [Data from L??nd, 1983 evaluated with monod model]
a_N     = 1,        #   []          Hill coefficient
KiN     = 604,          #   [mg/L]      n-inhibition constant for very high P-conc.   [Ref. F 0191]
```

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.

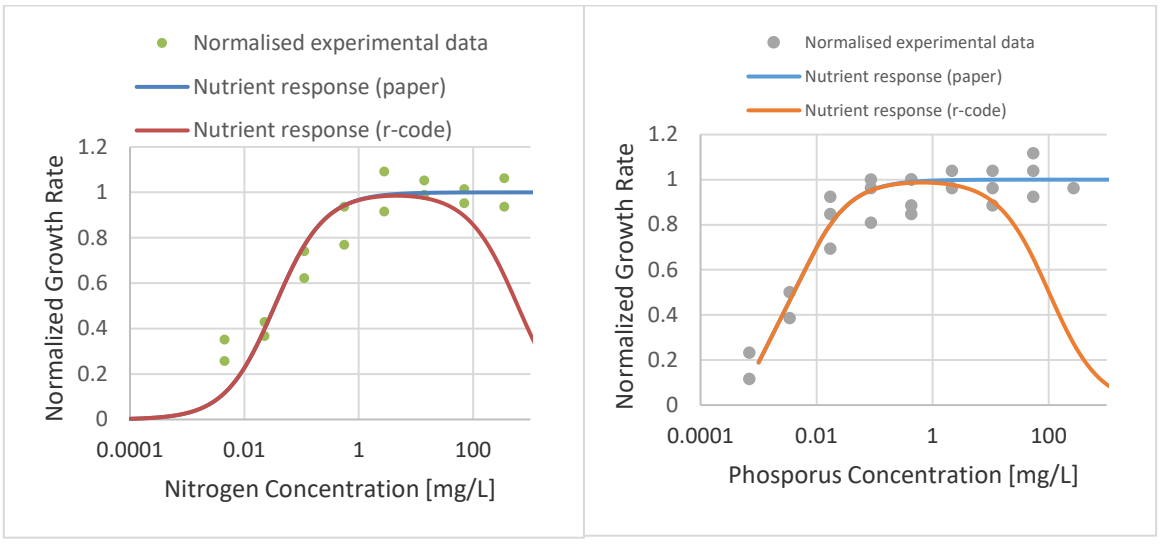


Figure 5: Growth rates of *L. minor* in dependence of nitrogen and phosphorus concentration as described in the paper and implemented in the R-code. Note that in Figure 4 and 5 in

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

$\hat{f}^{N}_{photo}: \mathbb{R}_{+} \rightarrow [0,1]$, $\hat{f}^{P}_{photo}: \mathbb{R}_{+} \rightarrow [0,1]$

$$\hat{f}^{N}_{photo}(N) = \frac{N}{N + N_{50}} \quad \text{respectively} \qquad \hat{f}^{P}_{photo}(P) = \frac{P}{P + P_{50}}$$

Default parameters

$$P_{50} = 0.0043 \text{ mg L}^{-1}, N_{50} = 0.034 \text{ mg L}^{-1}$$

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

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

Density dependence implemented in mmc3.r

```
281   # Biomass (crowding)
282   f_BM <- function(BM, BM50)
283   {
284   fact<-(BM50 - BM)/BM50
285   return(fact)
286   }
```

There is a typo in equation 6 since it should be $f_{photo}(D)$ instead $f_{photo}(I)$. 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)

$$D_L \quad 176\,\mathrm{g\_d.w./m^2}$$

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

```
107   #       - Density dependence -
108   BM50     = 176,    #  [g_dw/m?]            Cut off BM   [Ref. F 0191]
```

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 us the parameter value given in Monette *et al.* (2006).

Box 8: Density dependence

$\hat{f}_{photo}^{BM}: \mathbb{R}_+ \setminus \{0\} \rightarrow [0,1]$

$$\hat{f}_{photo}^{BM}(BM) = 1 - \frac{BM}{BM_L}$$

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 *Cint_unb.* The concentration response is modelled as a 3-parameter Hill function defined by the maximum inhibition E_{max} , the $EC50_{int}$ as the internal concentration resulting in 50 % effect and a slope parameter *b*. There is a typo in equation 7 since it should be $f_{photo}(Cint\_unb)$ and not $f_{photo}(E)$.

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_{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

$$f_{photo}(E) = 1 - E_{\max}\frac{C_{int\_unb}^{b}}{EC50_{\text{int}}^{b} + C_{int\_unb}^{b}}$$

Toxicity response function implemented in mmc3.r

```
287
288   # Effect
289   f_E <- function(b,EC50,Emax,C_active)
290   {
291   1-Emax*C_active^b/(EC50^b+C_active^b)
292   }
```

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

223  # Effect  (this is the delayed TD model)
224  dEdt <- k_E_in*f_E(b,EC50,Emax,C_int_u) - k_E_out * E
225
```

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_{max} and the slope b are dimensionless, whereas the $EC50_{int}$ 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

E_{max}	0.784
$EC50_{int}$	0.3
b	4.16

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

```
59  #       - Effect -
60  Conc      = 1,    #  [any]      Concentration of toxicant (may also be a table)
61  Emax      = .784,     #   maximum Effect
62  EC50      = 0.3,      #  [same as conc. data]      Midpoint of effect curve
63  b         = 4.16,     #  [-]          Slope of effect curve
```

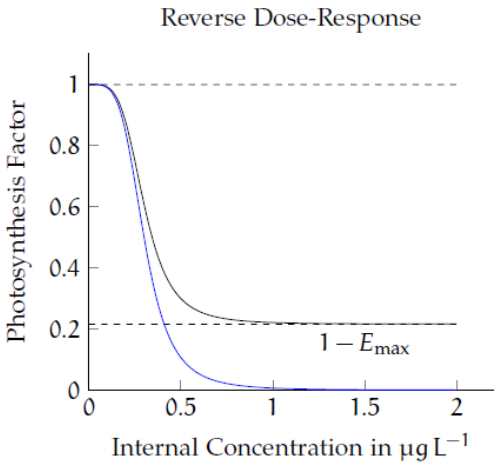


Figure 6: Reverse dose-response function for the parameters given in Equation 7 of Schmitt *et al.* (2013) for Metsulfuron-methyl (black line). For the blue line, Emax was set to one. Figure taken from Klein 2018.

The parameter E_{max}, $EC50_{int}$ 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_{max} to one by default and decide case by case if E_{max} should be included in the calibration.

Box 9: Concentration response function on photosynthesis (Toxicodynamics)

$\hat{f}_{photo}^{C_{int}}(C_{int}): \mathbb{R}_+ \rightarrow [1 - E_{max}, 1]$

$$\hat{f}_{photo}^{C_{int}}(C_{int\_unb}) = 1 - \frac{E_{max} \cdot {C_{int\_unb}}^b}{EC50_{int}^b + {C_{int\_unb}}^b}$$

The parameters $EC50_{int}$ [mass / volume], b [-], and E_{max} [-] are substance specific and have to calibrated. E_{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_{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

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

M_{int} = mass of substance in the plants [mass m^{-2} or mass per vessel]
Φ_{in}, Φ_{out} = substance fluxes (mass d^{-1}) into and out of the plant
k_{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 Φ_{in} and Φ_{out}, respectively. For the net flux, the concentration gradient outside and inside the plant, ΔC, 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: $\text{area} \cdot {}^{\text{length}}/_{\text{time}} \cdot {}^{\text{mass}}/_{\text{volume}} = {}^{\text{mass}}/_{\text{time}}$.

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_{int} [µg/L]

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

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

$$C_{int_{unb}}(t) = \frac{C_{int}(t)}{K_{p:w}}$$

$K_{p:w}$ 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_{int} in the notation by Schmitt *et al.* 2013. In equilibrium the internal unbound concentration is the same as the external concentration. Thus Kp:w can be written in the following ways:

$$K_{p:w} = C_{plant} / C_{water} = C_{int} / C_{int\_unb}$$

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 $EC50_{int}$ 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.

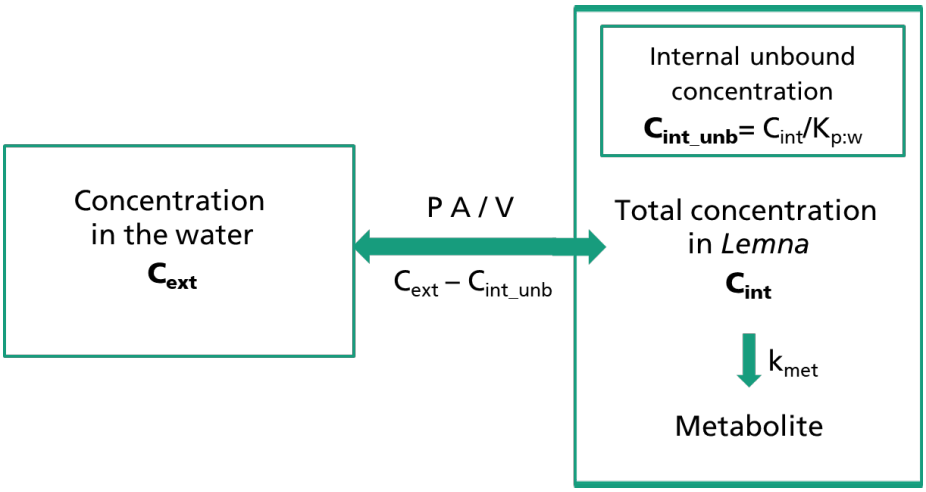


Figure 7: TK model concept of the *Lemna* model by Schmitt *et al.* 2013

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

```
195  # Calculate internal toxicant concentrations from amount in biomass
196  BM_fresh <- BM*BMw2BMd
197  C_int <- M_int/BM_fresh
198  C_int_u <- abs(C_int/Kbm)    # Unbound internal concentration
199

226  # TK part: Internal amount of toxicant
227  P_up_eff = P_up
228  if(P_Temp){P_up_eff = P_T(P_up,MolWeight, actTemp)}   # Temperature dependence of permeability
229  dM_intdt <- P_up_eff*AperBM*BM*(actConc-C_int_u) - C_int*BM_fresh*(k_resp_eff+k_loss)
230

236  # Calculation of temperature dependent permeability
237  P_T <- function(P_up,MolWeight, actTemp)
238  {
239  Eact <- 0.307*MolWeight/1.4+95          # Activation energy
240  Q10perm <- exp(10*Eact/0.008314/300^2)   # see Baur Publication 7
241  return(P_up * Q10perm^((actTemp-20)/10))
242  }
```

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_int_u using the partitioning coefficient Kbm (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_{int}. C_{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 metabolisation as described in equation 10.
2. The additional elimination term C_int*BM_fresh*(k_resp_eff + k_loss) is not given in the paper. It is needed to reduce the internal mass (C_int*BM_fresh) if the biomass is declining. This correction is not necessary if the TK is describe 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_up_eff is the fixed parameter permeability (P in the paper, P_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

Frond area/dry weight	1000	cm^2/g d.w.
Dry weight/frond	0.1	mg_d.w./frond
Fresh weight/dry weight	16.7	g f.w./g_d.w.

Conversation parameters implemented in mmc2.r (Schmitt *et al.* 2013)

```
68   AperBM    = 1000,    # [cm?/g_dw]   A_leaf / d_leaf = 1/d_leaf (for circular disc, d=0.05 cm) [Ref. HARLAN-022]

111  #       - Others -
112  mass_per_frond     = 0.1,    #  [g_dw/frond]  Dryweight per frond [Ref. HARLAN-022]
113  BMw2BMd     = 16.7    #  [g_fresh/g_dry]  Fresh- / dryweight [Ref. F 0191]
114
```

For the ratio of frond area to dry weight, AperBM = 1000 cm^2 g^{-1} dw, a reference is made in the paper to Landolt & Kandeler (1987) who estimated 40 mm^2 per frond from photographs. With the reported dry weight/frond ratio of 0.1 mg dw/frond, the 40 mm^2 per frond would result in 4 000 cm^2 g^{-1} dw. In the implementation, the 1000 cm^2 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]

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

This equation can be solved to estimate $K_{p:w}$ from the $log\ K_{ow}$:

$$K_{p:w} = 10^{0.73 \cdot logKow - 1.37} + 0.71$$

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

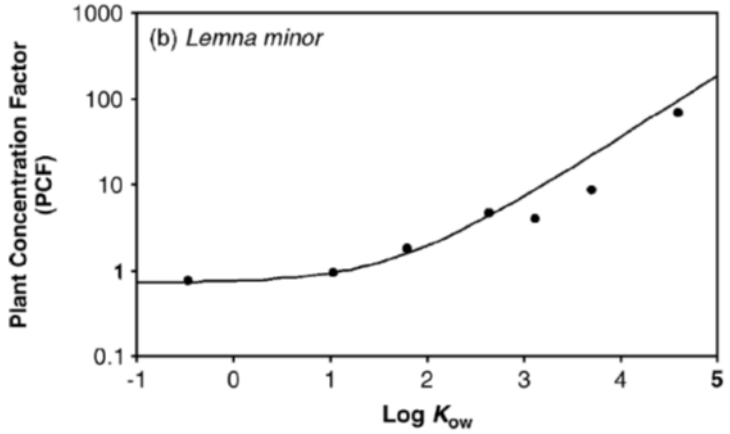


Figure 8: Water:Plant partitioning coefficient $K_{p:w}$ as a function of log K_{ow} (copied from Figure 3 in de Carvalho et al. 2007).

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

$$P = 0.0054 \text{ cm/d}$$

TK parameters implemented in mmc2.r

```
66    #         - Toxicokinetics -
67    P_up      = .0054,    # [cm/d]       Permeability for uptake

69    Kbm       = .75,    # []           Biomass(fw):water partition coefficient
```

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 *Lemna* is that in GUTS it is assumed that the biomass (in GUTS of the single organism) is constant while the biomass of the *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 *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 *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 lipophilc 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 *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 **internal mass** because in the R-code the differential equation is solved for mass and to have the same units for both state variables (*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_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*

$M_{int}: [0, t_n] \rightarrow \mathbb{R}_+$

$$\frac{d}{dt} M_{int}(t) = P \cdot A(t) \cdot \left(C_{ext}(t) - \frac{C_{int}(t)}{Kp{:}w} \right) - M_{int}(t)/Kp{:}w \cdot k_{met} - M_{int} \cdot (t)\, k_{loss} \cdot f_{loss}(t)$$

$$M_{int}(0) = 0$$

$$A(t) = BM(t) \cdot r_{A/DW}$$

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

$$C_{int}(t) = \frac{M_{int}(t)}{V(t)} = \frac{M_{int}(t) \cdot r_{FW/V}}{BM(t) \cdot r_{FW/DW}}$$

$M_{int}(t)$ = mass of the toxicant in the plant population [mass m^{-2}] or [mass per vessel]
P = permeability [cm d^{-1}]
$A(t)$ = total surface area of the plant population [cm² m^{-2}] or [cm² per vessel]
$V(t)$ = total volume of the population [cm³ m^{-2}] or [cm³ per vessel]
$C_{ext}(t)$ = external concentration in the water [mass/volume]
$C_{int}(t)$ = internal concentration [mass/volume]
$r_{A/DW}$ = area per dry weight ratio [cm²/g]
$r_{FW/DW}$ = fresh weight per dry weight ratio [-]
$r_{FW/V}$ = fresh weight density [g/cm³]
$K_{p:wt}$ = partitioning coefficient plant:water [-]
k_{met} = metabolisation rate [d^{-1}]

Default parameters

$$r_{A/DW} = 1000 \text{ cm}^2 \text{ g}^{-1}$$

$$r_{FW/DW} = 16.7$$

$$r_{FWV} = 1 \text{ g cm}^{-3}$$

$$r_{DW/FN} = 0.0001 \text{ g}$$

$$K_{p:w} = 1$$

$$k_{met} = 0 \text{ d}^{-1}$$

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 metabolisation rate k_{met} should only be calibrated if needed.

3 Summary

3.1 Variables used in the model

Table 1: Variables used in the model

Variable	Description	Start value	Unit
State variables (calculated by differential equation system)			
BM	Biomass (dry weight] of *Lemna* population	Study specific > 0	g dw m^{-2} (field) mg dw (lab)
M_{int}	Internal mass of toxicant in the Lemna population	Usually 0	Case specific, e.g. mg or µg m^{-2} (field) mg or µg (lab)
Help variables (can be calculated from state variables, no differential equations)			
V	Volume of *Lemna* population	Proportional to BM	cm^3 m^{-2} (field) cm^3 (lab)
A	Surface area of *Lemna* population	Proportional to BM	cm^2 m^{-2} (field) cm^2 (lab)
C_{int}	Internal concentration	$Mint/V$	Same as C_{ext}
C_{int_unb}	Internal unbound concentration	Proportional to C_{int}	Same as C_{ext}
FN	Frond number	Proportional to BM	m^{-2} (field) - (lab)
External variables (forcing functions, not affected by state variables but model inputs)			
C_{ext}	Concentration in the medium, e.g. measured concentration or PEC	Study specific, e.g $FOCUS_{sw}$ profile	Case specific, e,g. mg L^{-1} or µg- L^{-1}:
T	Temperature (for *Lemna* this could be water or air temperature)	Study specific, e.g. from FOCUSsw	°C
Rad	Global radiation	Study specific e.g. from FOCUSsw	kJ m^{-2} d^{-1}
N, P	Nitrogen and Phosphorus concentrations	Study specific	mg N L^{-1} mg P L^{-1}

3.2 Growth model for *Lemna* biomass

$$\frac{d}{dt}BM(t) = \left(k_{photo\_max} \cdot f_{photo}(t) - k_{loss} \cdot f_{loss}(t)\right) \cdot BM(t), \qquad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}$$

If $BM(t) < BM_{min}$ then set $BM(t)$ to BM_{min}.

Dependencies of photosynthesis

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

$$f_{loss}(t) = f^T_{loss}(t)$$

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

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

$$\hat{f}^N_{photo}(N) = \frac{N}{N + N_{50}} \quad \text{respectively} \qquad \hat{f}^P_{photo}(P) = \frac{P}{P + P_{50}}$$

$$\hat{f}^{BM}_{photo}(BM) = 1 - \frac{BM}{BM_L}$$

$$\hat{f}^{C_{int}}_{photo}(C_{int}) = 1 - \frac{E_{max} \cdot (C_{int}/Kp{:}\,w)^b}{EC50^b_{int} + (C_{int}/Kp{:}\,w)^b}$$

Dependencies of biomass loss

$$\hat{f}^T_{loss}(T) = Q_{10}^{\frac{T-T_{ref}}{10}}$$

3.3 Toxikokinetic model for internal mass

$$\frac{d}{dt}M_{int}(t) = P \cdot A(t) \cdot \left(C_{ext}(t) - \frac{C_{int}(t)}{kpw}\right) - M_{int}(t)/Kp{:}\,w \cdot k_{met} - M_{int} \cdot (t)\, k_{loss} \cdot f_{loss}(t)$$

$$A(t) = BM(t) \cdot r_{A/dw}$$

$$V(t) = BM(t) \cdot r_{fw/dw} \cdot \frac{1}{d_{fw/V}}$$

$$C_{int}(t) = \frac{M_{int}(t)}{V(t)} = \frac{M_{int}(t) \cdot d_{fw/V}}{BM(t) \cdot r_{fw/dw}}$$

3.4 Model parameters

Table 2: Default model parameters

Parameter name in equation system (section 3.2)	Parameter name in R code (Annex)	Default value	Unit	Description	Reference in Schmitt *et al.* 2013
k_{max_photo}	kphotomax	0.47	d^{-1}	Maximum photosynthesis rate	Lasfar (2007)
$k_{resp\text{-}ref}$	krespref	0.05	d^{-1}	Respiration resp. mortality rate at reference temperature	Claus (1972)
T_{min}	Tmin	8	°C	Minimum growth temperature	Lasfar (2007)
T_{max}	Tmax	40.5	°C	Maximum growth temperature	Lasfar (2007)
T_{opt}	Topt	26.7	°C	Optimum growth temperature	Lasfar (2007)
T_{ref}	Tref	25	°C	Reference temperature for respiration rate	Claus (1972)
Q_{10}	Q10	2		Q10 for respiration rate	Wangermann & Ashby (1951)
α	alpha	5.00E-05	$m^2\ d\ kj^{-1}$	Slope of radiation dependence	Hodgson (1970)
β	beta	0.025	-	Intercept of radiation dependence[1]	Hodgson (1970)
N_{50}	CN50	0.034	$mg\ L^{-1}$	N-conc. where growth rate is halved	Lüönd (1983)
P_{50}	CP50	0.0043	$mg\ L^{-1}$	N-conc. where growth rate is halved	Lüönd (1983)
BM_L	BMlimit	176	$g\_dw\ m^{-2}$	Limit density (carrying capacity)	Monette (2006)

Parameter name in equation system (section 3.2)	Parameter name in R code	Default value	Unit	Description	Reference in Schmitt et al. 2013
BM_{min}	BMmin	0	g_dw m^{-2}	Minimum biomass	
$r_{DW/FN}$	DW2FN	0.0001	g_dw $frond^{-1}$	Dry weight/frond(conversion factor, laboratory)	Determined for *L. gibba (*Schmitt *et al.* 2013, paper)
$r_{FW/DW}$	FW2DW	16.7	g fw g^{-1}_dw.	Fresh weight/ dry weight	Determined for *L. gibba* (Schmitt *et al.* 2013)
$r_{A/DW}$	A2BM	1000	cm^2 g^{-1} dw.	Surface area per dry weight	Landolt and Kandeler (1987)
Substance specific TKTD parameters used in Schmitt *et al.* (2013) for Metsufuron-methyl					
P	P	0.0054	cm/d	Permeability	Calibrated
$K_{p:w}$	Kp:w	0.75	-	Partitioning coefficient plant:water	Set to 1 by default, regression Carvalho *et al.* (2007)
k_{met}	kmet	0	d^{-1}	Metabolism rate	Set
E_{max}	Emax	0.784	-	Maximum effect	Calibrated
$EC50_{int}$	EC50	0.3	µg L^{-1}	Internal EC50	Calibrated
b	b	4.16	-	Slope	Calibrated

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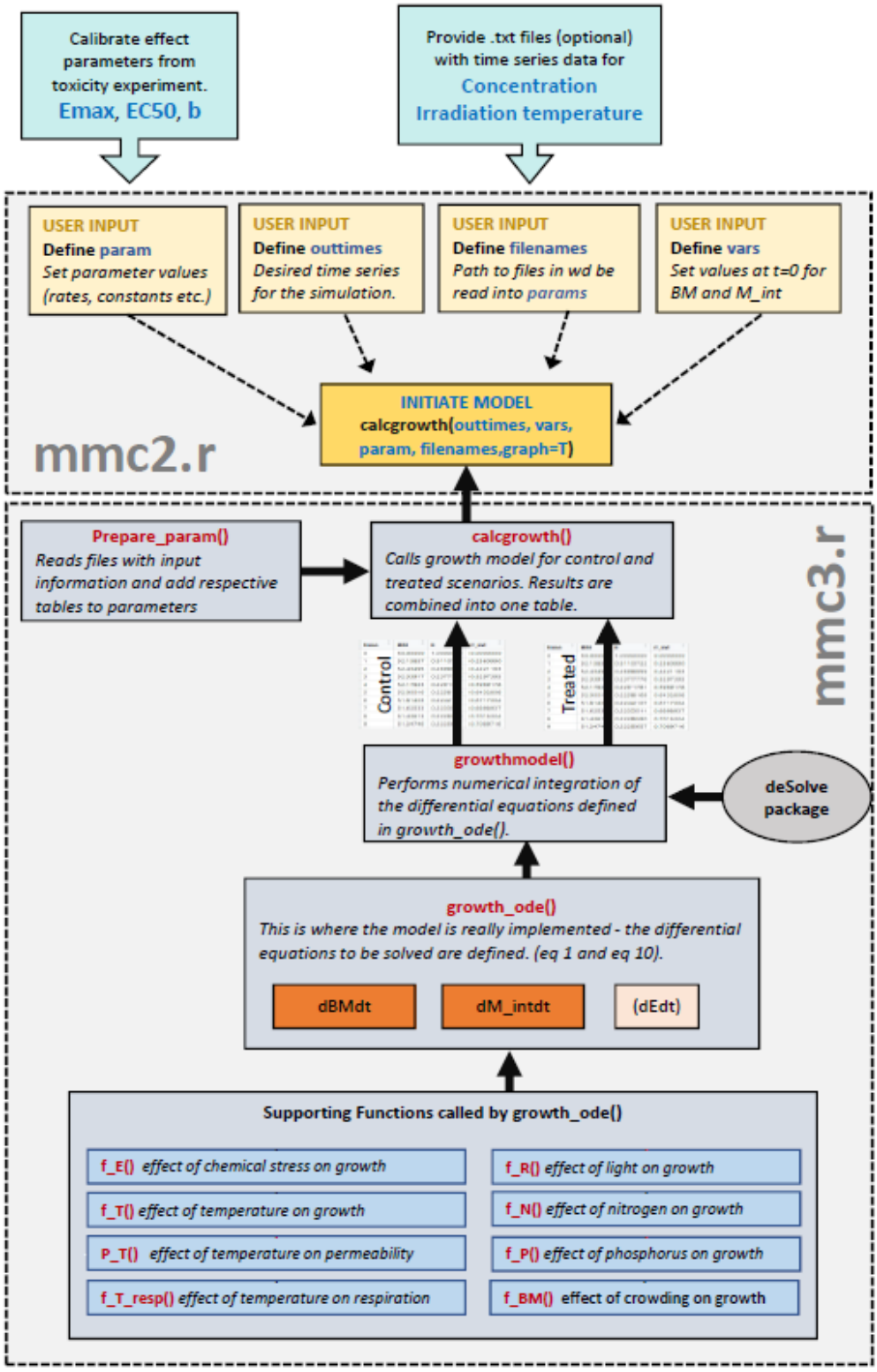
5 Annex

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_{int} [µg/L]

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

P = Permeability, A = surface area of the population, V = Volume of the population, C_{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: $C_{int_{unb}} = \frac{C_{int}}{K_{p:w}}$.

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

If $C_{int_{unb}}$ is proportional to C_{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_{int} [µg/L]

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

This equation can be solved to estimate $K_{p:w}$ from the *log* K_{ow}:

$$K_{p:w} = 10^{0.73 \cdot logKow - 1.37} + 0.71$$

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 $K_{pw} < 1$ then it yields $k_2 > k_1$.

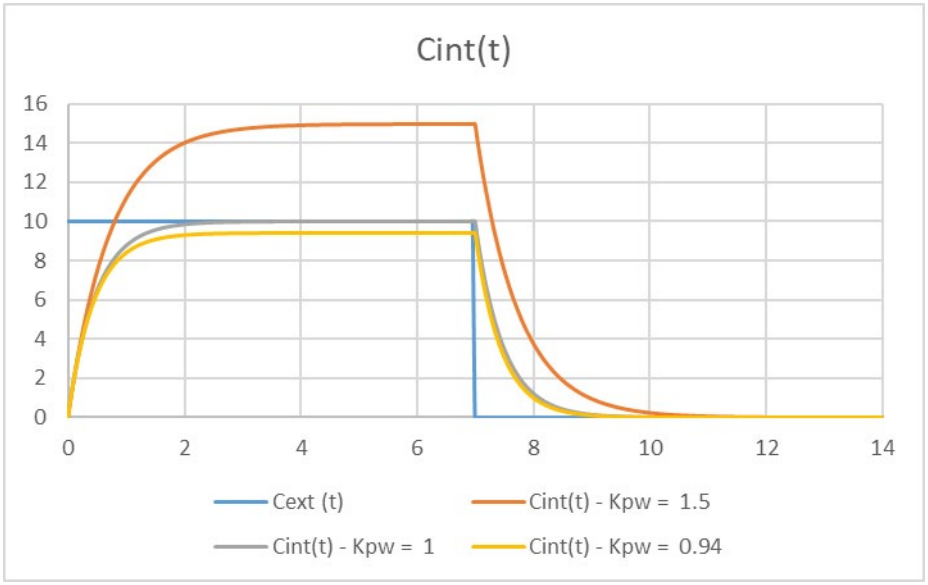
Example:

Set $k_1 = 2$ and try $K_{p:w} = 1.5$, $K_{p:w} = 1$ and $K_{p:w} = 0.94$.

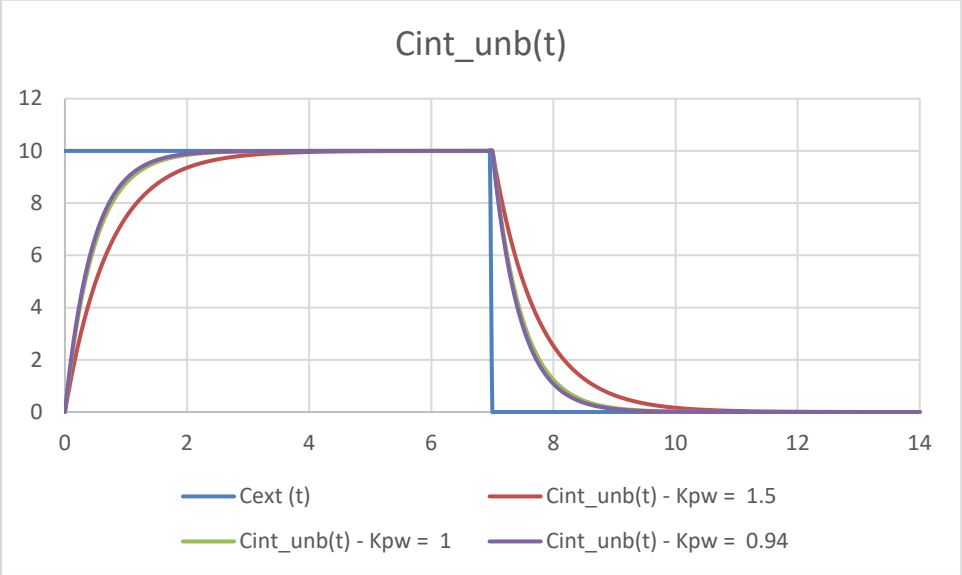
External concentration function is defined by

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

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 $C_{ext} \cdot K_{p:w}$.

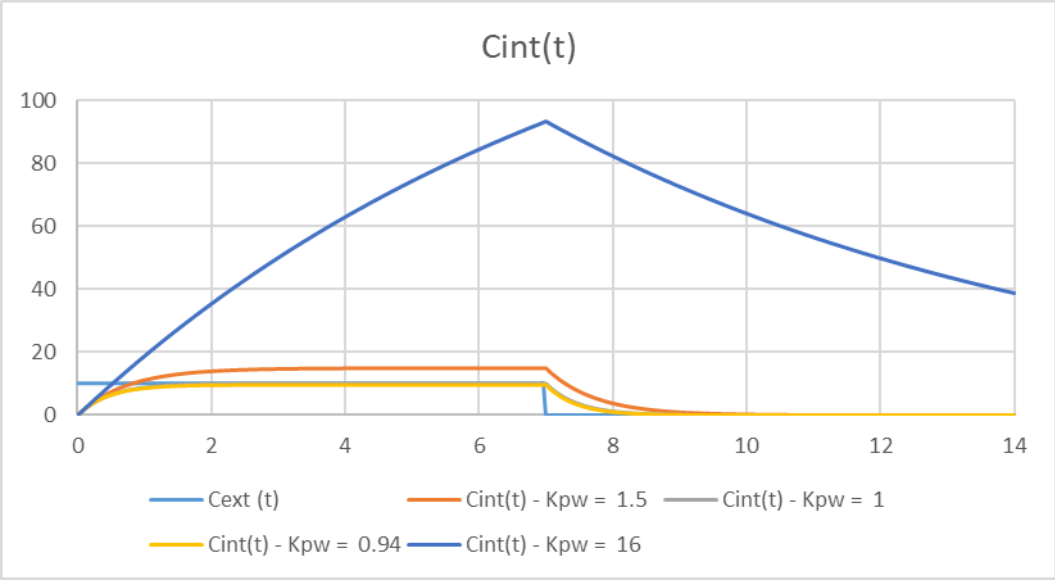


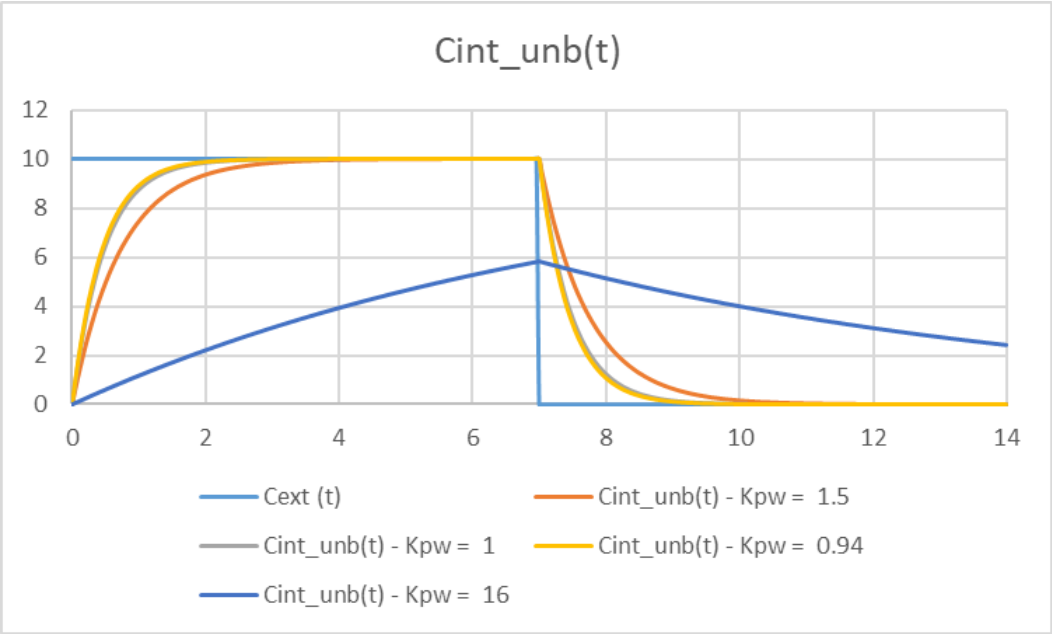
After transforming to unbound concentration by dividing by the respective $K_{p:w}$ value, we get the following curves. The plateau value is equal to the external concentration value. A higher $K_{p:w}$ leads to a slower uptake and to a slower elimination (in contrast to the faster increase of total 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):

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

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

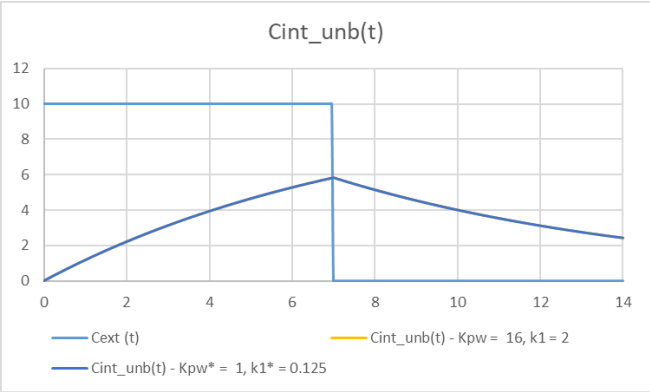
$$\xLeftrightarrow{C_{int_{unb}}(t)=\frac{C_{int}(t)}{K_{p:w}}} \frac{d}{dt}C_{int_{unb}}(t) = \frac{k_1}{K_{p:w}} \cdot C_{ext}(t) - k_2 \cdot C_{int_{unb}}(t)$$

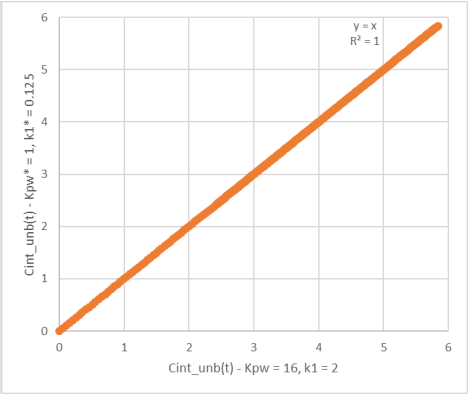
$$\xLeftrightarrow{k_2=\frac{k_1}{K_{p:w}}} \frac{d}{dt}C_{int_{unb}}(t) = \frac{k_1}{K_{p:w}}\left(\cdot C_{ext}(t) - \cdot C_{int_{unb}}(t)\right)$$

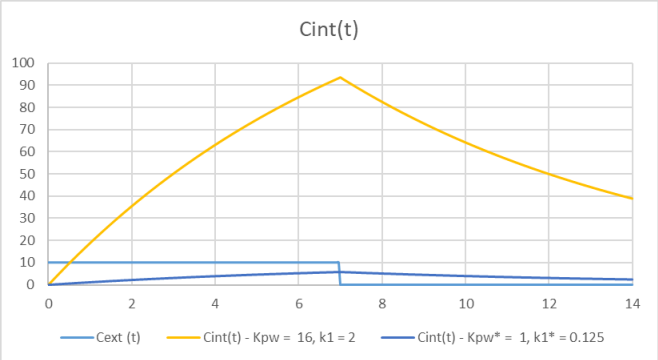
Additional remark 2: How to choose P to get the same internal unbound concentration as before using $K_{p:w}$

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 $k_{p:w}$ must not be smaller than the water content

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

$$
\begin{aligned}
& M_{int} = M_{int_{unb}} + M_{int_b} \\
\Leftrightarrow\ & M_{int} = BM_{FW} \cdot 0.94 \cdot C_{int_{unb}} + M_{int_b} \\
\Leftrightarrow\ & M_{int} = BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} + M_{int_b} \\
& \text{(definition of } C_{int_{unb}} \text{ according to Schmitt et al. 2013)} \\
\Leftrightarrow\ & M_{int_b} = M_{int} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} \\
\Leftrightarrow\ & M_{int_b} = C_{int} \cdot BM_{FW} - BM_{FW} \cdot 0.94 \cdot \frac{C_{int}}{K_{pw}} \\
\Leftrightarrow\ & M_{int_b} = C_{int} \cdot BM_{FW} \cdot \left(1 - \frac{0.94}{K_{pw}}\right)
\end{aligned}
$$

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:

$$M_{int_b} \geqslant 0 \Leftrightarrow C_{int} \cdot BM_{FW} \cdot \left(1 - \frac{0.94}{K_{pw}}\right) \geqslant 0$$

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.

$$1 - \frac{0.94}{K_{pw}} \geqslant 0 \Leftrightarrow -\frac{0.94}{K_{pw}} \geqslant -1 \Leftrightarrow \frac{0.94}{K_{pw}} \leqslant 1 \Leftrightarrow 0.94 \leqslant K_{pw}$$

5.3 Modelling laboratory tests

The *Lemna* model is suitable to describe both laboratory as well as field studies. Field studies are characterized by highly dynamic environmental conditions (temperature, light, nutrients, and exposure). Laboratory studies are usually shorter and only exponential growth is considered (without limit density). It is likely that the model will most often used as a Tier 2C, i.e. to model refined exposure tests in the laboratory. In addition, the calibration and validation of the TKTD model has to be done based on laboratory tests (EFSA PPR panel 2018).

The calculation of internal concentration is the same for both situations. However, the calculation of (dry) biomass in time is much simpler for growth under laboratory conditions. In principle, the basic growth equation is for both situation the same. In contrast to the field situation, the photosynthesis dependency function only considers the internal concentration (TD model) since temperature and light conditions can be assumed to be constant and nutrients should be available in surplus to allow exponential growth of the test duration.

There are two options:

1. The loss dependency function respectively the loss rate constant is set to zero.

$$\frac{d}{dt}BM(t) = k_{photo} \cdot f_{photo}^{C_{int}}(t) \cdot BM(t), \qquad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}$$

   The maximum photosynthesis rate k_{photo} should be set to the growth rate of the control or it can be included as an additional parameter to be fitted together with the TKTD parameters. The latter might be especially useful if growth rate in the lower test concentrations is higher than in the control but promotion by the test item is unlikely.
   The problem with this option is that the TKTD model is then fitted to effects on the growth rate (i.e. (photosynthesis rate – loss rate) while in the full *Lemna* model for field populations only the photosynthesis is inhibited.

2. The loss rate constant can be set to the reference rate (e.g. 0.05 d^{-1} at 25 °C as default value in Table 2.

$$\frac{d}{dt}BM(t) = (k_{photo} \cdot f_{photo}^{C_{int}}(t) - k_{resp-ref}) \cdot BM(t), \qquad BM(0) = BM_0 \in \mathbb{R}_+ \setminus \{0\}$$

   This would allow also modelling declines of biomass in the laboratory tests and would be consistent with the mode of action assumed in the full *Lemna* model. As in option 1, the photosynthesis rate can be determined from only the control data or it can be fitted together with the TKTD parameters.

We propose option 2, i.e. using a default loss rate of 0.05 d^{-1} for calibration and validation of the TKTD model based on laboratory tests if the model should finally be used also to simulated effects on field populations.

If only laboratory tests should be modelled (use of the model only as Tier 2C tool), the loss rate can be set to zero. In consequence, the toxicant affects not the growth rate instead the photosynthesis rate in the model which corresponds to the usual evaluation of growth inhibitions tests by calculation of e.g. E_rC_50 values.