Revisiting simplified DEBtox models for analysing ecotoxicity data*

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Abstract

Mechanistic effect models are gaining increasing interest in ecotoxicology and environmental risk assessment (ERA). For explaining effects on an individual's life-history traits (e.g., growth and reproduction), DEBtox is the leading toxicokinetic-toxicodynamic (TKTD) modelling approach. DEBtox is the application of a model based on Dynamic Energy Budget (DEB) theory to toxic effects, but the term is generally used to refer to practical simplified models from this theoretical framework. The DEBtox approach has remained relatively unchanged over the last few decades, but an updated model is urgently needed. The need for revision is triggered by three, relative recent, developments: the broad acceptance of simple TKTD models for survival (GUTS), the development and successful application of reserveless DEB models for invertebrates (DEBkiss), and the concrete interest in DEBtox from the field of ERA for pesticides in Europe. In this paper, an updated DEBtox model is presented that is based on DEBkiss, follows the TKTD formulation as established for GUTS (i.e., explicitly taking damage into consideration), and includes a starvation module that is essential for time-varying exposure (as some toxicant action mechanisms may induce starvation). The updated model is illustrated with a case study for springtails exposed to chlorpyrifos in food, and may find applications in science as well

 The paper was published as: Jager T. 2020. Revisiting simplified DEBtox models for analysing ecotoxicity data. Ecological Modelling 416:108904. https://doi.org/10.1016/j.ecolmodel.2019.108904.

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as in the regulatory arena.

Keywords: energy budget, DEBtox, DEBkiss, toxicokinetic-toxicodynamic modelling, ecotoxicity, environmental risk assessment

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1. Introduction

Mechanistic or process-based models are essential tools to understand toxic effects on organisms over time, at all levels of biological organisation (Ashauer and Escher, 2010; Grimm and Martin, 2013; Jager et al., 2006). At the individual level, mechanistic effect models take the form of toxicokinetic-toxicodynamic (TKTD) models. For effects on survival, the leading framework is the General Unified Threshold model for Survival (GUTS, Jager et al., 2011; Jager and Ashauer, 2018b). For sub-lethal effects, the only TKTD models with any track record in ecotoxicology are based on Dynamic Energy Budget (DEB) theory (Jusup et al., 2017; Kooijman, 2010). Various practical models have been derived from DEB theory for ecotoxicological applications, ranging from the full 'standard animal model' to several simplified versions (see overview in Jager et al., 2014). In general, these models can be referred to as DEBtox, though this term is usually reserved for the simplified DEB models that are specifically intended for analysis of laboratory ecotoxicity tests. These simplified DEBtox models have a considerable history, starting in 1996 with the presentation of a series of equations to analyse standard test results for *Daphnia magna* reproduction (Kooijman and Bedaux, 1996b) and fish growth (Kooijman and Bedaux, 1996a). This version also made it into regulatory guidance (OECD, 2006) under the heading of 'biology-based methods'.

Simplified DEBtox models have been very successful in explaining effects on multiple endpoints over the life cycle of an animal (see e.g., Jager et al., 2006). More recently, limited updates to the equations have been presented (Billoir et al., 2008; Jager and Zimmer, 2012), correcting some small errors in the original formulation but keeping the core of the model intact. However, three recent developments prompt a more thorough overhaul: the establishment of the GUTS framework for survival modelling (Jager et al., 2011; Jager and Ashauer, 2018b), the development of reserve-less DEBkiss models (Jager, 2018; Jager et al., 2013), and the concrete interest in using DEBtox for environmental risk assessment (ERA) of pesticides (EFSA, 2018).

The GUTS framework unifies virtually all TKTD models for the endpoint

survival, and has been judged "ready for use" by the European Food Safety Authority (EFSA) for risk assessment of pesticides in Europe (EFSA, 2018). GUTS does not only unify different models, it also unifies modellers by providing a common language and a common layout for TKTD modelling. For example, it explicitly distinguishes between toxicokinetics (TK) and damage dynamics. The internal concentration leads to (generally abstract) damage, which is repaired at a certain rate. It is the damage that ultimately drives the toxic effect. GUTS models can still be formulated to work in the absence of measured body residues, in the form of so-called reduced models. DEBtox models have always (implicitly) assumed that the property driving the toxic effect is the internal concentration, but the experience with GUTS has shown that this position is not tenable anymore.

DEBkiss is a simplified DEB model framework for animals that completely removes the reserve compartment; assimilates obtained from food are directly used to fuel metabolism, without any buffering. Even though DEBkiss was formulated as a consistent framework rather recently (Jager et al., 2013), reserve-less models are actually at the basis of DEB theory and its application in ecotoxicology (Kooijman and Metz, 1984). Removing the reserve simplifies the model and its application considerably, while still capturing the life history of many species. In the classical DEBtox models (Billoir et al., 2008; Jager and Zimmer, 2012; Kooijman and Bedaux, 1996b), reserve has always played a hidden role, usually in immediate steady-state with the food availability, and requiring an abstract parameter g that could not be fitted on the data (and was fixed to a 'not unreasonable' value). The demonstrated usefulness of DEBkiss (see list of publications at `http://www.debtox.info/debkiss_appl.html`) makes this framework a prime candidate as basis for an updated simplified DEBtox model.

TKTD models, including DEBtox, are gaining increasing interest for ERA of pesticides in Europe, with as most recent milestone the 2018 EFSA scientific opinion on TKTD models (EFSA, 2018). Even though DEBtox was judged not to be ready for use yet, its potential for application in ERA was recognised. It is good to note that the EFSA opinion does not single out a specific DEB model version, but it does state: "To facilitate wider use, DEBtox models should be made more accessible to non-advanced model users ... by promoting the use of simplified versions when applicable." Clearly, there is regulatory interest in simplified model versions. Several problematic issues were identified in the opinion, hampering acceptance of DEBtox; mainly a lack of suitable case studies (for pesticides and aquatic organisms,

with extrapolation to pulsed exposure scenarios), and a lack of user-friendly software. Furthermore, the EFSA opinion stresses the need for a separate evaluation of the physiological part of the model for each relevant species. This latter point seems to be rooted in a confusion about DEB model versions. The standard (non-simplified) DEB animal model can be used for ERA (as proposed by Baas et al., 2018; Zimmer et al., 2018) but cannot be parameterised completely from laboratory ecotoxicity data; species-specific defaults for the basic life history need to be taken from an on-line parameter collection (Marques et al., 2018). Clearly, such defaults would require regulatory evaluation. However, simplified DEBtox models do not need such defaults, and can usually be parameterised using only the results of a toxicity test (as long as observations on growth and reproduction over time are available).

The lack of pesticide-relevant case studies is, however, a more serious issue, and relates to an important weakness: almost all DEBtox applications have so far been performed with (presumed) constant exposure. The only examples with a pesticide and time-varying exposure that I am aware of is a study for *D. magna* exposed to the insecticide fenvalerate (Pieters et al., 2006). This study did show that DEBtox can be fitted to the effect patterns for a single pulse early in life, but this is insufficient basis for extrapolation to all kinds of exposure scenarios, for all life stages. In principle, TKTD models should be able to deal with time-varying exposure, in fact, that feature is one of the main drivers for their development and application (Ashauer and Escher, 2010; Jager et al., 2006). However, time-varying exposure requires some thought in the DEBtox context, as it can easily lead to a starvation situation (even in presence of abundant food). Toxicant-induced starvation can occur when a toxicant affects the assimilation rate or the maintenance costs of an organism. When we expose a fully-grown adult to such a toxicant, its assimilation rate will not be sufficient to cover its maintenance needs, which represents a starvation situation (the animal has to deviate from the basic rules for the energy budget). For a juvenile, this problem is less acute: it can decrease its growth rate and thereby free energy for maintenance. This specific starvation problem for adults will be relevant for time-varying food availability as well (see discussion in Van der Meer, 2016). Clearly, DEBtox would benefit from a dedicated and consistent starvation approach, which it currently lacks.

In this contribution, I present an updated DEBtox model, based on the DEBkiss framework, and consistent with the recent developments for GUTS.

To allow the model to operate consistently with time-varying conditions (food availability and toxicant exposure), I will also present a simple preliminary starvation module. This module is internally consistent (i.e., respects mass balance in the animal) but requires more detailed testing. The use of the model will be illustrated with a simple data set for constant exposure. However, in the near future, more useful data sets (with time-varying exposure) will become available, allowing for a more serious evaluation of the model's performance in extrapolating between different exposure situations. The presented DEBtox model is intended for use in ecotoxicological studies in general, and may find applications in science as well as in the regulatory arena.

2. Model description

The DEBkiss framework is described in detail elsewhere (Jager, 2018; Jager et al., 2013). However, a summary, as relevant for the current application, is provided in the supporting information. The supporting information also contains the full derivation of the model in compound parameters; below, only the final equations will be shown. The symbols used are explained in Table 1.

For model application in ecotoxicology and risk assessment, a few simplifications are generally justified, which allow deriving a compact model in the form of compound parameters. Primary parameters are the, rather abstract, parameters that are directly linked to metabolic processes (e.g., the volume-specific costs for somatic maintenance). Compound parameters are easy-to-interpret combinations of primary parameters (e.g., maximum body length and the von Bertalanffy growth rate constant) that have a direct link to measurable properties. The simplifications proposed are to focus on the juvenile/adult stages (i.e., ignore embryos), treat reproduction as a continuous process (rather than considering clutches of eggs and a reproduction buffer), and ignore the details of the feeding process (treat the scaled functional response as a parameter f between 0 and 1). Furthermore, I propose to include the process of maturity maintenance by default, but link it to the somatic maintenance rate in a specific manner to ensure that body size is a good proxy for maturity status (Jager and Zimmer, 2012). Finally, toxicant stress is limited to four metabolic processes: assimilation, maintenance (somatic and maturity), growth and reproduction. The mass flows of the resulting model are shown in Figure 1.

Symbol	Explanation	Unit	Value
	Variables and forcings		
C_w	external concentration (here food)	mg/kg	0-20 (n.e.)
D	scaled damage level	mg/kg	
f	scaled functional response (0-1)	–	1 (n.e.)
h	hazard rate	1/d	
L	animal body length	mm	
R	continuous reproduction rate	eggs/d	
R_c	cumulated continuous reproduction	eggs	
s_*	stress factor for specific process	–	
x_*	feedback factor for specific process	–	
	Basic parameters		
h_b	background hazard rate	1/d	2.65 (1.14-5.12) 10^{-3}
L_0	initial body length	mm	0.122 (n.e.)
L_m	maximum body length	mm	0.666 (0.654-0.678)
L_p	body length at puberty	mm	0.409 (0.399-0.424)
r_B	von Bertalanffy growth constant	1/d	0.0501 (0.0466-0.0539)
R_m	maximum reproduction rate	eggs/d	22.2 (20.6-24.3)
	Toxicity parameters		
b_b	effect strength energy budget	kg/mg	1.94 (1.03-4.34)
b_s	effect strength survival	kg/mg/d	4.63 (2.71-7.10) 10^{-3}
k_d	dominant rate constant	1/d	0.233 (0.155-0.398)
$\mathbf{S}$	vector for configuration pMoA	–	[0,0,0,1]
$\mathbf{X}$	vector for configuration feedbacks	–	[0,0,0,0]
z_b	threshold energy budget	mg/kg	8.60 (7.89-8.99)
z_s	threshold survival	mg/kg	6.71 (4.04-8.13)
	Extra parameters (losses with repro only)		
K_{RV}	partition coeff. egg-structure	kg/kg	1 (n.e.)
F_{BV}	egg dwt relative to structural dwt	kg/kg	0.007 (n.e.)
	Extra parameters (starvation only)		
y_P	product of yield factors starvation	–	0.64 (n.e.)
κ	allocation fraction to soma	–	0.8 (n.e.)

Table 1: Explanation of symbols used in the text, and parameter estimates from the fit in Figure 3. Several parameters are fixed (n.e. is not estimated), either to general defaults or to species-specific estimates (see supporting information). Note that starvation and losses with reproduction were not used for the model fit in Figure 3.

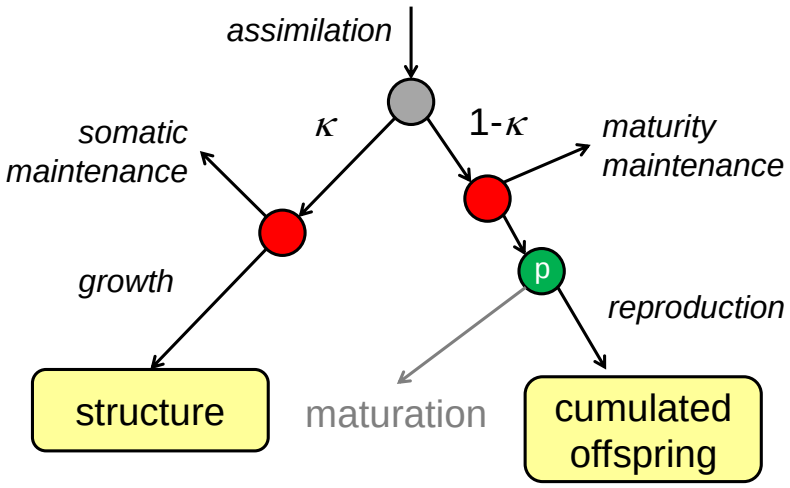

Figure 1: Schematic representation of the basic DEBkiss model used for analysis of ecotoxicity data. Yellow blocks are state variables. The grey circle denotes the κ-rule; a fixed split of assimilates between the soma and maturation/reproduction. Red circles denote primacy of maintenance over downstream processes. The green node 'p' marks a switch in allocation at puberty (start of offspring production).

2.1. Toxicokinetics and damage dynamics

Previous DEBtox models have largely ignored the damage concept and used the (scaled) internal concentration as driver for the toxic effect. The developments in the GUTS framework have shown that damage needs to be considered, and this property has now received a central position in that context (Jager and Ashauer, 2018b). Furthermore, in linking adverse-outcome pathways (AoP) to DEB models, damage was identified as integrating concept (Murphy et al., 2018). GUTS applies a generic damage compartment with first-order kinetics, linked to a one-compartment TK model. However, in the absence of information on TK (e.g., measured body residues), one will need to make use of a reduced model, which is simplified by combining TK and damage dynamics into a single compartment. The same strategy can be followed for DEBtox (see Figure 2). Below, I will only outline the equations for the reduced model, as DEBtox will usually be applied in situations without access to detailed TK information (the full model is provided in the supporting information).

For a non-growing and non-reproducing organism, scaled damage follows

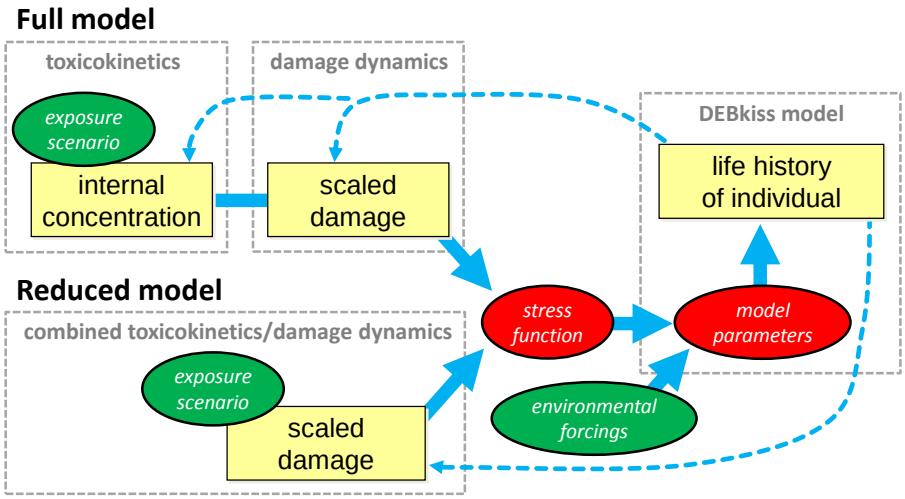

Figure 2: Schematic representation of the updated DEBtox model. The DEBkiss model is linked to scaled damage via a stress function. In the full model, there are separate compartments for toxicokinetics and damage, whereas in the reduced model, these two processes are combined in one compartment. Broken arrows represent feedbacks from the life-history traits to TK/damage dynamics (e.g., growth dilution).

simple first-order kinetics:

$$\frac{d}{dt}D = k_d(C_w - D) \tag{1}$$

Damage is scaled and has the unit of the external concentration. This equation only has a single parameter: the dominant rate constant k_d. Depending on the relative speed of the damage dynamics and TK, k_d needs to be interpreted as a TK elimination rate or as a damage repair rate (Table 2), or even as a combination of both (if both processes have a similar speed). For reduced GUTS models, in either case the same equation applies; the main reason for calling this state variable scaled damage instead of scaled internal concentration is in the interpretation of the rate constant (k_d estimated from toxicity data is not necessarily a TK elimination rate). However, in the context of DEBtox, the implications are more substantial. In this setting, animals will inevitably be growing and reproducing, which affects scaled damage dynamics (certainly when it is driven by TK). The life-history traits of the DEBkiss model are thus feeding back to the scaled damage module (Figure 2). This setup can become quite complicated as environmental stress (e.g., food limitation) will influence these feedbacks and thereby interact with

	Slow TK, fast damage repair	**Fast TK, slow damage repair**
Damage represents	An internal concentration of the parent compound, or a metabolite, at a target site	Some form of accumulated damage (generally kept abstract)
Feedback mechanism		
Surface:volume scaling of uptake (x_u)	Likely relevant, unless toxicity is driven by a metabolite whose formation is dominated by biotransformation	Likely irrelevant as damage accrual would not generally be surface-related
Surface:volume scaling of elimination (x_e)	Likely relevant, unless toxicity is driven by a chemical whose 'elimination' is dominated by biotransformation	Likely irrelevant as damage repair would not generally be surface-related
Growth dilution (x_G)	Always relevant due to the conservation laws for chemical mass	Relevance unknown; likely depends on the type of damage produced
Losses with reproduction (x_R)	Likely relevant, unless the chemical is not transferred to eggs	Unlikely to be relevant; even if damage is transferred to offspring, this is unlikely to represent a 'repair process' from the mother's perspective

Table 2: Explaining damage and the relevance of the different feedback mechanisms, depending on the relative speed of toxicokinetics (TK) and damage dynamics.

the toxicity. Furthermore, chemicals may affect growth and reproduction (see next section) and thereby affect their own damage dynamics again.

Three forms of feedback need to be considered: changes in the surface:volume ratio of the organism, growth dilution, and elimination with reproduction. The classical DEBtox models have focussed on the first two and have ignored the latter (probably because it needs additional parameters). When damage repair is fast (an implicit assumption in the classical DEBtox models), the damage equation represents TK, and these feedback processes generally make sense (Table 2). However, when TK is dominated by biotransformation, things already become more complex as biotransformation rates are unlikely to be governed by the surface:volume ratio. When damage repair is slow, the scaled-damage equation represents damage dynamics, and the realism of all these feedback processes is not so obvious anymore (Table 2). Given these complexities, I propose the following generic equation for scaled damage:

$$\frac{d}{dt}D = k_d(x_u C_w - x_e D) - (x_G + x_R)D \tag{2}$$

In this equation, the feedback processes can be modified by setting the four factors x_* to an appropriate value. Note that there are separate factors x_u and x_e for surface:volume scaling of uptake and elimination, respectively. Growth dilution and losses through reproduction each have their factor (x_G and x_R) that works in the same way (as an 'elimination' process, which is why they can be added).

The factors x_* should receive very specific values, when the associated process is deemed relevant, and those values will change over time. As a practical implementation, I propose to use a vector $\mathbf{X}$ with four switches: set to 1 when a feedback process is included, and 0 when it is excluded. The specific factors x_* of Eq. 2 can then be derived in the following manner:

$$[x_u, x_e, x_G, x_R] = \mathbf{X} \circ \left[\frac{L_m}{L}, \frac{L_m}{L}, \frac{3}{L}\frac{d}{dt}L, R\, F_{BV} K_{RV}\right] \tag{3}$$

$$x_u \to \max(1, x_u) \quad x_e \to \max(1, x_e) \tag{4}$$

The circle stands for element-wise multiplication (Hadamard product). For example, the factor for growth dilution (x_G) is derived by multiplying the third element of $\mathbf{X}$ (either 0 or 1) with the third element of the right-hand

vector in Eq. 3 (the relative volumetric growth rate). The 'max' operator ensures that x_u and x_e are set to one when their designated switch is set to zero.

The vector on the right-hand side of Eq. 3 contains the standard formulations for surface:volume scaling and growth dilution (Jager and Zimmer, 2012). To include losses with reproduction, two new parameters are introduced: the egg dry weight as fraction of the mother's dry weight (F_{BV}), and a partition coefficient for damage between egg material and structure (K_{RV}). This formulation follows from the assumption that the toxicant (or its damage) is transported with the flow of biomass (based on dry weight) to the eggs. It should be noted that F_{BV} will not generally be constant over time. However, starting with a reasonable fixed value is a good strategy for investigating the potential impact of losses with egg production.

Different settings for the vector $\mathbf{X}$ may be appropriate for different species-chemical combinations. The classical DEBtox models follow from $\mathbf{X} = [1, 1, 1, 0]$, and this has been used (without much discussion) in most published cases. However, for damage-dominated dynamics $\mathbf{X} = [0, 0, 0, 0]$ (as also used in GUTS) may be a better starting point; this configuration was for example used for the case study in the EFSA opinion (EFSA, 2018).

The simple TK equation of classical DEBtox is thus replaced by a configurable scaled-damage equation, with four switches for processes that can be set on or off. This freedom of choice in the model is not really practical, but there is currently no support for selecting one version for all situations. Standard toxicity tests (growth and/or reproduction over time under constant exposure) may not offer much possibility for distinguishing between the different possible configurations of the scaled-damage equation. The configuration will therefore also have little impact on the model fit. However, for extrapolations to untested conditions (e.g., limiting food levels or time-varying exposure), the impact may be considerable. It is thus conceivable that additional test conditions (e.g., different food levels or pulsed exposure) will provide better opportunities to select between the different options. Furthermore, additional endpoints such as survival may provide the necessary information, although this will rest on an assumption that the same form of damage drives the effects on all endpoints.

In the near future, more detailed data sets are likely to become available as DEBtox will be investigated for use in ERA. Eventually, that will provide more insight into which feedbacks are most appropriate for which species and which compound classes, and more insight in how to set up experiments to

provide information on the relevant switches. This update of the DEBtox TK equation is proposed for all DEB-based models used in ecotoxicology, including the standard DEB animal model. However, when a reserve compartment and/or reproduction buffer is included in the model, the potential effect of (the dynamics of) these compartments on TK and damage dynamics needs to be considered as well (see Van Haren et al., 1994).

2.2. Stress factor and modes of action

The scaled damage level is translated into a dimensionless stress level through a linear-with-threshold relationship:

$$s = b_b \max(0, D - z_b) \tag{5}$$

This is essentially the same equation as applied in classical DEBtox models, with one modification: the 'tolerance concentration' is replaced by its reciprocal (which is now an effect strength b_b). The reason for this (aesthetic) change in parameter definition is consistency with the survival models. It is good to stress that this linear-with-threshold relationship has little theoretical underpinning. It is practical as not all changes at the molecular or cellular level will translate into observable effects at the whole-organism level. Furthermore, a threshold allows us to ignore non-identified chemicals that are obviously present in every test system, and a linear increase makes intuitive sense (every additional molecule has the same increase in the stress level). This relationship works well in practice, and is also used in GUTS models for the hazard rate.

The threshold level is also often referred to as a no-effect concentration (Baas et al., 2018; Kooijman and Bedaux, 1996b), as it has the unit of an external concentration. However, the feedbacks in the damage equation (Eq. 2) complicate the link between the threshold parameter z_b and the maximum exposure concentration without effects on growth and reproduction. Furthermore, these feedbacks make this link dependent on environmental conditions (e.g., food availability). Therefore, z_b is best treated as a model parameter, and model simulations are needed to predict (no-)effect levels for relevant exposure conditions.

The stress level subsequently modifies the value of one or more primary model parameters. As stated above, the model in compound parameters offers access to four metabolic processes: assimilation, maintenance, growth and reproduction. In practice, changes in these processes (singly or in combination) suffice to explain the patterns observed in toxicity tests (Ashauer

and Jager, 2018). The affected metabolic process is generally referred to as a physiological mode of action (pMoA). Similarly to the feedback processes in Eq. 3, I propose to use a vector $\mathbf{S}$ with on-off switches to specify the pMoA in terms of these four metabolic processes. Specific stress factors s_* follow from this vector $\mathbf{S}$ and the value for s from Eq. 5:

$$[s_A, s_M, s_G, s_R] = s \times \mathbf{S} \tag{6}$$

$$s_A \rightarrow \min(1, s_A) \tag{7}$$

The extra operation on s_A, maximising its value to 1, is needed as s_A will be applied in the form of a linear decrease of a parameter (which should not become negative). In general, it is advisable to start from pMoA's that affect a single process, although combinations are certainly possible. For example, it seems that effects on growth costs are usually accompanied by an increase in reproduction costs (Ashauer and Jager, 2018). I here ignore the pMoA of 'hazard on reproduction' of the classical DEBtox models as the costs for reproduction is very similar and generally fits better. However, that pMoA can be easily implemented as a slightly different functional form of using s_R in the equations for growth and reproduction.

2.3. Effects on growth and reproduction

The relationship between the stress level and the primary parameter is assumed to be linear. However, there is freedom in selecting the primary parameter. For a decrease in assimilation, we can multiply the maximum specific assimilation rate by $1 - s$ or divide it by $1 + s$. The first assumes a linear decrease in the specific assimilation rate, whereas the second assumes a linear increase in the overhead costs for assimilation. Since this does not seem to be a critical decision for the model, I propose to follow the tradition in DEBtox modelling (see supporting information for details).

The resulting DEBkiss model in compound parameters, with the specific stress factors s_* included, is extremely compact. The equations for body length L and cumulative reproductive output R_c are:

$$\frac{d}{dt}L = r_B \frac{1+s_M}{1+s_G}\left(fL_m\frac{1-s_A}{1+s_M} - L\right) \quad \text{with } L(0) = L_0 \tag{8}$$

$$R(L \geq L_p) = \max\left(0, \frac{R_m}{1+s_R}\frac{fL_mL^2(1-s_A) - L_p^3(1+s_M)}{L_m^3 - L_p^3}\right) \tag{9}$$

$$R(L < L_p) = 0 \tag{10}$$

$$\frac{d}{dt}R_c = R \quad \text{with } R_c(0) = 0 \tag{11}$$

Any length measure can be used for L, as long as it is used consistently, and as long as the animal does not change shape during growth. If there are changes in shape, volumetric length (cubic root of body volume or weight, excluding any reproductive buffer or eggs) should be used. These equations are quite similar to the traditional DEBtox equations in compound parameters (Billoir et al., 2008; Kooijman and Bedaux, 1996b). The difference is that I here use real body length rather than scaled length, and the absence of the parameter g that governs the contribution of the reserve.

2.4. Adding effects on survival

Assuming that the same damage level affects both sub-lethal and lethal toxicity, a survival module from the GUTS framework can easily be added. The stochastic death version is most consistent with the approach for sub-lethal effects:

$$h = b_s \max(0, D - z_s) \tag{12}$$

$$\frac{d}{dt}S = -(h + h_b)S \quad \text{with } S(0) = 1 \tag{13}$$

This formulation matches the approach for sub-lethal effects as there is a linear-with-threshold relationship between damage and the effect on a model parameter (the hazard rate h). The alternative approach of individual tolerance is less intuitive in this context, as it has no analogue for continuous endpoints such as reproduction (which cannot be viewed as an all-or-nothing effect). An additional advantage of the stochastic-death formulation is that survival can be expressed in the form of a differential equation, which allows easy implementation in software alongside the equations for growth and reproduction.

2.5. Starvation module

Starvation may occur when we want to test, or extrapolate to, time-varying food availability. However, it is important to stress that toxicants that affect assimilation or maintenance can also induce starvation, even with *ad libitum* food provided in a laboratory setting. This is especially of concern when exposure is pulsed. Under constant exposure, individuals will generally grow to the maximum size that is sustainable under the stress conditions, and remain at that size. However, under pulsed exposure, there is the possibility for recovery, and, in between exposures, an individual can grow to a size that is too large to accommodate a subsequent stress on assimilation or maintenance.

In Eq. 8 above, dL/dt can become negative for certain body lengths L when f is low, and also when s_A or s_M are high. Even though shrinking is not unrealistic, if we simply use the same equation for growth and shrinking, the rate of shrinking will be incorrect (it implies that overhead costs paid during growth are available again when structure is being used to pay maintenance costs). Furthermore, organisms may avoid shrinking by redirecting resources from maturation/reproduction. Because DEBkiss does not have a reserve compartment, it is possible to include a consistent starvation module into the model with compound parameters as presented above. This does require two additional parameters, but reasonable defaults can be set.

The two-stage starvation strategy presented here is based on the assumption that an animal facing starvation will first modify κ in an attempt to fulfil the maintenance costs. If that is insufficient, structure is burnt to fill those needs (i.e., the animal shrinks). Starvation is triggered in the model above when dL/dt becomes negative. To keep the reproduction equation in the same form, it is practical to translate the change in κ into a virtual food level for reproduction:

$$f_R = \frac{f - \kappa \frac{L}{L_m} \frac{1+s_M}{1-s_A}}{1-\kappa} \tag{14}$$

If that food level is above zero, the $1-\kappa$ branch is sufficient to cover the maintenance needs. The f_R is used instead of f in the reproduction equation (leading to reduced egg production), and we can set dL/dt to zero (the animal can maintain its current body size). If f_R is below zero, this triggers the second stage of starvation: reproduction stops and the animal needs to

shrink. The shrinking equation in terms of compound parameters is:

$$\frac{d}{dt}L = \frac{r_B}{y_P}(1+s_M)\left(f\frac{L_m}{\kappa}\frac{1-s_A}{1+s_M} - L\right) \tag{15}$$

The parameter y_P is the product of two yield coefficients; it covers the overhead costs for building the structure in the first place (that are not recovered on shrinking), as well as the overhead costs for fuelling maintenance from structure. In the complete DEBkiss model, both yields are by default set to 0.8, so a starting value for y_P can be 0.64. Application of this equation in the model requires some careful consideration of the length measure used for L. For example, many arthropods will not be able to shrink in length due to their carapace, although they will shrink in terms of biomass.

It is important to note that κ is explicitly present in these two starvation equations, whereas it was hidden in the compound parameters of the basic model. There is, unfortunately, no way around this. When κ is high, most of the assimilation flux is already going to the soma (somatic maintenance and structural growth), which implies that there is little to gain from redirecting the $1-\kappa$ flux, and starvation will rapidly result in shrinking. An animal with a small κ can postpone shrinking for longer. The complete DEBkiss model has κ as a parameter, but to accurately identify it requires rather detailed biometric measurements on the species, such as relationships between body lengths and structural weight, dry-wet weight ratio, egg weight (e.g., Jager et al., 2013). For DEB applications, $\kappa = 0.8$ is considered a typical value (Jager et al., 2013; Kooijman, 2010), so this value may be used as a starting point. Furthermore, it may be possible to identify both κ and y_P from data on life history under a series of time-varying food scenarios; since these parameters are not chemical specific, they (in principle) need to be derived only once for a species.

This module represents just one plausible scenario for an organism to respond to starvation. Clearly, more strategies are possible, and different strategies will be needed for different species. For example, some species may be able to reduce their maintenance costs under starvation, or use their reproduction buffer (which is not considered in the present model). In any case, shrinking clearly cannot continue indefinitely; in practice, a limit to the amount of shrinking (or to the minimum body size) will be needed. Another thing to consider is that shrinking will lead to the reverse of growth dilution in the scaled-damage equation: concentration by shrinking. If growth dilution is included in Eq. 2, shrinking will lead to an increase in damage, which

will increase toxicity. For pMoAs that include effects on assimilation and maintenance, this turns into a positive feedback loop. Whether this also happens in real life remains to be investigated.

3. Case study

To illustrate the model and its application to experimental data, I provide a case study for the soil-dwelling springtail *Folsomia candida* exposed to chlorpyrifos in food. This data set was analysed earlier with the classical DEBtox model, amended with a receptor-kinetics module (Jager et al., 2007). Therefore, the toxicity parameters are not directly comparable between both analyses. The data set is truncated for survival and reproduction as these endpoints are affected by senescence later in life; a process that is not included here.

the model is fitted to data for body size, reproductive output, and survival simultaneously, by optimising an overall likelihood function. For survival, the likelihood contribution is calculated from the multinomial distribution (Jager et al., 2011). For body length and cumulative reproduction, the likelihood function is based on the normal distribution, after square root transformation (Jager and Zimmer, 2012). The observations on body length and cumulative reproduction are treated as independent, which is inappropriate as the same cohort of animals is followed over time. Unfortunately, there is no simple solution to deal with dependence. The confidence intervals will suffer most and therefore need to be treated as approximate. All fits were made with the BYOM platform in Matlab, using the DEBtox2019 package (which also includes a script to redo this case study): `http://www.debtox.info/byom.html`.

As a first step, five basic parameters are fitted to the two control treatments (regular and solvent control): L_p, L_m, r_B, R_m and h_b (parameter values with likelihood-based 95% confidence intervals in Table 1). Initial length L_0 was kept fixed (see supporting information). These basic parameters were subsequently kept fixed when fitting the toxicity parameters on the complete data set. This two-stage strategy ensures that the control fit is not compromised to compensate for a structural misfit in the treatments. Furthermore, fitting and analysing a model with 10 free parameters is technically challenging. Fitting only the five toxicity parameters makes it easier to run and compare a series of different model configurations (different damage configurations and different pMoAs). However, this two-stage procedure requires

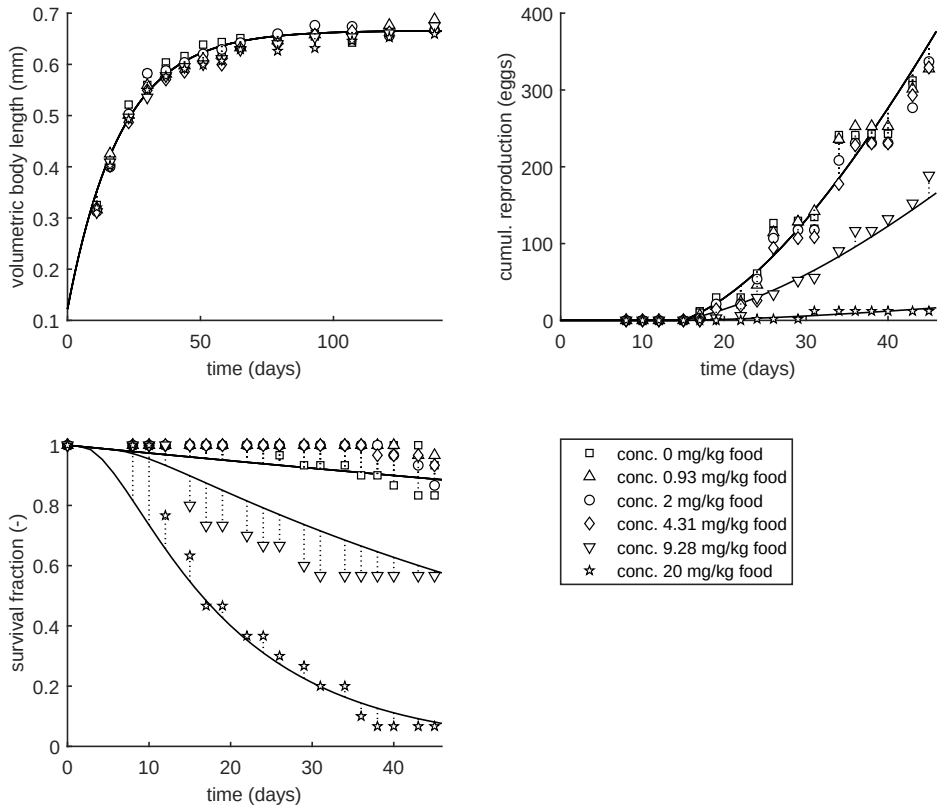

Figure 3: Best fit of the updated DEBtox model on the data for *Folsomia candida* exposed to chlorpyrifos in food. All data fitted simultaneously, using the pMoA 'costs for reproduction' and damage configuration without any feedbacks. Note the much longer time axis for the body-length data.

a good-quality control data set.

For this data set, there is no need to fit all possible pMoAs; as there is hardly any effect on body size, it is clear that this compound affects reproduction only ($\mathbf{S} = [0, 0, 0, 1]$). That still leaves a series of options for the damage configuration $\mathbf{X}$. Technically, there are 16 permutations of $\mathbf{X}$, although not all of them are equally plausible. Here, I fitted the four most obvious candidates (see Table 3). The configuration with most support is the fit without any feedback processes ($\mathbf{X} = [0, 0, 0, 0]$). This fit is shown in Figure 3 with the parameter values in Table 1 (including likelihood-based 95% confidence intervals). A damage configuration without feedbacks is perhaps not surprising, given that the compound is an acetyl-cholinesterase inhibitor. It is likely

Scaled damage representation	**X**	fit (MLL)	ΔAIC
Damage that is not diluted	[0,0,0,0]	930.2	0
Full TK (incl. losses with repro.)	[1,1,1,1]	932.5	4.7
Damage that is diluted	[0,0,1,0]	936.7	13
Classical DEBtox TK	[1,1,1,0]	938.3	16

Table 3: Four typical configurations for the damage module, sorted according to their corresponding model fit on the data set for the case study. Relative goodness-of-fit shown as minus log-likelihood (MLL) and difference in Akaike Information Criterion relative to the best fit (ΔAIC). All fits made with the pMoA for costs of reproduction.

that the scaled damage represents inhibited enzymes (which is why a receptor model was used in Jager et al., 2007), or the damage resulting from such an inhibition. However, a stronger identification of the most-likely damage configuration would be possible with more detailed experimental data (e.g., experiments at different food levels and/or with time-varying exposure).

Looking more closely at the fit, there seems to be some toxic effect on growth as well. Even though it is clearly insufficient to warrant one of the other single pMoAs, some care is needed. Since body size affects reproduction, as well as damage dynamics through the feedbacks (depending on the configuration of **X**), a good fit for the body-size data is important for a consistent overall model analysis.

4. Discussion

In view of the recent developments with GUTS and DEBkiss, and the current regulatory interest in TKTD models, an update of DEBtox is urgently required. In this contribution, I propose an updated set of simplified model equations, using easy-to-interpret compound parameters. In my opinion, TKTD models for practical applications need to be as simple and transparent as possible, especially if they (or model results) are to be used by non-experts. The proposed DEBtox update is simple enough to be parameterised entirely from a (somewhat extended) toxicity test; hence, there is no need for relying on species-specific default values. It is good to note that strict application of defaults for the basic parameters will be impossible in practice, as there are substantial differences in control response, for the same species, between laboratories and even between tests. For a proper analysis of life-history traits under stress, it is crucial that the model provides a close correspondence to the control, and therefore, some adaptation of the basic parameters to the

specific test situation will always be needed. The ability to use only the test data for model parameterisation simplifies data analysis, and also keeps the application of DEBtox in line with the way GUTS is used. Consistency with GUTS is further advanced by the move to use damage as the driver for toxic effects. In fact, the GUTS-SD model now results as a special case of DEBtox, for an animal that is not growing and not reproducing.

New modules have been derived to deal with losses due to reproduction and starvation (both toxicant-induced and due to food limitation). These additional modules require additional parameters, but these are only needed when these processes are deemed relevant (which will not be for all cases), and default values can be used as a starting point. The extended damage equation provides a flexible way of testing different feedbacks from the life-history traits to damage dynamics. This equation may also prove to be useful in other TKTD models (e.g., in GUTS for growing animals, and for the standard DEB model). Additional cross-fertilisation seems possible with individual-based population models, where a simple DEBtox module could serve as the individual-level module. For ERA applications, such a modular approach may be of particular interest: using the same module at different levels of biological organisation is efficient, provides consistency in risk assessment, and facilitates quality evaluation (see Jager and Ashauer, 2018a).

Of course, any simplification will come at a price. A specific disadvantage of models formulated with compound parameters is that the proposed set of equations cannot easily be extended, for example to include the embryo stage, a reproduction buffer, toxic effects on κ, following maturity (e.g., when L_p is not constant), or to predict (effects on) respiration rates. To this end, non-simplified models in primary parameters, such as standard DEB or DEBkiss, are needed. Application of these models, phrased in primary parameters, might be facilitated by working with compound parameters as inputs, which are then internally translated into primary parameters (see supporting information for the DEBkiss case). The DEBtox model presented here removes elements from DEB theory such as the reserve and maturity, which will have consequences for model behaviour. Whether this matters will depend on the purpose for which the model is to be used. However, for ecotoxicological applications, it is my expectation that the choice for a relevant TK/damage configuration will generally outweigh the choice of DEB model. Unfortunately, almost all case studies with DEB models and toxicants have so far been performed under constant exposure and constant environmental conditions. Therefore, there is currently little opportunity to

specify the limitations of the various models.

The main bottleneck for routine application of DEB-based models in ecotoxicology and ERA likely lies in the availability of suitable toxicity testing on life-history traits (see Ashauer and Jager, 2018). Toxicity tests (even those for scientific studies) are generally based on standard test protocols for regulatory purposes, which have clearly not been developed with the needs of TKTD modelling in mind. Proper parameterisation and testing of these models requires experiments of a design that is not restricted by the (rather arbitrary) rules of standard test protocols. Finally, in terms of testing strategy, it is important to stress that DEB-based data analyses usually highlight specific uncertainties in the data, uncertainties that require additional experimental work (that can then be limited in scope to elucidate a specific aspect). Therefore, it is not just the DEBtox model that needs to be updated: also the all-too-common mindset of doing the experiments first, and then dropping the data on the modeller's desk, needs to be changed to a more interactive and cyclical process.

5. Declaration of Competing Interest

None.

6. Acknowledgements

I would like to thank Roman Ashauer and Neil Sherborne for constructive comments on a draft of the manuscript. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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