Supporting Information for:
Revisiting simplified DEBtox models for analysing ecotoxicity data

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1 Basic DEBkiss model

The model description in this section is extracted from the full DEBkiss framework [12, 8]. It is not a complete description of the framework but focuses on the post-embryonic stages and on continuous reproduction (no reproduction buffer), as schematically shown in Figure 1. Furthermore, I include maturity maintenance here by default, and ignore details of the feeding process (the scaled functional response $f$ designates the food availability). The derivations in this section (including the model in compound parameters) are presented (in more detail) in the freely-available e-book [8]. This supporting information is meant to be readable as a standalone description of the model. Therefore, there is some overlap with the main text. Both the model version in compound parameters and the one in primary parameters are implemented into Matlab using the BYOM platform: http://www.debtox.info/byom.html (see the DEBtox2019 package).

Figure 1: Schematic diagram of the mass flows in a juvenile/adult DEBkiss animal. The parameter symbols are explained in Table 1. The node $p$ denotes the switch at puberty (start of reproductive investment; juvenile to adult). The other nodes represent a split of the assimilation fluxes. Maturation is shown in grey: this flux is not followed here but used as (implicit) sink for the flux $J_R$ before puberty is reached.

1.1 Symbols for the basic model

Set of symbols for the basic model, as relevant for this paper (subset of the symbol set for the complete DEBkiss model).
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_{Am}$</td>
<td>maximum area-specific assimilation rate</td>
<td>$ma/(l^2t)$</td>
<td>–</td>
</tr>
<tr>
<td>$J_M^v$</td>
<td>volume-specific maintenance costs</td>
<td>$ma/(l^2t)$</td>
<td>–</td>
</tr>
<tr>
<td>$W_{BO}$</td>
<td>assimilates in a single freshly-laid egg</td>
<td>$ma$</td>
<td>–</td>
</tr>
<tr>
<td>$L_p$</td>
<td>volumetric length at puberty</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$y_{AV}$</td>
<td>yield of assimilates on structure (starvation)</td>
<td>$ma/m$</td>
<td>0.8 mg/mg (dwt)</td>
</tr>
<tr>
<td>$y_{BA}$</td>
<td>yield of egg buffer on assimilates</td>
<td>$ma/ma$</td>
<td>0.95 mg/mg (dwt)</td>
</tr>
<tr>
<td>$y_{VA}$</td>
<td>yield of structure on assimilates (growth)</td>
<td>$m/ma$</td>
<td>0.8 mg/mg (dwt)</td>
</tr>
<tr>
<td>$\kappa$</td>
<td>fraction of assimilation flux for soma</td>
<td>–</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Conversions

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_V$</td>
<td>dry-weight density of structure</td>
<td>$m/l^3$</td>
<td>0.1-0.2 mg/mm$^3$</td>
</tr>
<tr>
<td>$\delta_M$</td>
<td>shape correction coefficient</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

### Fluxes and state variables

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$J_A$</td>
<td>mass flux for assimilation</td>
<td>$ma/t$</td>
<td>–</td>
</tr>
<tr>
<td>$J_I$</td>
<td>mass flux for maturity maintenance</td>
<td>$ma/t$</td>
<td>–</td>
</tr>
<tr>
<td>$J_M$</td>
<td>mass flux for somatic maintenance</td>
<td>$ma/t$</td>
<td>–</td>
</tr>
<tr>
<td>$J_R$</td>
<td>mass flux to reproduction buffer</td>
<td>$ma/t$</td>
<td>–</td>
</tr>
<tr>
<td>$J_V$</td>
<td>mass flux for structure</td>
<td>$m/t$</td>
<td>–</td>
</tr>
<tr>
<td>$R_c$</td>
<td>cumulative continuous reproduction</td>
<td>#</td>
<td>–</td>
</tr>
<tr>
<td>$W_V$</td>
<td>mass of structural body</td>
<td>$m$</td>
<td>–</td>
</tr>
</tbody>
</table>

### Other output and compound parameters

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f$</td>
<td>scaled functional response (0-1)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>$L$</td>
<td>volumetric body length</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$L_0$</td>
<td>initial volumetric body length</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$L_m$</td>
<td>maximum volumetric body length</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$L^M$</td>
<td>physical body length</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$L^M_s$</td>
<td>physical body length at specific stage</td>
<td>$l$</td>
<td>–</td>
</tr>
<tr>
<td>$r_B$</td>
<td>von Bertalanffy growth rate constant</td>
<td>$1/t$</td>
<td>–</td>
</tr>
<tr>
<td>$R$</td>
<td>continuous reproduction rate</td>
<td>#/t</td>
<td>–</td>
</tr>
<tr>
<td>$R_m$</td>
<td>maximum continuous reproduction rate</td>
<td>#/t</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 1: Explanation of symbols for the basic model, with dimensions given in mass ($m$ for body, $ma$ for assimilates), length ($l$ for organism), numbers (#), time (t). Suggested values for the yields (apart from $y_{AV}$) based on the typical values in [16].
1.2 Model in primary parameters

The basic model has two state variables: body dry weight $W_V$ and cumulative reproductive output $R_c$. Reproduction itself is a rate, but it is easier to compare to observations when cumulated (and treated as a state variable).

\[
\frac{d}{dt} W_V = J_V \quad \text{with} \quad W_V(0) = d_V L_0^3
\]

\[
\frac{d}{dt} R_c = R \quad \text{with} \quad R = \max\left(0, \frac{y_{BA}}{W_{B0}} J_R\right) \quad \text{and} \quad R_c(0) = 0
\]

The ‘max’ function on the reproduction rate $R$ is needed as the reproduction flux $J_R$ may become negative under time-varying food or stress levels.

The mass fluxes $J_*$ are calculated from body size expressed on the basis of volumetric length: the cubic root of body volume. The state variable for body dry mass relates to volumetric length as follows:

\[
L^3 = \frac{W_V}{d_V}
\]

The mass fluxes follow a simple set of rules. The assimilation flux is proportional to length squared (and a scaled functional response $f$), both maintenance fluxes are proportional to length cubed, but maturity maintenance $J_J$ does not increase further after puberty ($L \geq L_p$). As an appropriate simplification, the specific maturity maintenance is related to the specific somatic maintenance with a relationship that provides a close link between investment in maturity and body size (and hence allows using body size as a trigger for puberty). A fraction $\kappa$ of the assimilation flux goes to the soma, from which somatic maintenance is paid first; the remainder is used for structural growth with a certain yield factor $y_{VA}$. The $1 - \kappa$ fraction of assimilation is used for maturation (before puberty) and reproduction (after puberty). From the mass flux allocated to reproduction, maturity maintenance costs are paid first. In equations:

\[
J_A = f J_{A_m}^o L^2
\]

\[
J_M = J_M^o L^3
\]

\[
J_J = \frac{1 - \kappa}{\kappa} J_M^o \min(L^3, L_p^3)
\]

\[
J_V = y_{VA}(\kappa J_A - J_M)
\]

\[
J_R = (1 - \kappa) J_A - J_J \quad \text{(if} \quad L < L_p \text{then} \quad J_R = 0)
\]

If the flux allocated to the soma ($\kappa J_A$) is smaller than the maintenance needs ($J_M$), the animal encounters starvation. In the model equations above, the growth flux $J_V$ would become negative and the animal would shrink. In itself, shrinking is not unrealistic, but the way in which the animal shrinks, if we simply follow the equations specified above, violates basic thermodynamics. The assimilate flux available for growth ($\kappa J_A - J_M$) is
translated into structural biomass with an efficiency $y_{VA}$ that is smaller than one (in a chemical transformation there will always be overhead costs). Simply allowing this equation to become negative implies that structure is burnt to pay maintenance but that the overhead costs magically become available again to cover the maintenance needs as well (i.e., the yield of assimilates on burning structure would be larger than one). A separate issue is that it is biologically not very realistic for an animal to burn its structure at the first sign of starvation. It seems more likely that resources will be diverted from maturation/reproduction (the $1 - \kappa$ flux) to make sure maintenance needs are paid. A simple starvation module would thus consist of two stages. In the first stage, the total assimilation flux $J_A$ is enough to pay somatic maintenance costs, so the $1 - \kappa$ flux can be reduced (leading to a reduction or even a halt of reproduction). In the second stage, even the complete flux $J_A$ is insufficient to pay somatic maintenance costs, and the animal needs to shrink (using structure to cover somatic maintenance needs). In equations:

$$J_A \geq J_M : \quad J_V = 0, \text{ and for adults } J_R = \max(0, J_A - J_M - J_J)$$ \hspace{1cm} (9)

$$J_A < J_M : \quad J_V = \frac{(J_A - J_M)}{y_{AV}} \text{ and } J_R = 0$$ \hspace{1cm} (10)

Note that shrinking is governed by a new yield factor $y_{AV}$ that is smaller than one. This ensures that 1 mg of structure yields < 1 mg of assimilates to be used for maintenance.

The $1 - \kappa$ flux is not only used for reproduction, but also for maturity-maintenance costs. The present formulation assumes that maturity maintenance is paid for as long as possible from the $1 - \kappa$ flux, at the expense of reproduction. However, when the $1 - \kappa$ flux is insufficient to pay maturity maintenance, this flux can be reduced, and I ignore the potential consequences here. For juveniles, the $1 - \kappa$ flux is used for maturation, which is not explicitly followed in this simplified model. Here, I assume that the investment in maturation can be reduced to pay somatic maintenance costs, without further consequences (e.g., $L_p$ is unaffected). The extent to which this holds in reality remains to be investigated.

In the real world, starvation responses (and recovery after starvation) can be considerably more complex. For example, animals may be able to reduce their maintenance costs to some degree, or use structure to continue fuelling reproduction. Furthermore, I focussed on continuous reproduction, and thereby excluded a reproduction buffer. Such a buffer may easily be used to fuel maintenance needs under starvation.\(^1\) However, the present set of rules provides a good starting point.

In many cases, it is not the body dry mass $W_V$ that is measured in ecotoxicity test but some practical length measure (e.g., for Daphnia the distance between the eye and the base of the spike). Such a physical length measure $L_M$ can be related to the state variable $W_V$ by using the dry-mass density $d_V$ and a shape-correction coefficient $\delta_M$:

$$L_M = \frac{L}{\delta_M} \quad \text{where } L^3 = \frac{W_V}{d_V}$$ \hspace{1cm} (11)

\(^1\)For example, the results of [18] indicate that lipid droplets in *Daphnia magna* play the role of a reproduction buffer. Therefore, starvation time in adults depends on their position in the moult cycle at the start of starvation.
These two additional conversion parameters are species specific (and the shape corrector also depends on the type of length measure taken).

1.3 Model in compound parameters

The basic model, as presented in the previous section, uses primary parameters: parameters that are directly linked to a metabolic process. Such a direct link has advantages, for example when we want to apply a stressor on a process. However, there are also disadvantages. Firstly, the primary parameters are rather abstract, and their absolute value difficult to interpret. This also makes it difficult for users to come up with starting values for a fit, or to spot errors in the model output. Secondly, the model parameters are affected by the choice of the conversion factors \( \delta_M \) and \( d_V \). For many organisms, it will be tough to define these factors in a satisfactory manner. For daphnids, for example, this would require measurements of animal dry and wet weight, without contributions from eggs or reproduction buffer (we need values for structural biomass only). The e-book [8] includes an attempt to provide reasonable estimates for \textit{Daphnia magna}.

Fortunately, the value of these conversion factors is mainly relevant for the absolute value of the primary parameters, and not for model behaviour (with some notable exceptions). Therefore, it is possible to rewrite the model using compound parameters (easy-to-understand parameters that are themselves functions of primary ones). This trick underlies the original DEBtox models [15, 14, 2], but here I apply it to DEBkiss as well (which is simpler than for the original DEBtox models, which was derived from standard DEB with reserve compartment).

To derive an equation for changes in body length \( (dL/dt) \), we have to fill in the growth equation (Eq. 1) with the fluxes \( J_V \), \( J_A \) and \( J_M \) (note that \( L^3 = W_V/d_V \) and that I apply the chain rule for differentiation):

\[
\frac{d}{dt}(d_V L^3) = 3d_V L^2 \frac{d}{dt}L = y_{VA} \left( \kappa f J_{Am}^a L^2 - J_M^v L^3 \right) \quad (12)
\]

\[
\frac{d}{dt}L = y_{VA} 3d_V J_M^v \left( f \kappa J_{Am}^a / J_M^v - L \right) \quad (13)
\]

This equation takes the form of the traditional von Bertalanffy growth equation. When all parameters are constant, the von Bertalanffy curve results. Therefore, we can replace the two groups of primary parameters with more practical compound ones: the maximum length \( (L_m) \) and the growth rate constant \( (r_B) \):

\[
\frac{d}{dt}L = r_B (f L_m - L) \quad (14)
\]

\[
L_m = \kappa J_{Am}^a / J_M^v \quad \text{and} \quad r_B = y_{VA} 3d_V J_M^v \quad (15)
\]

We can similarly fill in the equation for the continuous reproduction rate with the respective fluxes:
Next, we can express the actual reproduction rate as a fraction of the maximum reproduction rate (at maximum food and at maximum body size), which results in an equation in compound parameters:

\[
R = \frac{Y_{BA}}{W_{B0}} \left( (1 - \kappa) f J_{Am}^a L^2 - \frac{1 - \kappa}{\kappa} J_{M}^v L_3^3 \right) - (16) \\
= \frac{Y_{BA}}{W_{B0}} \frac{1 - \kappa}{\kappa} J_{M}^v \left( \kappa f J_{Am}^a L^2 - L_3^3 \right) \quad (17) \\
= \frac{Y_{BA}}{W_{B0}} \frac{1 - \kappa}{\kappa} J_{M}^v \left( f L_m L^2 - L_3^3 \right) \quad (18)
\]

Four primary parameters are thus absorbed in the compound parameter \(R_m\).

The total model in compound parameters is thus extremely compact (excluding starvation for the moment):

\[
\frac{d}{dt} L = r_B (f L_m - L) \quad \text{with } L(0) = L_0 \\
R = \max \left( 0, R_m \frac{f L_m L^2 - L_3^3}{L_3^3 - L_3^3_p} \right) \quad \text{if } L < L_p \quad \text{then } R = 0 \quad (22)
\]

\[
\frac{d}{dt} R_c = R \quad \text{with } R_c(0) = 0 \quad (23)
\]

Note that \(L\) was so far used for volumetric length. However, we can easily see that any constant shape-correction factor on the length measures will drop out. Therefore, we can use these equations in unmodified form with other length measures (e.g., total body length) as long as we use that measure consistently in the model, and as long as \(L^M\) is always proportional to \(L\) (i.e., no changes in shape).
2 Toxicity

Most of these derivations (including the model in compound parameters) have been published in the e-book [8] (version 2.0). What is new in this contribution is the configuration of damage and the physiological mode of action in a flexible manner (using a vector with switches), and the consistent starvation module (Section 3). Furthermore, the presentation here is more streamlined than in the e-book, due to focus on application in ecotoxicology, and due to the constraints presented at the start of Section 1. This facilitates practical application and implementation into software.

2.1 Symbols for the toxicity module

Additional symbols used in this chapter are collected in Table 2. I will focus on aquatic organisms, and exposure through a water phase. However, these models can also be used (possibly requiring some modifications) for other exposure situations. Note that the symbols used here differ slightly from those in the main text, where the superscripts $w$ (to indicate parameters that are referenced to water) and $m$ (rate constant referenced to maximum size) were removed for readability. Here, that extra detail is needed to also present the full model, which uses damage referenced to structure (and hence receives a superscript $V$).

2.2 Toxicokinetics and damage

The classical DEBtox model starts with the one-compartment model for toxicokinetics (TK) and adds impact of changes in size by growth dilution (and ‘concentration’ of the body residue when the animals shrink) and by changes in the surface:volume ratio (as chemicals have to be taken up through a surface area of the body). This leads to the following equation for TK:

$$\frac{d}{dt}C_V = k_e^m \frac{L_m}{L} (K_{Vw} C_w - C_V) - C_V \frac{3}{L} \frac{d}{dt}L \quad (24)$$

The elimination rate is scaled with the surface:volume ratio in such a way that $k_e^m$ has the interpretation of the elimination rate at the maximum size. The last term is dilution by growth, expressed on length basis. This equation is subsequently turned into a scaled TK equation by dividing both sides of the ODE by the bioconcentration factor ($K_{Vw}$):

$$\frac{d}{dt}C_w^V = k_e^m \frac{L_m}{L} (C_w - C_V^w) - C_V^w \frac{3}{L} \frac{d}{dt}L \quad (25)$$

The scaled internal concentration $C_w^V$ is proportional to the true internal concentration $C_V$, but requires no knowledge of the bioconcentration factor. The scaled internal concentration has the dimension of an external concentration (hence the subscript $w$).

In classical DEBtox formulations, $C_w^V$ is directly linked to the toxic effect, which (implicitly) assumes that any damage dynamics would be fast. We could easily add a simple, scaled, first-order damage dynamics to the unscaled TK equation to derive the equivalent of the ‘full’ model as used for survival modelling in GUTS [9]:

8
Table 2: Explanation of additional symbols for the toxicity module, with dimensions given in mass (\(m\) for body dwt., \(m_a\) for assimilates dwt., and \(m_q\) for chemical mass), length (\(l_e\) for environment, \(l\) for organism), time (\(t\)).

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>(b^w_b)</td>
<td>effect strength energy budget, ref. water</td>
<td>(l^3/mq)</td>
<td>–</td>
</tr>
<tr>
<td>(b^w_s)</td>
<td>effect strength survival, ref. water</td>
<td>(l^3/mq/t)</td>
<td>–</td>
</tr>
<tr>
<td>(C_V)</td>
<td>internal concentration in organism</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(C^w_V)</td>
<td>scaled internal concentration, ref. water</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(C_w)</td>
<td>dissolved concentration in water</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(D^V)</td>
<td>damage level, referenced to structure</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(D^w)</td>
<td>damage level, referenced to water</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(F_{BV})</td>
<td>egg dry weight relative to structural dry weight</td>
<td>(m_a/m)</td>
<td>–</td>
</tr>
<tr>
<td>(h)</td>
<td>hazard rate due to toxic stress</td>
<td>(1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(h_b)</td>
<td>background hazard rate</td>
<td>(1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(k^m_d)</td>
<td>dominant rate constant (in fully-grown adult)</td>
<td>(1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(k^m_e)</td>
<td>elimination rate constant (in fully-grown adult)</td>
<td>(1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(k_r)</td>
<td>rate constant for damage repair</td>
<td>(1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(K_{RV})</td>
<td>partition coeff. repro buffer-structure</td>
<td>(m/m_a)</td>
<td>1</td>
</tr>
<tr>
<td>(K_{Vw})</td>
<td>partition coeff. structure-water</td>
<td>(l^2_e/m)</td>
<td>–</td>
</tr>
<tr>
<td>(S)</td>
<td>vector with switches to configure pMoA</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(s)</td>
<td>stress level on metabolic processes</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(s^*)</td>
<td>stress level on specific metabolic process</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(y_p)</td>
<td>product of yield coeffs. for growth and shrinking</td>
<td>–</td>
<td>0.64</td>
</tr>
<tr>
<td>(X)</td>
<td>vector with switches to configure damage eq.</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>(x^*)</td>
<td>feedback factor for specific process</td>
<td>– or (1/t)</td>
<td>–</td>
</tr>
<tr>
<td>(z^w_b)</td>
<td>threshold energy budget, ref. water</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
<tr>
<td>(z^w_s)</td>
<td>threshold survival, ref. water</td>
<td>(mq/l^3)</td>
<td>–</td>
</tr>
</tbody>
</table>

\[
\frac{d}{dt} C_V = k^m_e \frac{L_m}{L} (K_{Vw} C_w - C_V) - C_V \frac{3}{L} \frac{d}{dt} L
\]  
\[
\frac{d}{dt} D^V = k_r (C_V - D^V)
\]

Note that I here assume that damage is not diluted by growth (though this could easily be added). Damage is scaled with the (unknown) partition coefficient between structure and damage, which leaves \(D^V\) with the dimension of an internal concentration.

The effect of reproduction on TK has so far been ignored in DEBtox models. However, reproduction will affect TK when a toxicant is transferred to the offspring. This can be corrected by adding an additional factor to the TK equation:
\[
\frac{d}{dt} C_V = k_m^e \frac{L_m}{L} (K_{Vw} C_w - C_V) - C_V \frac{3}{L} \frac{d}{dt} L - C_V R F_{BV} K_{RV} \tag{28}
\]
\[
\frac{d}{dt} D^w = k_r (C_V - D^w) \tag{29}
\]
The last term in the ODE for \( C_V \) covers losses due to reproduction. This introduces two new parameters: the egg dry weight as fraction of the total body dry weight \( (W_B / W_V = F_{BV}) \), which we, as an approximation, may treat as a species-specific constant) and the partition coefficient between egg material and structure \( (K_{RV}, \text{for which we might use 1 as a default}) \).

This set of equations represents a very reasonable model for TK and damage dynamics. However, its application requires information on body residues, which is usually missing in ecotoxicity testing. Of course, a scaled TK equation can be linked to a scaled damage equation, but this requires two rate constants \( (k_m^e \text{ and } k_r) \) to be estimated from the toxicity data, which is usually impossible.

A more practical approach is to follow the use of ‘reduced models’ as defined for GUTS (see [9]). In reduced models, TK and damage dynamics are combined into a single one-compartment equation for scaled damage. This is useful for situations where body residues are not determined, which is the general situation for ecotoxicological applications. The scaled damage thus forms a one-compartment representation of an essentially two-or-more compartment system. When damage repair is very fast, the dynamics of scaled damage will be dominated by TK, and hence the scaled damage over time represents the kinetics of the body residue (of the parent compound or a relevant metabolite). When TK is very fast, the reduced model represents damage dynamics.

Application of reduced models in DEB-based applications is less straightforward than in GUTS as the animals are growing and reproducing (things that can generally be ignored for application to survival modelling). Growth and reproduction will affect TK, but their effects on damage dynamics are less obvious. Damage repair is unlikely to relate to the surface:volume ratio, damage is unlikely to be eliminated through reproduction, and may or may not be diluted by growth. Since we, \textit{a priori}, will usually not be in a situation where we know whether TK or damage dynamics dominate, this induces uncertainty about which feedback processes to include into the reduced model. Furthermore, the situation becomes more complex when TK is dominated by biotransformation rates, as such rates are unlikely to depend on the surface:volume ratio, and may affect only the uptake or only the elimination of a compound.

The solution to this conundrum is to define a configurable module for scaled damage dynamics as follows:
\[
\frac{d}{dt} D^w = k_d^m (x_u C_w - x_e D^w) - (x_G + x_R) D^w \tag{30}
\]
What is calculated in the ‘reduced model’ is scaled damage, referenced to the external concentration (here assumed to be water, hence the superscript \( w \)). Scaled damage is directly calculated from the external concentration, and also has the units of the external concentration.
Four feedback factors are included which cover the various possible feedbacks. The first two factors ($x_u$ and $x_e$) govern the scaling of the dominant rate constant with the surface:volume ratio. Two factors are used such that the scaling can also be applied on uptake or elimination only (instead of always on both, which was forced in Eq. 25). Scaling only one of these processes may be useful for cases where a compound is transformed. We can think of a situation where a chemical is taken up through a surface and ‘eliminated’ by biotransformation to a non-toxic metabolite. In that case, only uptake scales with the surface area. Conversely, we could have the situation where a toxic metabolite is formed by biotransformation, but eliminated passively through a surface area.

Each of the factors in Eq. 30 requires, when it is relevant for the overall dynamics, a specific value that will change over time. A practical way to implement that is with a vector $X$, containing four switches to set a feedback process on or off. This vector can be turned into values for $x_\ast$ in the following manner:

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

(31)

$$x_u \rightarrow \max(1, x_u)$$

$$x_e \rightarrow \max(1, x_e)$$

(32)

The vector $X$ is multiplied element-wise with a vector of feedback factors. Comparing Eq. 30 with Eq. 28 clarifies how this structure works. The ‘max’ operators on $x_u$ and $x_e$ are needed to make these factors 1 when their corresponding switch is set to zero. The various switches can be set to zero or one to exclude or include a particular process in the reduced model. The classical DEBtox equation results for $X = [1, 1, 1, 0]$.

<table>
<thead>
<tr>
<th>TK/damage representation</th>
<th>$X$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast damage repair</td>
<td></td>
</tr>
<tr>
<td>Classical DEBtox (no losses with repro)</td>
<td>[1, 1, 1, 0]</td>
</tr>
<tr>
<td>Non-transformed, losses with repro</td>
<td>[1, 1, 1, 1]</td>
</tr>
<tr>
<td>Activated chemical, no losses with repro</td>
<td>[0, 1, 1, 0]</td>
</tr>
<tr>
<td>Detoxified chemical, losses with repro</td>
<td>[1, 0, 1, 1]</td>
</tr>
<tr>
<td>Slow damage repair</td>
<td></td>
</tr>
<tr>
<td>Damage is diluted by growth</td>
<td>[0, 0, 1, 0]</td>
</tr>
<tr>
<td>Damage is not diluted by growth</td>
<td>[0, 0, 0, 0]</td>
</tr>
</tbody>
</table>

Table 3: Some example settings for the vector with switches $X$ in the reduced damage model.

In this document, I explicitly want to include the possibility of starvation, including shrinking (burning structure to pay somatic maintenance costs). For the model in primary parameters, a starvation strategy was already presented in Section 1.2; for the model in compound parameters, it will be presented in Section 3. The situation of shrinking requires a closer look at growth dilution. When the animal decreases in size, the concentration/density of the chemical/damage will increase. When the toxicant affects assimilation or maintenance, this can easily lead to a positive feedback loop: shrinking increases the
damage density, which increases the toxic effect, which leads to more shrinking, increasing damage density even further. Whether this is realistic is, at the moment, unclear. However, it is good to realise that the model can show this runaway behaviour, in its current formulation.

### 2.3 Modes of action and stress factors

Scaled damage $D^w$ needs to be linked to one or more metabolic processes.² To this end, I follow the classical DEBtox approach and define a linear-with-threshold relationship between the property that drives the effect (here: damage) and a primary parameter of the model. First, we define a linear stress factor:

$$ s = b^w_b \max(0, D^w - z^w_b) $$

The stress factor $s$ is zero when damage is below the threshold, and increases linearly when damage is above. The original DEBtox model did not use an ‘effect strength’ $b^w_b$, but rather the reciprocal ($c_T = 1/b^w_b$). This is a matter of taste, but the use of an effect strength is consistent with the killing rate as used in GUTS models. Note that $z^w_b$ has the unit of an external concentration (hence the superscript $w$), and $b^w_b$ has the units of the reciprocal of external concentration.

In principle, any primary DEB parameter can be affected by a chemical. However, it is practical to focus on four metabolic processes: assimilation, maintenance, growth and reproduction. Table 4 shows how the stress affects these parameters, using subscripts to $s$ for assimilation ($A$), somatic plus maturity maintenance ($M$), growth costs ($G$), and reproduction costs ($R$). The functional form by which the stress function $s$ affects the parameter follows the tradition in DEBtox models: multiplication with $1 - s$ or $1 + s$, or division by $1 + s$. There are no strong theoretical reasons to select a certain relationship; here, I therefore just follow the traditional selection in DEBtox models [15]. In practice, it is unlikely that the experimental data are strong enough to distinguish between various functional forms. However, it is good to stress that there is freedom here to try different forms (e.g., using $J^A_{Am}/(1 + s)$ for assimilation, as proposed by [17]).

<table>
<thead>
<tr>
<th>Target process</th>
<th>Target parameter(s)</th>
<th>Parameter under stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation or feeding</td>
<td>$J^A_{Am}$</td>
<td>$J^A_{Am}(1 - s_A)$</td>
</tr>
<tr>
<td>Maintenance costs</td>
<td>$J^M_v$ and $J^w_j$</td>
<td>$J^M_v(1 + s_M)$ and $J^w_j(1 + s_M)$</td>
</tr>
<tr>
<td>Growth costs</td>
<td>$y_{VA}$</td>
<td>$y_{VA}/(1 + s_G)$</td>
</tr>
<tr>
<td>Reproduction costs</td>
<td>$y_{BA}$</td>
<td>$y_{BA}/(1 + s_R)$</td>
</tr>
</tbody>
</table>

Table 4: Stress functions for processes that can be affected by a toxicant. The physiological mode of action (pMoA) is made up of one or more of these affected processes.

This selection of affected processes generally works well [1], although in several cases combinations are deemed appropriate (e.g., costs for growth and costs for reproduction).

²At this point, I assume that the reduced model is used, otherwise superscript $V$ would be used on $D$, $z_*$, and $b_*$. 
The target process ‘maintenance costs’ applies the same stress factor to both somatic and maturity maintenance costs. This is done to preserve the relationship between investment in maturity and structural body size (which also underlies the specific choice for the maturity maintenance costs, see Section 1.2). For the same reason, the target process ‘growth costs’ should be linked to the ‘costs for maturation’. Since maturity is not followed as a state variable in the simplest version of DEBkiss, there is no need to make this assumption explicit. When $L_p$ is found to shift with toxicant stress, it may be useful follow maturity explicitly (in conjunction with the model in primary parameters, see Section 5), and consider effects on maturation costs as well.

For the model formulation, I will use more specific stress factors with a subscript for each metabolic process. To facilitate flexible implementation, especially to allow a pMoA to affect multiple processes, I define a vector $S$ with switches to configure the pMoA. This is equivalent to the vector $X$ to configure the damage equation through Eq. 31. Specific stress factors $s^*$ follow from the vector $S$ and the value for $s$ from Eq. 33:

$$[s_A, s_M, s_G, s_R] = s \times S$$

$$s_A \rightarrow \min(1, s_A)$$

The extra operation on $s_A$, maximising its value to 1, is needed as it will be applied in the form of a linear decrease of a parameter (which should not become negative, see Table 4). Practical examples for the pMoA are shown in Table 5.

<table>
<thead>
<tr>
<th>pMoA</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assimilation or feeding</td>
<td>[1,0,0,0]</td>
</tr>
<tr>
<td>Maintenance costs (somatic and maturity)</td>
<td>[0,1,0,0]</td>
</tr>
<tr>
<td>Assimilation and maintenance</td>
<td>[1,1,0,0]</td>
</tr>
<tr>
<td>Growth costs</td>
<td>[0,0,1,0]</td>
</tr>
<tr>
<td>Reproduction costs</td>
<td>[0,0,0,1]</td>
</tr>
<tr>
<td>Growth and reproduction costs</td>
<td>[0,0,1,1]</td>
</tr>
</tbody>
</table>

Table 5: Examples for how the switch vector $S$ can be used to create pMoAs with one or more affected processes.

### 2.4 Effects on survival

Effects on survival can be included in the same analysis as the effects on growth and reproduction. Since we now include scaled damage, we can directly use the hazard equations from GUTS. The hazard rate is calculated as:

$$h = b^w_s \max(0, D^w_s - z^w_s)$$

The hazard rate is subsequently turned into a survival probability by integration (here in ODE form, which is suitable as the rest of the model is in ODE form as well):
\[
\frac{d}{dt} S = -(h + h_b) S \quad \text{with } S(0) = 1
\] (37)

Note that the hazard equation rests on the assumption that the same scaled damage level is affecting the energy budget and survival (i.e., the same \( k_m \) applies). It may, however, be that lethal and sub-lethal effects are triggered by a different type of damage, which may have different dynamics. At this moment, there is not enough experimental evidence to test this assumption.

So far, DEBtox applications have only used stochastic death (SD) for survival. A good reason for that is that SD is consistent with the formulation for sub-lethal effects: damage above a threshold leads to a linear change in a model parameter. The individual tolerance (IT) model assumes differences in the threshold amongst the individuals in the (test) population, and instant death above a threshold. For sub-lethal effects, such an all-or-nothing response is unrealistic. At the EC50 for reproduction, we do not see half of the individuals stopping reproduction while the rest continue to reproduce at control rate. Even though it seems inconsistent, it is of course possible to use an IT model for survival in a DEBtox model. However, it is good to note that the IT model cannot (generally) be represented as an ODE, which complicates implementation.

2.5 Effects on growth and reproduction: model in primary parameters

For the full model (as defined in Section 1), we don’t need to do anything else. We can define a pMoA as a switch vector \( S \) (Table 4), apply the stress factors \( s_* \) to the respective primary parameters (Table 4), and let the model run. As already explained, the downside of using the full model is dealing with abstract primary parameters and the need for a set of conversion factors and yield coefficients. The first issue can be tackled by an internal reformulation of model parameters: users of the model can work with compound parameters while, internally, the model runs with primary ones. This trick was used for the classical DEBtox model in [14] to allow for a straightforward (and error-free) application of stress factors on primary parameters. We have already seen in Section 1.3 that compound parameters can be derived from primary ones (e.g., Eq. 15). Such relationships can also be reversed: we can calculate the primary parameters from compound ones (and a set of conversion factors and yield coefficients):
\[
L_0 = \delta_M L^M_0 \\
L_p = \delta_M L^M_p \\
L_m = \delta_M L^M_m \\
J^v_M = \frac{3d_v}{y_{VA}} \\
J^a_{Am} = R_m \frac{W_{B0}}{y_{BA}} \frac{L_m}{L^3_m - L^3_p} + J^v_M L_m \\
\kappa = L_m \frac{J^v_M}{J^a_{Am}} \\
J^v_J = \frac{1 - \kappa}{\kappa} J^v_M
\]

Note that we now need to make a clear distinction between volumetric length measures \(L^*_v\) and actual length measures \(L^*_m\). Table 6 provides a summary of the parameters needed for this model version.

<table>
<thead>
<tr>
<th>Parameter group</th>
<th>Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forcings</td>
<td>(f, C_w)</td>
</tr>
<tr>
<td>User parameters: basic</td>
<td>(L^M, L^M_0, L^M_p, r_B, R_m, W_{B0})</td>
</tr>
<tr>
<td>Conversion factors and yields</td>
<td>(\delta_M, d_v, y_{VA}, y_{BA})</td>
</tr>
<tr>
<td>Switches for damage and pMoA configuration</td>
<td>(X, S)</td>
</tr>
<tr>
<td>User parameters: sub-lethal toxicity</td>
<td>(k^m_d, z^w_b, b^w_b)</td>
</tr>
<tr>
<td>User parameters: lethal toxicity</td>
<td>(h_b, z^w_s, b^w_s)</td>
</tr>
<tr>
<td>Additional: losses with repro only</td>
<td>(K_{RV})</td>
</tr>
</tbody>
</table>

Table 6: Input parameters used in the DEBtox model in primary parameters (compound parameters are translated into primary ones). In general, one would start from \(K_{RV} = 1\) and \(f = 1\) (this parameter can only be fitted if there is more than one food treatment). Parameters \(h_b, z^w_s\) and \(b^w_s\) are only used when survival data are available as well.

### 2.6 Effects on growth and reproduction: model in compound parameters

Starting with the equation for growth, we can use the filled-in form as presented in Eq. 13. In this equation, we can add the stress factors as in Table 4:

\[
\frac{d}{dt}L = \frac{y_{VA} J^v_M}{3d_v} \frac{1 + s_M}{1 + s_G} \left( f\kappa \frac{J^a_{Am}}{J^v_M} \frac{1 - s_A}{1 + s_M} - L \right) \\
= r_B \frac{1 + s_M}{1 + s_G} \left( fL_m \frac{1 - s_A}{1 + s_M} - L \right)
\]

15
For reproduction, including maturity maintenance, some more work is needed. Starting from Equation 16 and adding stress factors:

\[
R = \frac{y_{BA}}{W_{B0}} \frac{1}{1+s_R} \left( (1-\kappa)fJ^a_{Am}L^2(1-s_A) - \frac{1}{\kappa}J^v_M L^3_p(1+s_M) \right) = \frac{y_{BA}}{W_{B0}} \frac{1}{1+s_R} \frac{1-\kappa}{\kappa} J^v_M \left( f\kappa J^a_{Am}L^2(1-s_A) - L^3_p(1+s_M) \right) = \frac{y_{BA}}{W_{B0}} \frac{1}{1+s_R} \frac{1-\kappa}{\kappa} J^v_M \left( fL_mL^2(1-s_A) - L^3_p(1+s_M) \right)
\]

The maximum reproduction rate in the control is still the same as in Eq. 19. Therefore, we can write the reproduction equation as (adding the ‘max’ operator to prevent negative reproduction rates):

\[
R = \max \left( 0, \frac{R_m}{1+s_R} \frac{fL_mL^2(1-s_A) - L^3_p(1+s_M)}{L^3_m - L^3_p} \right)
\]

The final result is thus an extremely compact model:

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

\[
R = \max \left( 0, \frac{R_m}{1+s_R} \frac{fL_mL^2(1-s_A) - L^3_p(1+s_M)}{L^3_m - L^3_p} \right) \quad \text{if } L < L_p \text{ then } R = 0
\]

\[
\frac{d}{dt}R_c = R \quad \text{with } R_c(0) = 0
\]

The model, in this formulation, is quite similar to the original DEBtox equations of [15], with a few differences. The original model used scaled body length \( l = L/L_m \), which is avoided here (as we are interested in absolute length, and there is a potential for confusion as \( L_m \) will be affected by toxic stress as well). Furthermore, this model follows from DEBkiss, which does not have a reserve compartment. This implies that the parameter \( g \) from the original DEBtox equations does not occur here, which simplifies the equations somewhat. Furthermore, \( g \) was a nuisance as it could not be practically determined from experimental toxicity data and had to be fixed to a ‘not-unreasonable value’.

As already explained in Section 1.3, the model is expressed in volumetric lengths but any length measure can be used in these equations as long as it is used consistently. Any conversion factor will simply drop out.
3 Adding starvation response to the compound model

This module has not been published before; it has been derived specifically for this manuscript, but will be included in a future update of the DEBkiss e-book [8]. Two additional symbols are used in the derivation, which are presented in Table 7.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Explanation</th>
<th>Dimension</th>
<th>Sugg. value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$f_R$</td>
<td>virtual food level for reproduction (starvation)</td>
<td>$-$</td>
<td>$-$</td>
</tr>
<tr>
<td>$f^*$</td>
<td>minimum food level to prevent shrinking</td>
<td>$-$</td>
<td>$-$</td>
</tr>
</tbody>
</table>

Table 7: Explanation of additional symbols for the starvation module.

Disadvantage of using the model in compound parameters, as derived in the previous section, is that we do not have direct access to the mass fluxes anymore. In most cases, this should not concern us. However, this does become problematic for starvation. In Section 1.2, I presented a consistent starvation strategy in Eq. 9 and 10. However, without access to the mass fluxes $J_s$, this is impossible to translate into the model formulation using compound parameters. However, by cheating a little, it can be done in DEBkiss (as it lacks a reserve). The price to pay is that we need to set a value for $\kappa$, and to make assumptions on the value of the product of the two yield factors: $y_{VA} \times y_{AV}$.

Starvation occurs when the ODE for growth threatens to become negative. At that point, I assumed that the organism is able to redirect resources from the $1 - \kappa$ flux to meet the maintenance requirements to prevent shrinking (at the expense of a reduction in reproduction rate). To prevent shrinking (so to keep $dL/dt = 0$), at a certain body size and stress level, the organism needs a certain amount of resources $f^*$:

\[
0 = r_B \frac{1 + s_M}{1 + s_G} \left( f^* L_m \frac{1 - s_A}{1 + s_M} - L \right) \tag{54}
\]

\[
f^* L_m \frac{1 - s_A}{1 + s_M} = L \tag{55}
\]

\[
f^* = \frac{L}{L_m} \frac{1 + s_M}{1 - s_A} \tag{56}
\]

When starvation occurs, the real food level is insufficient to meet these demands, so $f < f^*$. Now $\kappa$ needs to change to prevent shrinking. The assimilation flux of resources was given by:

\[
J_A = f J_{Am} L^2 \tag{57}
\]

The flux to the soma (somatic maintenance and growth) is:

\[
J_M + J_G = \kappa J_{Am} L^2 \tag{58}
\]

\[3\]From this equation, it follows that a fully-grown adult ($L = L_m$) will enter a starvation situation as soon as $f < 1$ or at any stress on assimilation or maintenance. Exposing adults to a toxicant could thus rapidly induce starvation.
The $\kappa$ flux ($J_M + J_G$), required $f^*$, so $\kappa$ needs to be multiplied by a factor of $f^*/f$ to prevent shrinking. The flux into the $1 - \kappa$ branch is thereby decreased to:

$$J_J + J_R = \left(1 - \kappa \frac{f^*}{f}\right) f J_{Am} L^2$$

(59)

To minimise changes to the reproduction equation (which does not include $\kappa$ explicitly), it turns out to be practical to work with a virtual food level for the $J_J + J_R$ (or $1 - \kappa$) flux. This virtual food level is given the symbol $f_R$:

$$(1 - \kappa)f_R = \left(1 - \kappa \frac{f^*}{f}\right) f$$

(60)

$$f_R = \frac{1 - \kappa f^*}{1 - \kappa}$$

(61)

$$f_R = \frac{f - \kappa f^*}{1 - \kappa}$$

(62)

This $f_R$ can be used in the standard reproduction equation, capturing the effect of a change in $\kappa$, such that $\kappa$ can be left to its original value in the equations. Some values of $f_R$ will lead to negative reproduction rates (when the flux $J_J + J_R$ is insufficient to pay maturity maintenance), but this is captured by the ‘max’ operator in Eq. 52.

If starvation becomes more severe, there will come a point where $f_R$ starts to become negative. This implies that the $1 - \kappa$ flux does not have sufficient assimilates to fuel somatic maintenance. If $f_R$ threatens to become negative, the organism will need to shrink: burn structural biomass to pay somatic maintenance costs. We already encountered the shrinking equation (note that $J_V$ is here negative):

$$J_V = (J_A - J_M)/y_{AV}$$

(64)

Starting from this equation for $J_V$, we can go through the same steps as for the growth equation in Section 2.6, and including stress factors.\(^4\)

$$3d_V L^2 \frac{d}{dt} L = \frac{1}{y_{AV}} \left( f J_{Am} (1 - s_A) L^2 - J_M^V (1 + s_M) L^3 \right)$$

(65)

$$\frac{d}{dt} L = \frac{J_M^V (1 + s_M)}{3d_V y_{AV}} \left( f J_{Am}^V \frac{1 - s_A}{J_M^V + s_M} - L \right)$$

(66)

We would like to use the compound parameters $r_B$ and $L_m$ again, but the result is not as nice as for the growth equation ($\kappa$ cannot be avoided):

\(^4\)Growth and shrinking are probably rather unrelated processes. Therefore, I think it is logical to assume that any stress on growth costs will not affect the shrinking process. Therefore, there is no stress factor $s_G$ on the yield of assimilates on burning structure.
\[
\frac{dL}{dt} = r_B \frac{y_{VA} y_{AV}}{(1 + s_M)} \left( f \frac{L_m}{\kappa} \frac{1 - s_A}{1 + s_M} - L \right)
\]

(67)

3.1 Summary of starvation module

The starvation module is triggered when the standard calculation for \(\frac{dL}{dt}\) leads to negative growth. The first step is to calculate a virtual food level for reproduction:

\[
f_R = \frac{f - \kappa \frac{L_m}{1 - s_A}}{1 - \kappa}
\]

(68)

As long as \(f_R \geq 0\), use this \(f_R\) in the reproduction equation, and set \(\frac{dL}{dt} = 0\). When \(f_R < 0\), set \(R = 0\) and shrink (using \(y_P = y_{VA} y_{AV}\)):

\[
\frac{dL}{dt} = \frac{r_B}{y_P} (1 + s_M) \left( f \frac{L_m}{\kappa} \frac{1 - s_A}{1 + s_M} - L \right)
\]

(69)

Clearly, shrinking cannot continue indefinitely. The degree of shrinking that an animal can sustain is species specific. In practice, some limit will need to be set, or the degree of shrinking linked to survival in some manner.

The simple starvation module presented here thus requires knowledge of \(\kappa\), which is a primary parameter. This is unfortunate. However, we can use the general default \(\kappa = 0.8\) as a starting point. It might even be possible to fit \(\kappa\) using data on starvation responses. For shrinking, we additionally need the product of the two yield coefficients: \(y_{VA} \times y_{AV}\). By default, both factors are set at 0.8 in debkiss, so 0.64 is a good place to start.

The total set of model parameters for the compound model is now summarised in Table 8. For explaining growth and reproduction under ad libitum feeding (standard ecotoxicity test conditions), we need to fit 8 model parameters. If we have toxicity data at different, constant food levels, we need to fit one \(f\) for each extra food treatment. Fitting survival requires 3 extra parameters. To include chemical losses with reproduction and deal with starvation, we need to provide values for two parameters in each case. These parameters can generally be fixed to defaults or derived from general biological information (especially the relative egg weight \(F_{BV}\)).
<table>
<thead>
<tr>
<th>Parameter group</th>
<th>Symbols</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forcings</td>
<td>$f, C_w$</td>
</tr>
<tr>
<td>User parameters: basic</td>
<td>$L_m, L_0, L_p, r_B, R_m$</td>
</tr>
<tr>
<td>Switches for damage and pMoA configuration</td>
<td>$X, S$</td>
</tr>
<tr>
<td>User parameters: toxicity sub-lethal</td>
<td>$k_d^m, z_w^b, b_w^b$</td>
</tr>
<tr>
<td>User parameters: toxicity lethal</td>
<td>$h_b, z_s^w, b_s^w$</td>
</tr>
<tr>
<td>Additional: for losses with repro only</td>
<td>$F_{BV}, K_{RV}$</td>
</tr>
<tr>
<td>Additional: for starvation only</td>
<td>$\kappa, y_P$</td>
</tr>
</tbody>
</table>

Table 8: Various parameters used in the proposed standard DEBtox model. In general, one would start by fixing $K_{RV} = 1$ (same chemical affinity in egg and structure), $f = 1$ (*ad libitum* feeding), $\kappa = 0.8$, $y_P = 0.64$, and $F_{BV}$ set to a reasonable value for the species of interest (dividing egg dry weight by a representative (structural) dry weight for reproducing mothers).
4 Complete compound model

In pseudo code, the set of derivatives (as input function for an ODE-solver) looks like this:

% calculate stress factor and hazard rate
\[ s = b^w_w \max(0, D^w - z^w_b) \] \hspace{1cm} (70)
\[ h = b^w_s \max(0, D^w - z^w_s) \] \hspace{1cm} (71)

% translate s into a set of specific stresses sA, sM, sG and sR
\[ [s_A, s_M, s_G, s_R] = s \times S \] \hspace{1cm} (72)
\[ s_A \rightarrow \min(1, s_A) \] \hspace{1cm} (73)

% calculate derivative for body length
\[ \frac{d}{dt} L = r_B \frac{1 + s_M}{1 + s_G} \left( f_{Lm} \frac{1 - s_A}{1 + s_M} - L \right) \] \hspace{1cm} (74)

f_{R} = f \hspace{1cm} by default, the virtual food level for repro equals f
if \( \frac{dL}{dt} < 0 \) % starvation, so calculate virtual food level for repro
\[ f_{R} = f - \kappa \frac{L_{m}}{1 + s_M} \] \hspace{1cm} (75)

if \( f_{R} \geq 0 \) % the 1-kappa branch can pay maintenance
\[ \frac{d}{dt} L = 0 \] \hspace{1cm} (76)

otherwise % calculate shrinking rate
\[ \frac{d}{dt} L = \frac{r_B}{y_{P}} (1 + s_M) \left( f_{Lm} \frac{1 - s_A}{\kappa} \frac{1}{1 + s_M} - L \right) \] \hspace{1cm} (77)

end
end
R = 0 \hspace{1cm} by default, reproduction is zero
if \( L \geq L_p \) % calculate reproduction rate
\[ R = \max \left( 0, \frac{R_m}{1 + s_R} \frac{f_{R} L_{m} L^2 (1 - s_A) - L_p^3 (1 + s_M)}{L_{m}^3 - L_p^3} \right) \] \hspace{1cm} (78)
end
% calculate change in cumulative reproduction rate
\[ \frac{d}{dt} R_{c} = R \hspace{1cm} with \hspace{1cm} R_{c}(0) = 0 \] \hspace{1cm} (79)
% calculate survival probability

\[
\frac{d}{dt} S = -(h + h_b) S \quad \text{with } S(0) = 1
\]  

(80)

% define the feedback factors from the settings in \( X \)

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

(81)

\[
x_u \to \max(1, x_u) \quad x_e \to \max(1, x_e)
\]

(82)

% calculate scaled damage:

\[
\frac{d}{dt} D^w = k_d^m (x_u C_w - x_e D^w) - (x_G + x_R) D^w
\]

(83)
5 Which model to use

In this document, I presented two formulations of the DEBkiss model for analysing toxicity data. One uses the model in primary parameters, although the user can be presented with easy-to-interpret compound parameters (see Section 2.5). The other is built with compound parameters (as summarised in Section 4). Clearly, the latter model is more compact and requires fewer parameters. Several of these additional parameters are difficult to establish for a species. Fortunately, their value is not usually relevant, as evidenced from the fact that we can create a model in compound parameters that does not need these parameters. What they are needed for is to identify $\kappa$, and, as shown in Section 3, $\kappa$ popped up in the compound model for the starvation response only. However, it is difficult to require all users of (results of) a model to be familiar with the detailed role that each parameter plays. For this reason, I would say that the model in compound parameters is the best place to start, and only move to the primary-parameter model if the compound model performs unsatisfactorily. The model with primary parameters is easier to extend when some of the model assumptions need to be modified, and some potential extensions are discussed below.

Adding a reproduction buffer. For the application of DEBkiss to ecotoxicity data, I made the simplifying assumption that reproduction is a continuous process. However, in many species (incl. daphnids and springtails), offspring are produced in clutches, which implies that the continuous allocation to reproduction needs to be collected into a reproduction buffer. The complete DEBkiss model includes such a buffer [8], which also requires a (species-specific) set of rules for its behaviour. It is good to note that a reproduction buffer, and its handling rules, might also affect toxicokinetics (as it affects size and composition of the individual). Furthermore, such a reproduction buffer might also be used to fuel somatic maintenance costs under starvation, postponing negative effects for adults. The e-book [8] contains equations for these processes, but the resulting model will be more complex to parameterise and to apply in practice (discontinuous reproduction also requires due care in fitting the model to observations).

Adding maturity. In Figure 1, maturation was shown in grey; the process is there, but only as a sink for the $1 - \kappa$ flux in juveniles. However, we can easily add maturity as a state variable in the DEBkiss model. This can be explored when body length at puberty does not remain constant under different treatments. Instead of setting puberty at a fixed body size, it can be set at a fixed cumulative investment into maturation (as is done in the standard DEB animal model). In addition, the simplifying rule to link specific maturity maintenance to somatic maintenance (see Eq. 6) can be changed, and specific maturity maintenance treated as a parameter to be fitted ($J^h_J$, multiplied by the maturity level rather than body size). The e-book [8] provides more details.

Adding pMoAs. Chemicals may affect any of the primary parameters of the DEBkiss model. For the model in compound parameters, a restriction to four metabolic processes was made. An obvious candidate for an additional pMoA would be $\kappa$. Effects on $\kappa$ have
been suggested for the effects of parasites (e.g., [5]) and kairomones (see [7], Page 56). Effects patterns associated with changes in $\kappa$ seem to be accompanied by changes in $L_p$. Therefore, such a pMoA is best investigated in the primary-parameter model with added state variable for maturity (see previous point). This also holds if one would like to try out effects on either somatic or maturity maintenance by itself. Such pMoAs break down the relationship between body size and investment in maturation, and DEB theory would predict a shift in $L_p$.

Adding respiration or feeding data. We may have additional endpoints in a toxicity test besides body size and reproductive output. For example, respiration and feeding rates may have been measured. Such additional endpoints can be very helpful to select the most appropriate pMoA (e.g., to distinguish between effects on assimilation or maintenance). Since the model in primary parameters has direct access to all mass flows, it is easy to produce estimates for such additional endpoints. The e-book [8] provides more details.

Adding yolk-feeding stages. For the compound-parameter model, the embryo stage was left out; this stage would be hard (and perhaps even impossible) to include consistently. However, the primary-parameter DEBkiss model explicitly includes this stage. A detailed case study for Atlantic cod was presented recently [13], excluding toxicant stress. Including toxicants requires further thought, as TK will be more complex (e.g., the surface area for uptake in an egg is not related to the structural biomass of the embryo, and chemicals will also partition into the yolk compartment).
6 List of assumptions

Model assumptions, relevant for the updated DEBtox model presented here, are summarised in Table 9. Additional assumptions for the toxicant-effects module in Table 10.

1. There are two types of biomass: assimilates and structural body components (food is not explicitly considered). Each type has a constant composition. They can be converted into each other with a certain constant efficiency. The state variables of the organism are the mass of the structural body, and the cumulated reproductive output.

2. We consider only post-embryonic stages here, which means that the animal has two life stages: a juvenile that feeds but does not reproduce, and an adult that reproduces. The transition from juvenile to adult (puberty) is triggered by a critical structural body size.

3. Feeding is included by setting/fitting a scaled functional response (0 means complete starvation and 1 ad libitum feeding).

4. The assimilation rate is proportional to the surface area of the animal. Food is instantly translated into assimilates that are directly used to fuel metabolic processes.

5. The flow of assimilates is split into a constant fraction $\kappa$ for somatic maintenance and structural growth (the soma), and $1 - \kappa$ for maturation, maturity maintenance, and reproduction. From the $\kappa$ flow, somatic maintenance costs are paid first. Only structural biomass requires maintenance, which is proportional to its volume. The remainder of this flow is used for growth (with certain efficiency).

6. From the $1 - \kappa$ flow, maturity maintenance costs are paid first. Maturity maintenance is proportional to structural volume up till puberty; after puberty, it is fixed to the level at puberty. After paying maturity maintenance, the remainder is used for egg production in adults. For juveniles, the remainder is burnt to increase complexity of the organism (maturation) and is not followed. Transformation of allocated assimilates to eggs comes with a certain (high) efficiency. Here, reproduction is treated as a continuous process (also leading to fractional eggs being produced in a time interval). For the compound model, egg weight is constant.

7. If feeding is insufficient to pay somatic maintenance costs, the organism first diverts energy from the $1 - \kappa$ flux of assimilates, thereby reducing the flux to reproduction/maturation and maturity maintenance (this latter process remains dominant over the downstream sinks, but can be reduced to zero if the $1 - \kappa$ flux is insufficient to meet its demands). If reduction of the $1 - \kappa$ flux is insufficient, structure is converted into assimilates to pay somatic maintenance (shrinking). The investment into maturity and maturity maintenance can be reduced without relevant consequences for the life-history.

Table 9: The list of assumptions for the basic DEBkiss model as used here.
1. Exposure to toxicants produces damage. In the ‘reduced model’ scaled damage in the organism is treated as one homogeneous (well-mixed) compartment. The accrual flux of damage is proportional to the external concentration, and the repair flux is proportional to the damage level. Damage dynamics may or may not be affected by changes in the surface:volume ratio, dilution by growth, or losses with reproduction.

2. When the damage level exceeds a threshold, the value of one or more of the primary parameters changes proportional to the amount by which damage exceeds the threshold. For survival, the affected parameter is the hazard rate, and survival will have its own threshold and proportionality (the effects strength). Survival is assumed to be driven by the same type of damage as sub-lethal effects. Note that this assumption of proportionality implies recovery: when damage decreases, so will the effect on the model parameter.

3. Background mortality can be represented by a constant hazard rate (leading to a exponential decrease in survival probability over time).

Table 10: The list of assumptions for the toxicant extension of DEBkiss.
7 Fixed values for springtails

For the fit in the main text on the data for *Folsomia candida*, two species-specific parameters were kept fixed: the initial volumetric length $L_0$ and the relative weight of an egg $F_{BV}$. The latter is only needed for the feedback configurations that include losses with reproduction.

The initial fresh weight of the 1-day old animals used in the toxicity test was reported by the original authors to be $18 \mu g$ [3]. This seems to be very large. For the original DEBtox analyses with this data set [10, 11], I assumed that a mistake in the units was made, so that it should have been $18 \text{ng}$. This translates into a volumetric length of $26 \mu m$ (which looks reasonable enough in the graphs). However, this is rather small given a reported egg diameter of $80-110 \mu m$ [4].

More recently, Hamda performed detailed studies on *F. candida* in a DEB context [6]. He reported an egg dry weight $W_{B0}$ of $0.41 \mu g$ and a dry-weight density $d_V$ of $0.28 \text{mg/mm}^3$ for adults. Assuming the same $d_V$ holds for eggs, it is possible to translate the egg diameter of $80-110 \mu m$ from [4] to a dry weight of $0.08-0.2 \mu g$. Given the uncertainties in these translations (and potential differences between strains), these numbers are quite comparable. Looking at egg fresh weight, we get estimates of $0.3-0.7 \mu g$ [4] and $1.5 \mu g$ [6]. This makes it highly likely that the initial fresh weight reported by [3] was a factor of 10 off, and it should have been $1.8 \mu g$ for 1-day old juveniles. This can be translated into $L_0 = 0.122 \text{mm}$, assuming a wet-weight density of $1 \text{mg/mm}^3$. This is the value used for the fit in the main text.

Interestingly, quite large differences in initial body weight do not have a strong influence on the visual model fit. This partly relates to the fact that I fit the data as volumetric length: taking the cubic root of estimated volume implies that a difference of a factor of 1000 on weights translates into a factor of 10 on length. Another aspect is that the initial growth rate on length basis is largely independent of the initial size. This can be seen from Eq. 14: at small values of $L$, the growth rate $dL/dt$ is independent of $L$. A somewhat small $L_0$ can thus be compensated by a somewhat larger value for $r_B$, producing a rather similar fit on the body-size data. Since growth leads to dilution of damage (depending on the configuration for the damage equation applied), there may be more important consequences for the response to toxicants.

The unrealistically small initial body length in the original DEBtox analyses for this data set [10, 11] actually yields a somewhat better fit on the initial part of the growth curve. This may relate to the fact that *F. candida* may not grow according to the von Bertalanffy growth curve early in life. Hamda [6] found that early growth was retarded for some time (12.6 days at 20°C), possibly due to different behaviour (the urge to disperse rather than spend the maximum amount of time on feeding). Initial slow growth could be rather common for many species, and there may well be substantial consequences for the analysis and prediction of toxicity [19]. Unfortunately, the data set used for the case study in this paper does not have the information to support or reject the possibility for initial slow growth.

The egg fresh weight was estimated as $1.5 \mu g$, based on the results of [6]. The reproductive period used for the model fit is between the observations at day 23 and day 44, in
which time the mothers weigh some 180 µg (fresh weight) on average across all treatment. This leads to a relative egg weight $F_{BV}$ of 0.008 mg/mg.
References


