Supporting information for: Using the standard DEB animal model for toxicokinetic-toxicodynamic analysis

Tjalling Jager*, Benoit Goussen, André Gergs

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*DEBtox Research, Stevensweert, The Netherlands (tjalling@debtox.nl, http://www.debtox.nl/)

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1 Model specification of the basic DEB model

The standard DEB animal model (hereafter, stdDEB) is well defined [23, 34, 27], although there is a broad diversity in how it is presented in various places. This relates to the breadth of the theory and its applications; some are better served by a specific scaling or parameters with a different unit. However, as a result, it is not so easy to identify the set of equations in powers (energy flows in J/d) and volumetric length (in cm) as used for the add-my-pet (AmP) library [30]. One of the few places where these equations are concisely listed is the coffee mug handed out to the participants of the 2017 DEB symposium in Tromsø (Figure 1). This formulation forms the basis of the model description below.

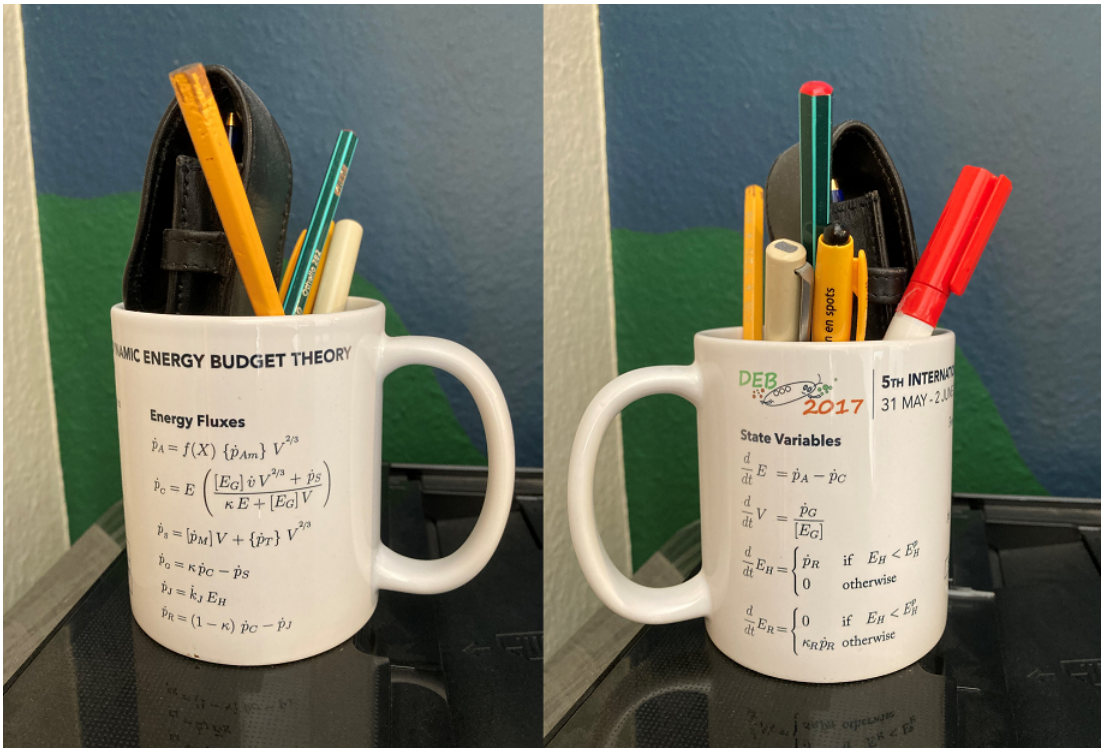

Figure 1: The coffee mug from the Tromsø DEB symposium, with the concise list of the equations for the standard DEB animal model.

The energy flows in the stdDEB model are shown in Figure 2. Symbols for the basic model (in absence of chemical stress) are explained in Table 1. The notation of DEB theory [23] is used in this document for the basic model. The TKTD modules follow the notation as used for the most-recent simplified DEB-TKTD model, referred to as DEBtox2019 [10]. This should not hurt much in this context, and maintains consistency with both standard DEB and DEBtox models. In the model definition, the 'coffee mug' formulation is followed, with a few exceptions:

1. Volumetric length L is used as a state variable, rather than structural volume V. However, this is a simple translation since $L = V^{1/3}$.

2. Following reproduction rate $\dot{R}$ in eggs/day rather than as investment into a reproduction buffer E_R. Note that the same is done for the AmP entries. The cumulative egg production R_c is used as a state variable in the implementation.

3. Adding a parameter κ_H as a 'maturation efficiency'. This parameter is set to 1 in absence of stress, and then has no influence, but provides a handle to allow applying a stress factor to maturation (see the technical background document for [9], and [21]).

In the following sections, the model formulation is presented in detail. For the implementation in Matlab-BYOM (http://www.debtox.info/byom.html), and connection to the TKTD module, several decisions needed to be made that are related to specific properties of stdDEB. These decisions are placed in boxes for emphasis.

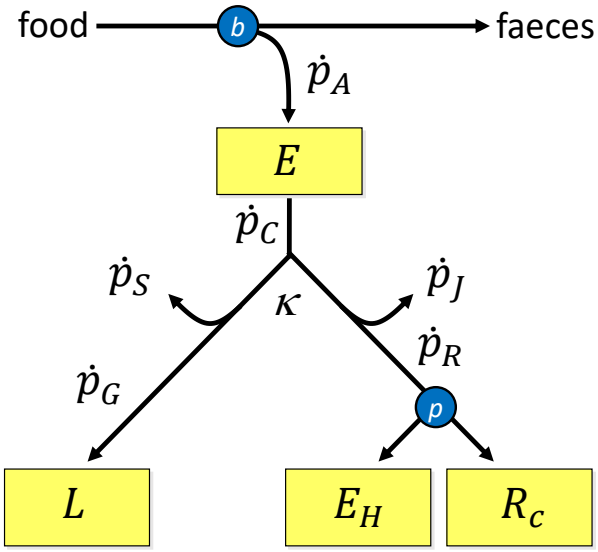

Figure 2: Schematic representation of the energy flows in stdDEB, specified as powers ($\dot{p}_*$ in Joules per day). Node 'b' indicates birth, and node 'p' puberty. These switches are triggered by specific threshold for maturity (E_H).

1.1 Powers and state variables

Specification of the powers for stdDEB (all in J/d), as shown in Figure 2:

Symbol	Explanation	Unit	Sugg. value
	Primary parameters		
T_A	Arrhenius temperature	K	8000
$\{\dot{p}_{Am}\}$	Maximum area-specific assimilation rate	J/(cm^2 d)	22.5 z
$[\dot{p}_M]$	Volume-specific somatic maintenance costs	J/(cm^3 d)	18
$\{\dot{p}_T\}$	Surface-specific somatic maintenance costs	J/(cm^2 d)	0
$\dot{k}_J$	maturity maintenance rate constant	1/d	0.002
$[E_G]$	Volume-specific costs for growth	J/cm^3	2800
E_H^b	Maturity level at birth	J	0.275 z^3
E_H^j	Maturity level at metamorphosis (for abj model)	J	–
E_H^p	Maturity level at puberty	J	166 z^3
$\dot{v}$	Energy conductance	cm/d	0.02
κ	Allocation fraction to soma	–	0.8
κ_R	Reproduction efficiency	–	0.95
κ_H	Maturation efficiency	–	1
	Parameters for starvation module		
y_P	Product of two yield factors (for shrinking)	J/J	0.64
	Forcings, environmental conditions		
f	Scaled functional response	–	1
T_{ref}	Reference temperature (20°C)	K	293.15
T	Actual temperature	K	–
	Conversions		
d_V	Dry-weight density of structure	g/cm^3	–
$\delta_{\mathcal{M}}$	Shape correction coefficient	–	–
	Powers		
$\dot{p}_A$	Energy flux for assimilation	J/d	
$\dot{p}_C$	Mobilised energy flux from reserve	J/d	
$\dot{p}_G$	Energy flux for structural growth	J/d	
$\dot{p}_J$	Energy flux for maturity maintenance	J/d	
$\dot{p}_R$	Energy flux to maturation or reproduction	J/d	
$\dot{p}_S$	Energy flux for somatic maintenance	J/d	
	State variables		
E	Reserve energy	J	
E_H	Cumulated energy investment into maturity	J	
L	Volumetric body length	cm	
R_c	Cumulated reproduction rate	eggs	

Table 1: Explanation of symbols for the basic model. Typical values for the standard model, with a zoom factor z, at 20°C, from [23] (Table 8.1). The zoom factor scales the animal, its maximum volumetric body length being $L_m = zL_m^{ref}$ with $L_m^{ref} = 1$ cm.

$$\dot{p}_A = f\{\dot{p}_{Am}\}L^2 \quad \text{assimilation} \tag{1}$$
$$\dot{p}_S = [\dot{p}_M]L^3 + \{\dot{p}_T\}L^2 \quad \text{somatic maintenance} \tag{2}$$
$$\dot{p}_C = E\frac{[E_G]\dot{v}L^2 + \dot{p}_S}{\kappa E + [E_G]L^3} \quad \text{reserve mobilisation} \tag{3}$$
$$\dot{p}_G = \kappa\dot{p}_C - \dot{p}_S \quad \text{growth} \tag{4}$$
$$\dot{p}_J = \dot{k}_J E_H \quad \text{maturity maintenance} \tag{5}$$
$$\dot{p}_R = (1-\kappa)\dot{p}_C - \dot{p}_J \quad \text{maturation/reproduction} \tag{6}$$

Specification of the state variables for stdDEB under unstressed conditions:

$$\frac{d}{dt}E = \dot{p}_A - \dot{p}_C \quad \text{reserve} \tag{7}$$
$$\frac{d}{dt}L = \frac{1}{3L^2}\frac{\dot{p}_G}{[E_G]} \quad \text{structure} \tag{8}$$
$$\frac{d}{dt}E_H = \begin{cases} \kappa_H\dot{p}_R & \text{when } E_H < E_H^p \\ 0 & \text{otherwise} \end{cases} \quad \text{maturity} \tag{9}$$
$$\frac{d}{dt}R_c = \dot{R} = \begin{cases} 0 & \text{when } E_H < E_H^p \\ \frac{\kappa_R}{E_0}\dot{p}_R & \text{otherwise} \end{cases} \quad \text{cumulative reproduction} \tag{10}$$

There is one non-standard symbol introduced above: κ_H. This is set to 1, so it does not have an effect. However, this is required to have a parameter for the mode of action 'costs of maturation', since maturation can potentially be affected by toxicant stress (see Section 2.3). This parameter was introduced in the technical background document for the 'DEBtox e-book' [9] to have a counterpart in the $1-\kappa$ branch for the mode of action 'costs for growth' (see also [21]).

It is important to note that the initial reserve in the fresh egg (E_0) in Eq. 10 is not a model parameter. It follows from the other parameters by the 'maternal effects' rule. This rule states that the mother produces an egg with such a level of reserve that the embryo hits the maturity level at birth (E_H^b) with a scaled reserve density (e) that equals the scaled reserve density of the mother at egg formation. This implies that well-fed mothers produce well-fed offspring, and that egg size will decrease with food limitation (or assimilation stress) of the mother. This is a rather awkward rule, which requires awkward code to implement (see next section). For each value of the parameters, we need to numerically find the egg costs. Furthermore, this would need to be done continuously, as the mother's reserve density may fluctuate over time as a result of variation in food availability and/or changes in assimilation or reserve mobilisation as a result of toxicant stress.

There is, however, little empirical support for this specific maternal effects rule as a general rule for all animals. As reviewed by Bernardo [5], there are species that follow this pattern, species that do the exact opposite (e.g., *Daphnia magna*, see [6]), and species in which egg size does not seem to respond at all to changes in the mother's nutritional

status. The 'stylised fact' underlying this rule in stdDEB [34] thus has plenty of exceptions. Furthermore, the review of Bernardo points at the large variation in egg size for the eggs produced by a single mother, even within a single clutch. Therefore, it is defensible to deviate from this rule in a stdDEB-TKTD implementation. This issue is dealt with in more detail in Section 1.2.

Some handy conversions to often-used compound parameters (see [27]):

$$e = \frac{[E]}{[E_m]} \qquad \text{scaled reserve density} \tag{11}$$

$$[E_m] = \frac{\{\dot{p}_{Am}\}}{\dot{v}} \qquad \text{maximum reserve density} \tag{12}$$

$$\dot{k}_M = \frac{[\dot{p}_M]}{[E_G]} \qquad \text{somatic maintenance rate constant} \tag{13}$$

$$g = \frac{[E_G]}{\kappa[E_m]} \qquad \text{energy-investment ratio} \tag{14}$$

$$\dot{r}_B = \frac{1}{3}\frac{\dot{k}_M g}{e+g} \qquad \text{von Bertalanffy growth constant} \tag{15}$$

$$L_m = \kappa\frac{\{\dot{p}_{Am}\}}{[\dot{p}_M]} \qquad \text{maximum volumetric length} \tag{16}$$

1.2 Initial values and egg costs

For use as a DEB-TKTD model, we will often need to start the model at birth. Birth takes place at a fixed maturity level $E_H = E_H^b$, where E_H^b is a constant model parameter. However, we do not know the value of the other state variables, length at birth L_b and the reserve at birth E_b, before running the model. These states at birth depend on the initial reserve in the egg E_0, which is *not* a model parameter. Thus, the question of calculating the costs of the egg E_0 for the reproduction rate $\dot{R}$ (Eq. 10) is closely related to the question of the initial states at birth, L_b and E_b.

The maternal effects rule of DEB theory states that the egg costs are such that the embryo will be born with the same reserve density that its mother had at the moment of egg formation. If we know the model parameters, we can thus calculate the E_0 that leads to a certain reserve density e when $E_H = E_H^b$. However, there is no explicit solution to the ODEs for the embryo. Kooijman [22] provides a routine to solve the system to obtain the initial amount of reserve, and the age and length at birth. This routine applies scaled variables but still requires numerical procedures to solve. Rather than using this routine, we here opt for simply simulating the (unscaled) model for the embryo. This is more transparent and even allows for the possibility to add toxic effects or other dynamic stress to the embryo stage in the future.

Trying to find an exact value for E_0 and the states at birth, for every relevant reserve density of the mother, would be extremely calculation intensive. Instead, we propose to

work with a lookup table. For a given set of basic model parameters, we can simulate a range of egg costs E_0, and collect the relevant values at birth: e_b, E_b and L_b. Note that scaled reserve density is given by:

$$e = \frac{[E]}{[E_m]} = \frac{E}{[E_m]L^3} = \frac{E\dot{v}}{\{\dot{p}_{Am}\}L^3} \tag{17}$$

For any given scaled reserve density e of the mother, we can thus interpolate in that table to find the associated egg cost E_0 to calculate the reproduction rate. The relationship between E_0 and e seems to be rather smooth (and close to linear), so interpolation will be accurate (and even limited extrapolation should not be problematic). However, we need to make sure that the table covers the relevant range of e values. This same table can then also be used to look up initial values at birth for different data sets (with potentially different f). This table only depends on the basic DEB model parameters, and not on the TKTD parameters. Therefore, assuming that we calibrate the model in steps (fit basic parameters first, and keep them fixed while fitting the TKTD parameters), the table would not have to be recalculated in the second step.

For the egg costs to calculate the reproduction rate, we need to consider how to apply the maternal effects rule in detail. Most importantly, we decided to ignore the potential impact of toxic effects on the developing egg for the egg costs. Egg costs are thus calculated using the unstressed values for the basic DEB parameters. The alternative would be unworkable, as it would require simulation of the TK and TD of the egg over its development, for each time point where we need to know the egg costs. Furthermore, it would assume that the mother can anticipate the complex dynamics of the toxic effects over egg development when producing an egg (see also discussion in [9], Section 3.2). This still leaves some options for the implementation of the maternal effects rule.

Decisions 1: Do we apply the maternal rule strictly? If so, egg costs (E_0) would vary over the course of a toxicity test with scaled reserve density (e), and thus respond to changes in f and toxicant stress (for some pMoAs). Alternatively, we could base egg costs on $f = 1$, or use the f that was established for the specific data set. The implementation in BYOM *allows the user to select one of these three options. For the case study, we base egg costs on the input value of f, and we suggest this as a default. This implies that egg costs do not change over the course of the toxicity test, but will be affected by the value for f that we select. The* BYOM *implementation does not consider toxic effects on the embryo during egg development for the egg costs. Egg costs are thus calculated using the unstressed parameter values.*

For the starting values of a toxicity test, we need to think about the f of the mothers that were used to spawn the eggs/neonates to start the test with. That value for f will affect the egg reserve E_0, and thereby the initial values at birth. We can either use a fixed value here (e.g., $f = 1$) or the same value as used for the test.

Decisions 2: For the initial values at birth, do we use $f = 1$ (eggs that start the toxicity test originate from well-fed mothers) or the specific f for the data set? The implementation in BYOM *allows the user to select one of these two options. For the case study, initial values are based on the f established for the data set, and we suggest this as a default.*

Starting the model at another point than the start of embryonic development or birth requires additional simulations (for each choice of basic model parameters). If we want to start at a certain length $L(0) > L_b$, we would need simulations to obtain the maturity level $E_H(0)$ and the reserve $E(0)$ at that new starting point. This required due consideration for the code as there may be one or more life-cycle events between L_b and $L(0)$ (i.e., metamorphosis and/or puberty).

Decisions 3: By default, the model analysis starts at birth. As additional option, implemented in the BYOM *package, the user can start at a specified physical length $L(0) > L_b$. Any reproduction before $L(0)$ is ignored, but any acceleration is included. For the period between birth and the start of the experiment, the value of f for the specific data set is used.*

State	Initial value embryo	Initial value birth	initial value post birth
E	E_0	E_b	$E(0)$
E_H	0	E_H^b	$E_H(0)$
L	10^{-6} cm	L_b	$L(0)$
R_c	0	0	0

Table 2: Initial values for the embryo simulations and for analysing toxicity-test data (from birth). Note that the formulation of the ODE for size in terms of body length implies that we cannot use $L = 0$ as a starting value (as long as the value is very small, model results will be insensitive to the exact value).

Simulating the embryonic stage is complicated by the fact that we don't know how long we need to simulate. Age at birth is not fixed but depends on E_0. Furthermore, we don't know where to start looking for E_0; we could use the AmP implied property as starting values, but the 'correct' value for E_0 will shift when we refit (some of the) basic model parameters. We can obtain a useful starting point by looking at a special case that *can* be solved analytically: assume that we have an infinitely large egg and that maintenance costs can be ignored. How long does it take for the embryo to reach the puberty threshold for birth, and how much reserve has it used? This question is solved in Section 4.4. The resulting age at birth will be too short, and the reserve used will underestimate E_0. Nevertheless, it is a robust place to start the search.

1.3 Temperature correction

Temperature affects life-history traits, and especially so for ectothermic animals. In DEB theory, the default assumption is that all times and rate constants (all parameters with

a dimension that includes 'time') scale in the same way with temperature. Temperature thus stretches or compresses the time axis of the life cycle. We can use the Arrhenius relationship to scale from a reference temperature T_{ref} to the actual temperature T (both in Kelvin). All physiological rate constants have to be multiplied by a factor F_T:

$$F_T = \exp\left(\frac{T_A}{T_{ref}} - \frac{T_A}{T}\right) \tag{18}$$

where T_A is the Arrhenius temperature in Kelvin. Lika and co-workers [28] suggest a value of 8000 K as typical value.

Note: it is currently unclear whether and how toxicological rate constants (i.e., k_d and b_s) scale with temperature. In fitting the model, this only requires consideration if we combine or compare data sets at different temperatures. However, this aspect becomes hugely important if we want to extrapolate laboratory-observed effects to more field-relevant temperature scenarios.

1.4 Starvation

Starvation is the situation where the energy allocated to the soma is insufficient to pay somatic maintenance costs. Under these conditions, the animal would need to change its allocation rules (temporarily). The response to starvation is highly species specific, and no 'standard' DEB module is available. For ecotoxicology, a starvation module is needed, especially under pulsed exposure (see [10]), since toxicant stress can induce starvation, even under abundant food availability. We here suggest implementing the same module as for the DEBtox2019 model, for simplicity, and for the sake of consistency between both model approaches. However, other strategies can be included while keeping the structure of stdDEB-TKTD intact.

Under starvation (when $\dot{p}_G < 0$), assume that a starvation response is triggered that includes three stages [12].

Stage 1 As long as $\dot{p}_C > \dot{p}_S + \dot{p}_J$, the additional flux needed to pay $\dot{p}_S$ is taken from $\dot{p}_R$. Growth stops ($\frac{d}{dt}L = 0$). Maturity maintenance is thus being paid, and some maturation or reproduction is possible with whatever remains after both maintenance fluxes are paid. Effectively, this implies a change in κ, just enough to pay somatic maintenance, but without change in reserve mobilisation.

$$\dot{p}_R = \dot{p}_C - \dot{p}_J - \dot{p}_S \tag{19}$$

$$\dot{p}_G = 0 \tag{20}$$

Stage 2 As long as $\dot{p}_C \geq \dot{p}_S$, the additional flux needed to pay $\dot{p}_S$ is taken from $\dot{p}_R$. Growth stops ($\frac{d}{dt}L = 0$). Maturity maintenance can not be fully paid anymore, but gets what's left in the $1 - \kappa$ branch. No maturation or reproduction anymore.

$$\dot{p}_R = 0 \tag{21}$$
$$\dot{p}_J = \dot{p}_C - \dot{p}_S \tag{22}$$
$$\dot{p}_G = 0 \tag{23}$$

Stage 3 When $\dot{p}_C < \dot{p}_S$, the animal will pay somatic maintenance from structure (shrinking) and $\dot{p}_R = 0$. Maturity maintenance is no longer paid. Shrinking comes with a certain efficiency, but the growth overheads cannot be recovered. Assuming that the yield for growth and shrinking can both be taken as 0.8, we thus get a total factor of 0.64. This can be implemented as an increase in $\dot{p}_G$, even though it mechanistically acts as a decrease on the growth costs $[E_G]$ (which, under starvation, gets a different interpretation). A unit of structure costs $[E_G]$ Joule to build, of which a part is overhead costs. A fraction y_{VE} of those costs are actually retained in the biomass; the fraction $1 - y_{VE}$ is lost and cannot be recovered under shrinking. When burning this unit of structure to pay maintenance, we again have to pay overhead costs y_{EV}. So we can regain a fraction of the invested energy, equalling the product of these two yields ($y_P = y_{VE}y_{EV}$), for maintenance work.[1] Note that $\dot{p}_G$ is now negative.

$$\dot{p}_R = 0 \tag{24}$$
$$\dot{p}_J = 0 \tag{25}$$
$$\dot{p}_G = \frac{\dot{p}_C - \dot{p}_S}{y_P} \tag{26}$$

An individual cannot shrink indefinitely, so some limit to shrinking may be necessary (or shrinking should increase the hazard rate, see Section 2.2). However, treatments that induce strong starvation should be treated with great care, or may even be excluded from the TKTD analysis. Given the uncertainties about the actual starvation strategy of a species, and given the potential for interaction with TK and TD, it is likely that more complex models need to be considered in case that severe starvation is relevant for the question at hand.

These rules have the consequence that animals will shrink even though there is still a considerable amount of reserve present. However, an *ad hoc* increase in reserve mobilisation under starvation seems unrealistic, given the surface-specific mechanism proposed for reserve dynamics (see [29]). The reserve plays a somewhat awkward role in the starvation process, since adults can make less use of it than juveniles (see [35]). A fully-grown adult, raised on a constant f, will hit the starvation point as soon as f decreases a tiny bit, since all of the energy allocated to soma is already needed for somatic maintenance. A juvenile has the possibility of decreasing growth, and thereby can pay somatic maintenance under food limitation for longer, without deviating from the rules.

[1] The product $[E_G]y_P$ is the joules of energy for paying maintenance, that can be obtained by burning a cm^3 of structure.

Decisions 4: At this moment, the starvation module is set up analogous to that for DEB*tox2019. Reserve mobilisation is not affected under starvation; the required energy for somatic maintenance is taken from the* $1-\kappa$ *branch, and if that is insufficient, from burning structure. Starvation does not lead to increase in the hazard rate, nor to rejuvenation (i.e., decrease in* E_H*). Maturity maintenance can be reduced (even to zero) without consequences for the individual.*

We propose this strategy as the default situation for stdDEB-TKTD. However, for specific species, specific starvation strategies may be outlined. The stdDEB framework offers more flexibility for defining starvation strategies than the simplified and compound-parameter formulated DEBtox2019.

Note that, in stdDEB-TKTD, we do not (yet) consider the reproduction buffer. If such a buffer would be included, it would make sense to modify the starvation rules, such that the buffer (before its contents are irreversibly allocated to eggs) can be used to cover maintenance needs. A simple modification would be to continue the Stage 1 rule as long as there is reserve in the reproduction buffer. We can allow $\dot{p}_R$ to become negative, such that the buffer decreases. This is equivalent to the rules suggested for the DEBkiss framework ([12], Section 4.1.1).

1.5 The abj-model

Some species accelerate their growth over the initial part of the life cycle. Several possible causes for such accelerating growth curves can be put forward within a DEB context [24, 26]. One of these scenarios involves a period of V1-morphic growth between birth and a maturity level in between birth and puberty, termed 'metamorphosis'. For a V1-morph, surface area scales with volume to the power 1, rather than a power of 2/3 for the standard model. Two parameters of stdDEB depend on the surface:volume ratio, namely $\{\dot{p}_{Am}\}$ and $\dot{v}$. These parameters thus need to be multiplied by a factor δ that depends on length for a certain period of their life. A practical implementation is as follows:

$$\delta = \begin{cases} 1 & \text{if } E_H < E_H^b \\ L/L_b & \text{if } E_H^b \leq E_H < E_H^j \\ L_j/L_b & \text{if } E_H \geq E_H^j \end{cases} . \tag{27}$$

$$\dot{v} \to \delta \dot{v} \tag{28}$$

$$\{\dot{p}_{Am}\} \to \delta \{\dot{p}_{Am}\} \tag{29}$$

A problem with these equations is that E_H^j is a constant model parameter, but scaling of $\dot{v}$ and $\{\dot{p}_{Am}\}$ requires knowledge of L_j. The L_j is not generally constant, as it will depend on food level f and can change with chemical stress. The only solution that we see is to simulate the model from birth to metamorphosis, stop the model, collect L_j and restart the model with L_j as input for the remaining part of the time vector. This is the solution that is implemented in the BYOM package.

The acceleration requires some thought when shrinking can occur. An animal may shrink below the size at metamorphosis again. We propose to have this metamorphosis only once. If an animal shrinks below L_j again, there will be no new acceleration phase. However, for some species and some data sets, it may be needed to deviate from this rule.

Decisions 5: Metamorphosis and puberty can occur only once in the life time of an individual. Shrinking below L_j (or rejuvenation below E_H^j) does not trigger deceleration; shrinking below L_p does not turn the adult into a juvenile. After a shrinking episode, continuing growth beyond L_j or L_p does not trigger a new life-stage event. This is incorporated in the BYOM *implementation by not allowing E_H to decrease.*

It is good to note that the standard model is nested within the abj-model: the standard model is derived by setting $E_H^j = E_H^b$. In the BYOM code, acceleration is disabled by setting $E_H^j = 0$, as E_H^b may be re-fitted.

2 The TKTD module

The TKTD module is lifted from DEBtox2019 [10]. Additional symbols are explained in Table 3. The notation of this module here follows [10], so without the dot above symbols for rate constants.

2.1 Damage dynamics

For DEBtox2019, a flexible damage configuration was proposed for reduced models[2], with the following generic equation for scaled damage:

$$\frac{d}{dt}D_w = k_d(x_u C_w - x_e D_w) - (x_G + x_R)D_w \tag{30}$$

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 (or damage accrual) and elimination (or damage repair), respectively. Growth dilution and losses through reproduction each have their factor (x_G and x_R) that work 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. A practical implementation is 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. 30 can then be derived in the following manner:

$$[x_u, x_e, x_G, x_R] = \mathbf{X} \circ \left[\frac{L_m}{L}, \frac{L_m}{L}, \frac{3}{L}\frac{d}{dt}L, \dot{R}\, F_{BV} K_{RV}\right] \tag{31}$$

$$\text{if } \mathbf{X}(1) = 0 \text{ then } x_u = 1 \quad \text{if } \mathbf{X}(2) = 0 \text{ then } x_e = 1 \tag{32}$$

[2] In reducedTKTD models, toxicokinetics and damage dynamics are lumped in a single compartment with first-order kinetics. In so-called full models, separate modules for toxicokinetics and damage dynamics will be used. See also Figure 2 in [10].

Sym.	Explanation	example unit
	Toxicity parameters	
k_d	dominant rate constant	1/d
$\mathbf{S}$	switch vector for configuring pMoA	[-]
$\mathbf{X}$	switch vector for damage feedbacks	[-]
z_b	effect threshold sub-lethal effects	mg/L
b_b	effect strength sub-lethal effects	L/mg
z_s	effect threshold lethal effects	mg/L
b_s	effect strength lethal effects	L/mg/d
h_b	background hazard rate for lethal effects	1/d
	Additional State variables	
D_w	scaled damage level	mg/L
S	survival probability	[-]
	Additional intermediate variables	
x_*	specific feedback process for process *	[-] or 1/d
s	stress level	[-]
s_*	specific stress level for process *	[-]
h	hazard rate for lethal effects	1/d
	Additional parameters for losses with reproduction	
K_{RV}	partition coefficient egg-(total) body	1 g/g
F_{BV}	egg dwt, relative to mother's (total) dwt	g/g
	Forcing functions	
C_w	external concentration	mg/L

Table 3: Symbols used in the TKTD and survival module. The choice of unit for the external concentration affects the units of other parameters as well (the ones with mg and L in their units). This choice is up to the user. However, it is strongly advised to keep 'days' as the unit for time. This is especially needed when using the parameter-space explorer in BYOM, since the default search ranges assume that the time vector is in days. The AmP library also uses days as the standard.

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. 31 (the relative volumetric growth rate). The vector on the right-hand side of Eq. 31 contains the standard formulations for surface:volume scaling and growth dilution [20].

The L_m that is needed in this relationship, could, in principle, be calculated from the model parameters. However, we may want to calibrate on multiple data sets, with slightly different parameters, or compare the calibrated model parameters to a validation data set. Clearly, the scaling should not lead to different values of the rate constants for individuals that have the same size (even though their maximum size differs). Therefore, it makes sense to use a fixed reference length for L_m in this relationship. The additional rules in Eq. 32 are needed since the factors x_u and x_e need to be set to 1 to drop out of Eq. 30. In [10], a 'max' operator was used here, minimising x_u and x_e to 1. That works as long

as L is always smaller than L_m. However, if a reference L_m can be set by the user, and basic parameters re-fitted, it is safer to explicitly make these factors one when they are not needed.

Using the same damage module for stdDEB-TKTD, as is, leads to a somewhat awkward mechanistic interpretation, as only the state variable L for structural length is used for the feedbacks, and reserve is ignored. For readability of the list below, we here take the perspective of a case where an internal concentration drives the toxic effect (fast damage dynamics), although the same issues arise for the case where damage drives toxicity. The list of interpretation issues:

1. When uptake and/or elimination scales with a surface:volume ratio, this ratio is based on structural body volume only. This would hold if there is no toxicant present in the reserve, and toxicant exchange takes place via a structural surface area. It also holds when the toxicant is evenly distributed over structure and reserve (such that the concentration in both is the same), equilibration between structure and reserve is instant, and exchange takes place across a surface area that relates to the total body surface (so including the contribution of reserve). It also holds, approximately, when the reserve makes up only a very small fraction of the total body volume.

2. When there is dilution by growth, only the changes in structural body volume apply. Changes in reserve mass do not affect the internal toxicant concentration. This holds if there is no toxicant present in the reserve at all, but also in the case where reserve density is constant and equilibration between structure and reserve is instant. At a constant reserve density, the relative change in structural volume equals the relative change in total volume.

3. Any V1-acceleration (see Section 1.5) does not affect the scaling of the rate constants. In other words, the scaling with L is maintained, even though one could argue that in V1-morphs, there should be no scaling since surface area is proportional to volume. However, metabolic scaling does not necessarily imply changes in morphology.

An even distribution of a chemical across structure and reserve (as in issue 1 above) is perhaps not such a stretch of the imagination, as reserve has almost the same properties as structure in AmP entries (mainly reflecting the defaults used).

The losses with reproduction require some further consideration for stdDEB. Since eggs are treated as 'wrapped reserve', the interpretation that all toxicant is associated with structure would be troublesome to reconcile with this feedback mechanism. If there is no toxicant in the reserve, it also cannot be eliminated via egg production. Note that in DEBtox2019, two new parameters were introduced to include losses with reproduction: the egg dry weight as fraction of the mother's dry weight (F_{BV}), and a partition coefficient for the chemical (or its damage) between egg material and structure (K_{RV}). The V in the subscript refers to structure. This was appropriate for DEBtox2019 as all biomass is treated as structure in DEBkiss-derived models. However, for stdDEB-TKTD, these parameters require some more thought. Given the uncertainty about how the toxicant (and its damage) is distributed over structure and reserve, we for now propose to refer these parameters to

the total body mass. Therefore, F_{BV} would be the dry mass of an egg, relative to the total dry mass of the mother. And K_{RV} the partition coefficient between the egg biomass and the total dry mass of the mother.

By default, we can set $K_{RV} = 1$, which implies that eggs will have the same chemical/damage density as the mother's body. For stdDEB-TKTD, we can set F_{BV} to a reasonable constant for the species, just as done in DEBtox2019. The AmP implied properties could help with that. However, in stdDEB-TKTD, we have direct access to the amount of reserve and structure, both in the mother and in the egg. Therefore, F_{BV} could also be calculated as function of time, using conversions of the state variables to wet weight (see Section 3.2). It seems, however, that such a detailed calculation would not add much in a TKTD analysis. Therefore, this is not (yet) implemented in the BYOM package. However, for cases where losses with reproduction is identified as an important feedback mechanism, due care is needed as the model formulation for this process is approximate at best.

TK/damage representation	**X**
Fast damage repair	
Classical DEBtox (no losses with repro)	[1,1,1,0]
Activated chemical, no losses with repro	[0,1,1,0]
Detoxified chemical, no losses with repro	[1,0,1,0]
Slow damage repair	
Damage is diluted by growth	[0,0,1,0]
Damage is not diluted by growth	[0,0,0,0]

Table 4: Some example settings for the vector with switches **X** in the reduced damage model. For fast damage repair, toxicokinetics will drive the toxic effects, whereas with slow damage repair, it will be the damage dynamics. The vector **X** =[0,0,0,0] is proposed as default.

Decisions 6: The TKTD *module strictly follows the structure of the simplified model. Thus, reserve is ignored for damage dynamics; all feedbacks are based on structural length L. For losses with reproduction, we likewise implement a fixed factor F_{BV}, set to a not-unreasonable value. This should suffice to explore the importance of this feedback route. These choices can easily lead to logical inconsistencies in the model interpretation, but alternatives require further study (and will likely require more parameters).*

More elaborate TK models have been proposed for DEB models, allowing the toxicant to partition over structure and reserve (see, e.g., [36, 2] and the technical background document of [9]). This allows for a fully consistent TK module, and is very relevant when the toxicokinetics of the parent compound is driving the toxic effect. However, when biotransformation plays a role, and even more so when damage dynamics is driving the toxicity, such a partitioning is less obvious. Furthermore, it is not clear whether such extensions would be testable in principle, and whether they would be essential for the analysis of ecotoxicity data. Therefore, we refrain from *a priori* including them into the model for now.

2.2 Stress, hazard and survival

The stress function takes the damage level D_w, as established from the toxicokinetics and damage-dynamics module from the previous section, and translates it into dimensionless stress. This stress is defined such that a stress level of zero implies no stress.

$$s = b_b \max(0, D_w - z_b) \tag{33}$$

This is 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). This change was introduced with DEBtox2019 [10] to have a similar interpretation as b_s for survival effect (although the units will be different).

The hazard rate due to toxicant stress is calculated in an analogous fashion:

$$h = b_s \max(0, D_w - z_s) \tag{34}$$

The survival probability requires its own state variable S. We do not use the DEB ageing module here (see [23], Section 6.1). Therefore, only a descriptive background hazard h_b is considered:

$$\frac{d}{dt}S = -(h + h_b)S \quad \text{with } S(0) = 1 \tag{35}$$

Normally, h_b is taken as constant. However, there will be cases where background mortality increases over time. We can therefore extend h_b to include a Weibull pattern over time:

$$h_b(t) \rightarrow a\, h_b^a\, t^{a-1} \tag{36}$$

When $a = 1$, the regular constant background mortality emerges. For $a > 1$, background mortality increases over time. This is a descriptive model extension to provide a better fit to control data in some cases.

Note that we here, following DEBtox2019 [10], only consider stochastic death as a death mechanism, and ignore the alternative of individual tolerance [13, 15]. The reason lies in consistency with the sub-lethal effects. For effects on growth and reproduction, we do not consider differences in sensitivity between individuals. All individuals are identical, and damage above a threshold increases or decreases a DEB model parameter. A pure individual tolerance approach would be illogical for growth and reproduction, as it implies that each individual is either growing/reproducing at the control rate or not at all (individual tolerance does not allow for gradual responses). A mixed model can be proposed, where the effect is graded while individuals also differ in their threshold value. However, we will not often see data sets that are strong enough to parametrise such a model. Furthermore, individual tolerance will then cause substantial difficulties for implementation and interpretation: when sensitive individuals die because of the treatment, less-sensitive animals remain, which then leads to an increase in the mean growth and reproduction rates. Such a model would thus require something like an individual-based modelling approach. We therefore consider stochastic death to be the most logical and consistent death mechanism for DEB-based TKTD analysis.

2.3 Modes of action

The modes of action will be defined analogous to those for DEBtox2019. Even though more pMoAs can be defined for stdDEB (e.g., a change in κ [19]), we will stay close to the classical DEBtox formulation [25] here. Note that maturation costs are here included with the pMoA for growth costs. The reason is to keep this pMoA close to how it works in DEBtox2019. In DEBtox2019, length at puberty is constant, which is not the case in stdDEB (only for a specific choice of $\dot{k}_J$). Increasing growth costs alone will decrease the length at puberty (building structure becomes more expensive, relative to maturing). To limit this change in puberty length, we propose to let maturation costs be modified by growth stress to the same extent (see [9], Section 3.2). For similar reasons, maturity maintenance has been coupled to somatic maintenance. For the stdDEB model, these links are not necessary, but using them as defaults ensures consistency with the simplified models.

Specific stress factors s_* follow from this vector $\mathbf{S}$ and the value for s from Eq. 33:

$$[s_A, s_M, s_G, s_R, s_H] = s \times \mathbf{S} \tag{37}$$

$$s_A \to \min(1, s_A) \tag{38}$$

The specification of this vector is illustrated in Table 5. 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).

pMoA	$\mathbf{S}$	change in target parameter(s)
Assimilation or feeding	[1,0,0,0,0]	$\{\dot{p}_{Am}\} \to (1 - s_A)\{\dot{p}_{Am}\}$
Maintenance costs	[0,1,0,0,0]	$[\dot{p}_M] \to (1 + s_M)[\dot{p}_M], \dot{k}_J \to (1 + s_M)\dot{k}_J$
Growth/maturation costs	[0,0,1,0,0]	$[E_G] \to (1 + s_G)[E_G], \kappa_H \to \kappa_H/(1 + s_G)$
Reproduction costs	[0,0,0,1,0]	$\kappa_R \to \kappa_R/(1 + s_R)$
Hazards to embryo	[0,0,0,0,1]	$\kappa_R \to \exp(-s_H)\kappa_R$

Table 5: Examples for how the switch vector $\mathbf{S}$ can be used to create standard pMoAs. The same pMoAs were defined for DEBtox2019, and the table shows their analogues for stdDEB-TKTD. Note that the pMoAs can easily be combined by putting more than one 1 in $\mathbf{S}$.

3 Auxiliary theory and statistics

Auxiliary theory deals with the translation of the (rather abstract) state variables of the model to real-world observable properties. Statistics deals with the evaluation of the differences between the model output and the observed properties. Both are essential elements of model application, but quite separate from the basic model formulation.

3.1 Body size as length

In many cases, body size is determined as an actual body length. The state variable L, however, is a volumetric length (cubic root of structural volume). We can convert L into a physical length L_w with a shape-correction coefficient:

$$L_w = \frac{L}{\delta_{\mathcal{M}}} \tag{39}$$

The coefficient $\delta_{\mathcal{M}}$ depends on which actual length measure is taken; in any case that measure should not be influenced by reserve density. Further, $\delta_{\mathcal{M}}$ is generally assumed to be constant, which implies that the species does not change in shape over ontogeny. For some species, taking $\delta_{\mathcal{M}}$ as function of size or developmental stage is appropriate (see e.g., [17]).

3.2 Body size as wet weight

We may encounter cases where body size is measured as volume or wet weight (or even dry weight). These measures are generally more robust, but add a complication for the calculations. Since volume and weight have contributions from structure as well as reserve (unlike length, which can often be assumed to be determined by structure only). Furthermore, the reserve state variable is followed in terms of energy, which then has to be translated to volume or weight.

If we can ignore the contribution of a reproduction buffer to body volume, we can sum the contributions of structure and reserve (see [23], Page 81):

$$V_w = L^3(1 + \omega_V e) \quad \text{with} \quad \omega_V = \frac{[E_m]}{d_E}\frac{w_E}{\mu_E} \tag{40}$$

Scaled reserve density e relates to unscaled reserve according to:

$$e = \frac{E}{[E_m]L^3} \tag{41}$$

With these equations, the state variables L and E can thus be combined into a total body volume V_w. We can translate this into wet weight by assuming a wet-weight density of 1 g/cm^3, and into dry weight by using the dry-weight density d_V.

These equations include several conversion parameters. Looking at the AmP entries, we can see that the density of reserve often equals the density of structure, so $d_E = d_V$. This reflects the suggested defaults [28], since data sets only rarely contain the information necessary to determine these parameters. We can calculate the molecular weight of the reserve w_E from the chemical indices for carbon, hydrogen, oxygen and nitrogen in reserve, and the molecular weights of these elements. The chemical indices seem to be the same for most AmP entries, following the standard animal (1:1.8:0.5:0.15, see [28]). The chemical potential of reserve also does not seem to change between entries, but that value is slightly different from that in [28].

If we do not start the model simulation at birth, it would be handy if the user can specify the initial body size in volume or wet weight as well. Within the model code,

Symbol	Explanation	Unit	Sugg. value
	Conversions		
d_E	Dry-weight density of reserve	g/cm^3	d_V
d_V	Dry-weight density of structure	g/cm^3	–
w_E	Molecular weight of reserve	g/C-mol	23.9
μ_E	Chemical potential of reserve	J/C-mol	$550 \cdot 10^3$
	Model output		
ω_V	contribution of reserve to volume	–	
V_w	total volume	cm^3	

Table 6: Explanation of additional symbols when using body volume or weight as model ouptut for the basic model. Note that w_E is calculated from the chemical indices for reserve and the molecular weights of the elements.

this then needs to be translated into an initial volumetric length, which remains the state variable in the model. From Eq. 40, we can derive the initial volumetric length from an initial value of body volume:

$$L(0) = \left(\frac{V_w(0)}{1 + \omega_V e}\right)^{1/3} \tag{42}$$

Here, we need to make an assumption for scaled reserve density e at the start of the experiment. Since we do not start at birth, we cannot use e_b from the maternal effects rule. In fact, we need to assume something about the food level that the organism experiences between birth and the start of the experiment. This was already done with 'Decisions 3' In Section 1.2, where we assumed that the animal experiences a constant food level of f (the same value as assumed for the experiment) between birth and the start of the experiment. The only additional assumption would be that the time between birth and the start of the experiment is long enough for the animal's reserve to reach steady state with the food density, so $e = f$.

Decisions 7: To translate a user-supplied initial body volume (or wet weight) into an initial volumetric length (the state variable), assume that scaled reserve density at the start of the experiment equals scaled food level ($e = f$).

The same relationships can also be used to translate the energetic egg costs E_0 to an egg volume or wet weight V_{w0}:

$$V_{w0} = E_0 \frac{w_E}{d_E \mu_E} = E_0 \frac{\omega_V}{[E_m]} \tag{43}$$

This result could also be used for the feedback 'losses with reproduction' to calculate F_{BV} continuously as V_{w0}/V_w.

3.3 Reproduction as eggs or neonates

The state variable for reproduction in the model is the continuously cumulated egg production, implying instantaneous translation from the investment flux $\dot{p}_R$ into eggs. In reality, egg production from invested reserve will take some time, and many species will collect the investment over some time period to spawn a clutch of eggs. It is possible to expand DEB models with a reproduction buffer, collecting the investment $\dot{p}_R$ until spawning (e.g., [2, 32]). However, this requires another state variable, rules for spawning (also under stress), rules for potential use of reproduction buffer under starvation, consideration of the reproduction buffer in TK and body weight, and more complexity in the code (due to the discontinuities in the model at spawning). Furthermore, the AmP entries currently do not consider the reproduction buffer and, therefore, their values cannot be used in a model that includes such a buffer. Therefore, we have not (yet) included a reproduction buffer into the current model formulation. However, clutch-wise spawning has the potential to lead to bias in model analyses [18], especially for pulsed exposure. The reason is that rapid effects on reproduction will be delayed by the reproduction buffer. The dynamics of the effects on egg production are not directly representing the dynamics of the effects on the energy flux $\dot{p}_R$. Furthermore, it is good to realise that the time resolution of reproductive observations is limited by the spawning cycle; the time points were no reproduction is observed basically carry no information on the investment into reproduction.

Many aquatic invertebrate test species do not release an egg clutch, but incubate the eggs in a brood pouch (e.g., cladocerans, amphipods and mysids). The eventual neonate release is then scored as 'reproduction'. This causes an additional delay between effects on reproductive investment and the observed reproduction that needs to be considered [18].

3.4 Statistics

The statistical framework that we propose follows the work laid down for simplified models [20]. Therefore, the subject is treated here in a cursory manner only. Furthermore, other statistical frameworks can be used, without changes to the basic model structure (see e.g., [31]).

Likelihood functions are drawn up for each endpoint, and combined assuming that the endpoints are independent. For survival, the multinomial distribution is used to design the likelihood function [4, 13, 15]. This is an appropriate distribution for the data, as the data are for a single discrete (irreversible) event in the individual's life. The log-likelihood function for a parameter set θ and a data set X is (first considering a single exposure treatment C_w):

$$\ell(\theta|X) = \sum_i x_i \ln p_i(C_w, \theta) \tag{44}$$

The individual observations for each time interval between observations i are denoted as x_i (the numbers of deaths in interval i). The (unconditional) death probability for each interval is p_i. The unconditional death probabilities can be calculated from the model output for survival probability at the start of each interval, and the observed deaths follow

from the observed number of survivors at the start of each interval. The log-likelihood contributions from each treatment can be summed as they are independent.

For continuous endpoints, we use the general likelihood function as proposed by [20], following from the normal distribution. The log-likelihood ℓ of a parameter set θ, given a data set Y with N individual observations, can be calculated from the sum-of-squares (SSQ) as follows:

$$\ell(\theta|Y) = -\frac{N}{2} \ln \mathrm{SSQ}(\theta; Y) + C \tag{45}$$

$$\mathrm{SSQ}(\theta; Y) = \sum_{i=1}^{N} \left(\hat{Y}_i(\theta) - Y_i \right)^2 \tag{46}$$

Where C is a proportionality constant that can be ignored, since we only need to know the likelihood up to a proportionality (all inference is based on likelihood *ratios*). Furthermore, note that $\hat{Y}_i(\theta)$ is the model prediction for the data point Y_i. This likelihood function follows from a series of assumptions. Most importantly, it assumes that the residuals follow from independent normal distributions with a constant variance. Independence is often violated in practice as we follow the same cohort of individuals over time, and also because reproduction is cumulated over time. Note that in deriving this likelihood function, residual variance is treated as a nuisance parameter and is 'profiled out' (in effect, it is estimated from the data). This has the advantage that the likelihood function does not require any additional parameters to be fitted from the data. Furthermore, the normal-distribution based likelihood function above assume that individual replicates are used as observed values. Jager and Zimmer [20] also provide approximate likelihood functions for the case where means are to be fitted.

Transformation of model and data can be used to bring the residuals more in line with normality and homoscedasticity. Log-transformation would lead to the following SSQ:

$$\mathrm{SSQ}(\theta; Y) = \sum_{i=1}^{N} \left(\ln \hat{Y}_i(\theta) - \ln Y_i \right)^2 \tag{47}$$

Log-transformation places more emphasis on small values for the observed endpoints. For example, compared to non-transformation, log-transformation for body size will result in a model fit with a closer correspondence to the observations on small individuals, at the expense of the observations on large individuals. Since residual variance tend to increase with the mean, this generally works in the right direction. However, log-transformation may be too strong, and lead to poor fits for large individuals. Square-root transformation offers a milder form of transformation:

$$\mathrm{SSQ}(\theta; Y) = \sum_{i=1}^{N} \left(\hat{Y}_i(\theta)^{1/2} - \ln Y_i^{1/2} \right)^2 \tag{48}$$

Similarly, we can use other power transformations, when deemed appropriate. These transformations are offered in BYOM, in the form of the flexible Box-Cox transformation, and

can be set individually for each set of observations (e.g., a different transformation for body size data than for cumulative reproduction data).

For the case study in the main text, an additional constraint was used in the fit, in the form of a zero-variate data point for the egg dry weight. This data point was provided with a normal distribution with a certain mean μ and standard deviation σ. For a model prediction $\hat{Y}$ we can calculate the following probability density $f(\hat{Y})$, using the familiar equation for the normal distribution:

$$f(\hat{Y}(\theta)) = \frac{1}{\sigma\sqrt{2\pi}} \exp\left(-\frac{1}{2}\left(\frac{\hat{Y}(\theta) - \mu}{\sigma}\right)^2\right) \tag{49}$$

This probability density can be used as a log-likelihood contribution as follows:

$$\ell(\theta) = \ln f(\hat{Y}(\theta)) \tag{50}$$

and added to the other likelihood contributions (again assuming independence).

4 Miscellaneous issues

4.1 AmP

Deriving the basic parameters of stdDEB (see Table 1) is not a trivial matter. However, this task is facilitated by the add-my-pet (AmP, see [30]) library, which contains stdDEB parameter values and implied properties for an enormous range of species. This is a living library, where entries are regularly being added and updated, with contributions from a broad group of international scientists. Matlab-based code is available to extract the most-likely parameter set from all available observations on the life history of the species in a structured manner (see the AmP portal https://www.bio.vu.nl/thb/deb/deblab/add_my_pet/index.html). Since experimental data on a species may well be insufficient to identify all parameters, 'pseudo-data' are added, representing the 'generalised animal' (see [28]). Each entry is evaluated by a board of curators before it is placed on the website. Using the AmP library for chemical risk assessment purposes was proposed by [3, 37]. However, there are limitations to this approach, as already mentioned in the main text. The main issue is that, in almost all cases, some parameters will need to be re-fitted to provide a close correspondence between the model and the control response in the toxicity test. At this moment, it is unclear what a good strategy would be for re-fitting.

The paper of Kooijman *et al* [27] provides guidance for fitting the standard DEB model to data. Depending on the type of data available, that paper explains which (compound) parameters can be identified. Even though this is very practical, it is not directly applicable as a strategy for deciding which AmP parameters to re-fit on a specific data set. Firstly, the model formulation in [27] is a scaled model version: the parameter for the assimilation rate is scaled out (which strongly improves the identifiability of the remaining parameters). Secondly, it uses a number of compound parameters (g and $\dot{k}_M$) not present in the current formulation. And finally, identifying parameters from data is not the same question as

deciding which AmP parameters should be re-fitted. If we cannot identify a parameter from the control data set, should then we leave this parameter at the value of the AmP entry? Since the basic DEB parameters are highly interdependent in their effect on the life history, this is not a simple 'yes'.

Despite these issues, we can still obtain valuable pointers from the paper of Kooijman *et al* [27]. The AmP parameters that typically cannot be estimated from growth and reproduction data, from one or small range of food levels, would be (restricted to the parameters considered in the present formulation): T_A, $\{\dot{p}_{AM}\}$ (unless maximum size can be fixed), κ_R, $\{\dot{p}_T\}$, k_J, and typically E_G and δ_M (unless length and volume/weight is available). This leaves $\dot{v}$, $[\dot{p}_M]$, κ and the maturity thresholds E_H^* as prime candidates for re-fitting. With the relationships for the compound parameters g and $\dot{k}_m$ (see conversions on Page 7 of this document), this latter list is consistent with the suggestions for the scaled DEB model [27]. This could be used as a default list for re-estimation for TKTD modelling, and these are also the parameters re-fitted for the present case study (see main text, Table 1).

More research is needed to refine the strategy for adapting the AmP entry to a specific toxicity data set. A promising direction to explore would, for example, be to re-fit all basic parameters to the controls of the toxicity test, but use the AmP values as prior distributions (in the Bayesian sense). Thereby, all parameters are allowed to change, but the fit is penalised if the parameters stray too far from the AmP values. Such a strategy would guard against large deviations from the AmP entry. The parameters in the AmP entries are currently provided without confidence intervals; such intervals would help designing the priors. Methods to derive confidence intervals for stdDEB parameters have been developed [31], although these do not (yet) consider the correlations between the parameters.

For TKTD modelling, we need a close correspondence between the basic model and the control response for a specific experimental test, while the AmP entry provides a compromise between all available sources of information for a species. The AmP entry for *Folsomia candida* [8], as used for the case study here, is clearly such a compromise between various, not entirely consistent, data sets. The fits on body size and reproductive output shown in the entry are not very convincing. Furthermore, some of the implied properties are dubious. For example, the parametrisation implies that 85% of the body weight of the animal is comprised of reserve, which seems unrealistic. It is furthermore unclear why a dry-weight density d_V of 0.17 g/cm^3 is used ([7] derived a value of 0.28 g/cm^3 for this species). This illustrates that at least some of the entries do not yet provide a solid basis for TKTD analysis.

4.2 DEBtool code for TKTD modelling

The DEBtool Matlab platform also contains code for analysis of toxicity data, linked to the AmP library (see AmPtox at https://amptox.debtheory.org/docs/index.html, accessed October 2021). There is no presentation of the underlying equations and assumptions. However, from scanning through the code we can already make a few observations in relation to stdDEB-TKTD.

1. The AmPtox code assumes that toxicity is driven by the toxicokinetics of the parent compound, and thus does not consider 'damage'. It therefore only applies a single feedback setting, namely $[1, 1, 1, 0]$, as also used for the classical DEBtox models [25, 20]. For our stdDEB-TKTD, we explicitly consider other configurations as well.

2. The feedbacks in AmPtox are using structural length, just as done for stdDEB-TKTD. Some files use the maximum length under stress for scaling uptake/elimination, which is likely an error (scaling should be done with reference to a constant maximum length).

3. The AmPtox code applies the same pMoAs as proposed for stdDEB-TKTD. The only difference is that in AmPtox, costs for growth is not linked to costs for maturation as done in Table 5.

4. The AmPtox code applies a scaled version of the standard animal model, rather than the unscaled model in primary parameters as used for stdDEB-TKTD. Therefore, some conversions are required in the code.

5. The AmPtox code does not consider toxicant-induced starvation, metabolic acceleration, or time-varying exposure.

6. The AmPtox code applies the maternal effects rule, also considering toxic stress on the embryo. The embryo is implicitly assumed to have the same stressed parameter values as the mother at the time of egg formation, and these parameters remain constant over egg development. However, the egg costs are not calculated from reserve density e of the mother, but from the stress level (for effects on assimilation) or assuming $e = 1$ (for the other pMoAs). Our stdDEB-TKTD ignores toxic effects on the embryo for the egg costs and bases egg costs on e, f or $f = 1$.

4.3 Quick comparison to DEBtox2019

Table 7 compares the here-described version of stdDEB-TKTD to DEBtox2019 [10], in general terms, focussing on the model structure. The simplified model lacks a reserve compartment and does not follow maturity as a state variable. This simplifies the implementation and application considerably. The use of compound parameters also makes it easier to work with, and the simplified model can generally be fitted using only the data from the toxicity test. The downside is that the simplifications may limit biological realism, and limit the flexibility of the model (e.g., it restricts the number of pMoAs and model extensions that can be tried).

The simplified model, for life stages post birth, can actually be viewed as a special case of the standard DEB animal model (the embryo stage *is* treated quite differently). It follows when $v \to \infty$ (so reserve becomes vanishingly small) and for a special choice of $\dot{k}_J$ (so that body size is a good proxy for maturity status). Additionally, the simplified model has constant costs for a single egg, hidden in the parameter for the maximum reproduction rate.

Table 7: Comparison of DEBtox2019 and stdDEB-TKTD.

Issue	**stdDEB-TKTD**	**DEBtox2019**
Parameters	Primary parameters, abstract but with a direct link to the underlying energy flows.	Compound parameters, easy to interpret and with a direct link to observable properties, but no direct link to underlying energy flows.
Parametrisation	Basic parameters must be taken from AmP, as the control data alone do not suffice. Some of them would need to be refitted to the controls.	Basic parameters are generally fitted on controls. AmP cannot be used, though the 'implied properties' can be used as starting values or to constrain the fit.
Reserve	Reserve standard. However, the (change in) reserve is not (yet) considered in the TKTD module.	No reserve. This version is based on DEBkiss, which removes the reserve compartment.
Egg costs	Maternal effects rule used to calculate egg costs (a model output). However, the implementation supplies several simplifying options.	The cost for an egg is constant. This parameter is hidden in the maximum reproduction rate.
Initial values	Initial values for length, reserve and maturity are needed (model outputs).	Only body length needs an initial value (a model parameter).
Puberty	Investment into reproduction starts at a fixed maturity threshold.	Investment into reproduction starts at a fixed body length.
Metamorphosis	Acceleration stops at a fixed maturity threshold.	Acceleration stops a fixed body length.

Note that Table 7 only compares two model versions, at the rather extreme ends of the simple-complex range (see [33]).

4.4 Derivation of initial values for eggs

We can get a first guess for the eggs costs and time to birth by considering an egg with an infinitely large reserve, and assume that all maintenance costs can be ignored. This is similar to foetal development (see also [22]), but also to the embryonic development in DEBkiss [16]. The state variables are then given by (here: $\kappa_H = 1$ and $f = 0$, so $\dot{p}_A = 0$):

$$\frac{d}{dt}E = -\dot{p}_C \tag{51}$$

$$\frac{d}{dt}L = \frac{1}{3L^2}\frac{\dot{p}_G}{[E_G]} \tag{52}$$

$$\frac{d}{dt}E_H = \dot{p}_R \tag{53}$$

The powers (ignoring somatic and maturity maintenance):

$$\dot{p}_C = E\frac{[E_G]\dot{v}L^2}{\kappa E + [E_G]L^3} \tag{54}$$

$$\dot{p}_G = \kappa\dot{p}_C \tag{55}$$

$$\dot{p}_R = (1-\kappa)\dot{p}_C \tag{56}$$

Start with $\dot{p}_C$:

$$\dot{p}_C = \frac{E[E_G]\dot{v}L^2}{\kappa E + [E_G]L^3} \quad \text{when } E \to \infty: \tag{57}$$

$$\dot{p}_C = \frac{[E_G]\dot{v}L^2}{\kappa} \tag{58}$$

Next $\dot{p}_G$ and include it into the ODE for L:

$$\dot{p}_G = \kappa\dot{p}_C \qquad \text{fill in } \dot{p}_C: \tag{59}$$

$$\dot{p}_G = [E_G]\dot{v}L^2 \tag{60}$$

$$\frac{d}{dt}L = \frac{1}{3L^2}\frac{\dot{p}_G}{[E_G]} \qquad \text{fill in } \dot{p}_G: \tag{61}$$

$$\frac{d}{dt}L = \frac{\dot{v}}{3} \qquad \text{solve for } L(0) \approx 0: \tag{62}$$

$$L = \frac{\dot{v}}{3}t \tag{63}$$

Next $\dot{p}_R$ and include it into the ODE for E_H:

$$\dot{p}_R = (1-\kappa)\dot{p}_C \qquad \text{fill in } \dot{p}_C: \tag{64}$$

$$\dot{p}_R = (1-\kappa)\frac{[E_G]\dot{v}L^2}{\kappa} \qquad \text{fill in } L: \tag{65}$$

$$\dot{p}_R = \frac{1-\kappa}{\kappa}[E_G]\frac{\dot{v}^3}{9}t^2 \tag{66}$$

$$\frac{d}{dt}E_H = \dot{p}_R \qquad \text{fill in } \dot{p}_R: \tag{67}$$

$$\frac{d}{dt}E_H = \frac{1-\kappa}{\kappa}[E_G]\frac{\dot{v}^3}{9}t^2 \qquad \text{solve for } L(0) = 0: \tag{68}$$

$$E_H = \frac{1-\kappa}{\kappa}[E_G]\frac{\dot{v}^3}{27}t^3 \qquad \text{solve } t_b \text{ where } E_H = E_H^b: \tag{69}$$

$$t_b = \left(E_H^b \frac{\kappa}{1-\kappa}\frac{27}{[E_G]\dot{v}^3}\right)^{1/3} \tag{70}$$

Next the ODE for E:

$$\frac{d}{dt}E = -\dot{p}_C \qquad \text{fill in } \dot{p}_i: \tag{71}$$

$$\frac{d}{dt}E = -\frac{[E_G]\dot{v}L^2}{\kappa} \qquad \text{fill in } L: \tag{72}$$

$$\frac{d}{dt}E = -\frac{[E_G]\dot{v}^3}{9\kappa}t^2 \qquad \text{solve for } E(0) = E_0: \tag{73}$$

$$E = E_0 - \frac{[E_G]\dot{v}^3}{27\kappa}t^3 \quad \text{calculate } \Delta E \text{ to reach } t_b: \tag{74}$$

$$\Delta E = \frac{[E_G]\dot{v}^3}{27\kappa}t_b^3 \tag{75}$$

We can now use t_b as lower limit for the end of the time vector for simulation, as eggs will develop slower than a foetus without maintenance. We can furthermore use ΔE as lower limit for E_0, as eggs will need more reserve to complete their development.

An advantage of using these estimates rather than the implied properties of AmP is when we start re-fitting basic model parameters. That would rapidly lead to a situation where the AmP E_0 is far out of range. From this initial estimate, we need to fill a table with values of E_0 with the corresponding e_b and values for all the states at birth. The implementation in BYOM proceeds through a number of steps. In simplified form:

1. Our first guess for E_0 may be too low to allow birth. Go through a loop, increasing E_0 by 25% of the initial guess, until we have an E_0 that allows birth. Calculate corresponding e_b.

2. Increase that E_0 by a small amount (1% of the initial guess) and calculate corresponding e_b. With these two values, we can calculate the slope of E_0 versus e_b. Use that slope to estimate a change in E_0 that leads to an 8% change in e_b. If our e_b at this point is 0.7 or less, take half that step.

3. Decrease E_0 in a loop until $e_b < 0.3$. Every time that birth takes place, collect the state variables at birth, and calculate a new step size that would lead to 8% change in e_b (or half that when $e_b < 0.7$). A safety is included: if birth does not occur because the egg reserve runs out, the step size is decreased, a maximum of 3 times. This should ensure that our lowest value in the table is close to 0.3 or to the critical value for reaching birth.

4. Increase E_0 in a loop until $e_b > 1.5$. Every time that birth takes place, collect the state variables at birth, and calculate a new step size that would lead to 12% change in e_b (or half that when $e_b < 0.7$).

The 8 and 12% are tuned to generally (in the tested cases) arrive at a range that has steps of approximately 0.1 for e_b, and smaller steps for smaller values. The aim is to reach a table with approximately 20 values spanning $0.3 < e_b < 1.5$. This should be enough for most purposes.

An events function is used to catch the point where the embryo no longer has enough reserve to sustain further development. This will occur when scaled reserve density e hits the scaled length $l = L/L_m$, with:

$$e = \frac{E\dot{v}}{\{\dot{p}_{Am}\}L^3} \quad \text{and} \quad L_m = \kappa\frac{\{\dot{p}_{Am}\}}{[\dot{p}_M]} \tag{76}$$

For some combinations of parameter values, reaching $e_b = 0.3$ will not be possible. Before birth is reached, $e < l$ and the embryo starves (and, presumably, maturity will no longer increase). This is especially occurring for species with acceleration. Before metamorphosis, L_m, as implied by the model parameters, is relatively small; much smaller than the real ultimate body length. Therefore, scaled length l is relatively large at birth, and can be larger than 0.3 in some cases.

It is important to note that this table of e_b versus E_0 only changes when we fit basic DEB parameters, or possibly when we change temperature. If we fit the toxicant parameters in isolation, while keeping basic parameters fixed, there is no need to continuously recalculate this table. In the BYOM package, the code is set up in this fashion, so there is no recalculation of the table when fitting the toxicity parameters. This ensures that reasonable calculation times are feasible.

Note: when $\dot{k}_J = \dot{k}_M$, we can derive the exact same results. Key is that E_H/L^3 is constant under that condition. The investment into maturation is a constant fraction of what is used for growth, so $\dot{p}_R/\dot{p}_G$ is constant.

$$\frac{\dot{p}_R}{\dot{p}_G} = \frac{(1-\kappa)\dot{p}_C - \dot{p}_J}{\kappa\dot{p}_C - \dot{p}_M} \qquad \text{using } \dot{k}_M \text{ and } \dot{k}_J = \dot{k}_M\text{:} \tag{77}$$

$$= \frac{(1-\kappa)\dot{p}_C - \dot{k}_M E_H}{\kappa\dot{p}_C - \dot{k}_M[E_G]L^3} \qquad \text{this remains constant when:} \tag{78}$$

$$E_H = \frac{1-\kappa}{\kappa}[E_G]L^3 \tag{79}$$

This is a logical result. The $1-\kappa$ branch receives a fraction $(1-\kappa)/\kappa$ of what goes into the κ branch. And $[E_G]L^3$ is the energy invested to make L^3 of structure. Because the equation for dL/dt leads to the same result as given above (the maintenance drops out as it is in $\dot{p}_C$ and $\dot{p}_G$), we already can see Eq. 69 emerging.

5 Details for the case study

The files used to make the calculations for the case study are part of the BYOM package for stdDEB-TKTD version 1.0. that can be obtained from: http://www.debtox.info/byom.html. Please note that the parameter-space explorer takes a lot of calculation time, especially when also creating profile likelihoods. Calculation time can be drastically reduced by using parallel processing (requiring Matlab's parallel computing toolbox), and a computer with plenty of (fast) physical cores.

5.1 Details for the basic fit in main text

Figure 3 shows the profile likelihoods for the fitted basic parameters. The profiles are well defined, so there are no identification issues. The profile likelihoods serve to obtain the 95% confidence intervals on the basic model parameters. Figure 4 shows the profile for the fitted background hazard rate h_b, which also is well defined. Figure 5 shows the end result for the zero-variate data point, included in the fit: the initial dry weight of an egg. It is on the high side of the confidence interval for the data point, but still very reasonable. The zero-variate data point served to constrain the basic fit, such that the parameters cannot imply unrealistic egg weights.

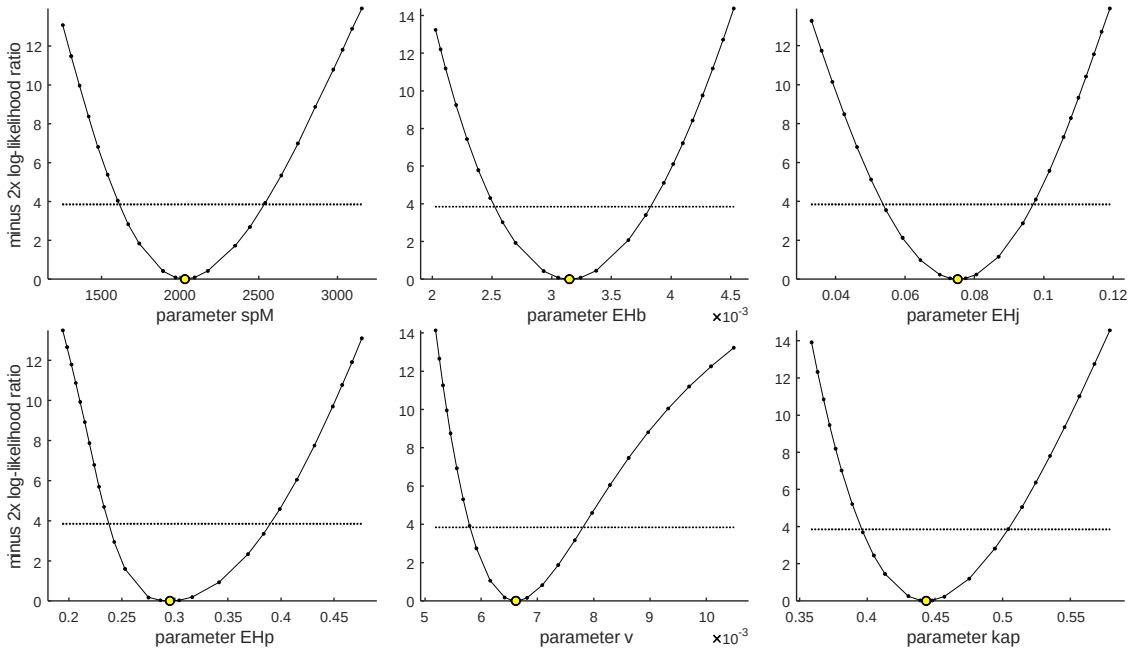

Figure 3: Profile likelihoods for the fit of the basic parameters to the control data for growth and reproduction. Horizontal line is the cut-off to obtain 95% confidence intervals.

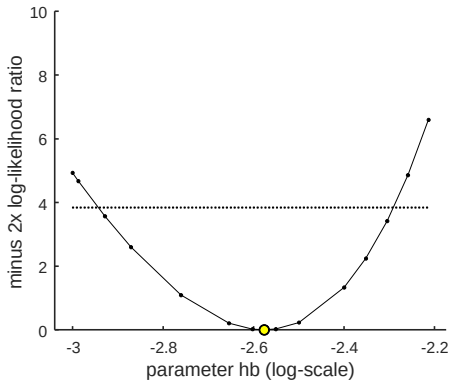

Figure 4: Profile likelihoods for the fit of the background hazard rate to the control mortality data. Horizontal line is the cut-off to obtain 95% confidence intervals.

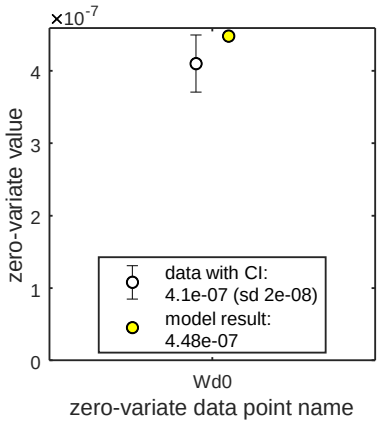

Figure 5: Final result for the zero-variate data point.

5.2 Details for the toxicity fit in main text

The toxicity parameters where fitted using the parameter-space explorer [11]. This algorithm attempts to map parameter space to find the best fitting parameter set, as well as a sample from parameter space for error propagation.

The parameter-space plot is shown in Figure 6, which shows that all five TKTD parameters are identifiable from the data set. On the diagonal, profile likelihoods for the individual parameters are shown, which are used to derive the 95% confidence intervals on the parameters.

Figure 7 shows the same fit as in the main text, but in more detail, with each endpoint and each treatment in a separate panel. The intervals on the curve are generated from the sample in Figure 6 (see [11]). This does not include the uncertainty in the basis parameters, and hence the controls and low-exposure treatments do not have a green area around them.

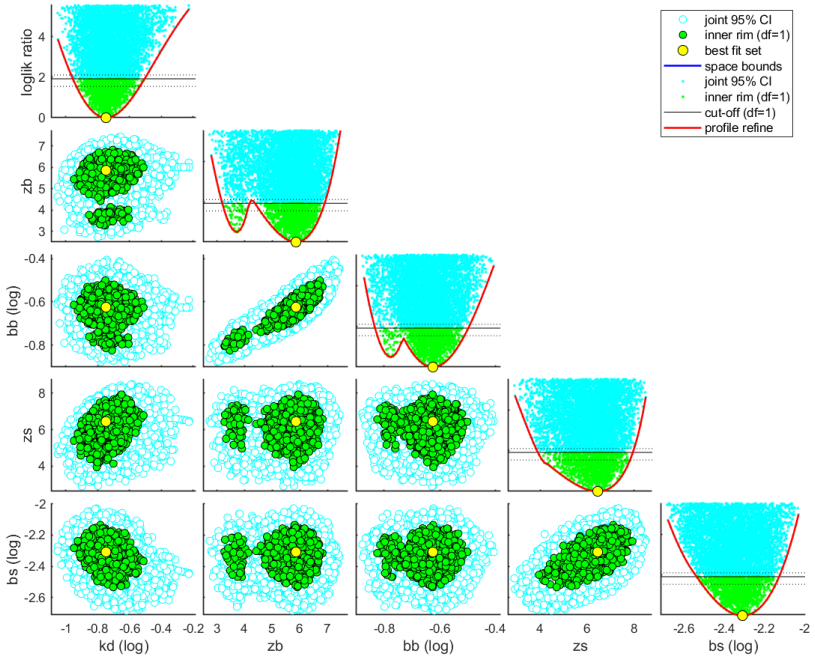

Figure 6: Parameter-space plot for the fit of the TKTD parameters to the complete data set. Panels show the sample from parameter space from various directions. On the diagonal, the profile likelihoods for the individual parameters are plotted.

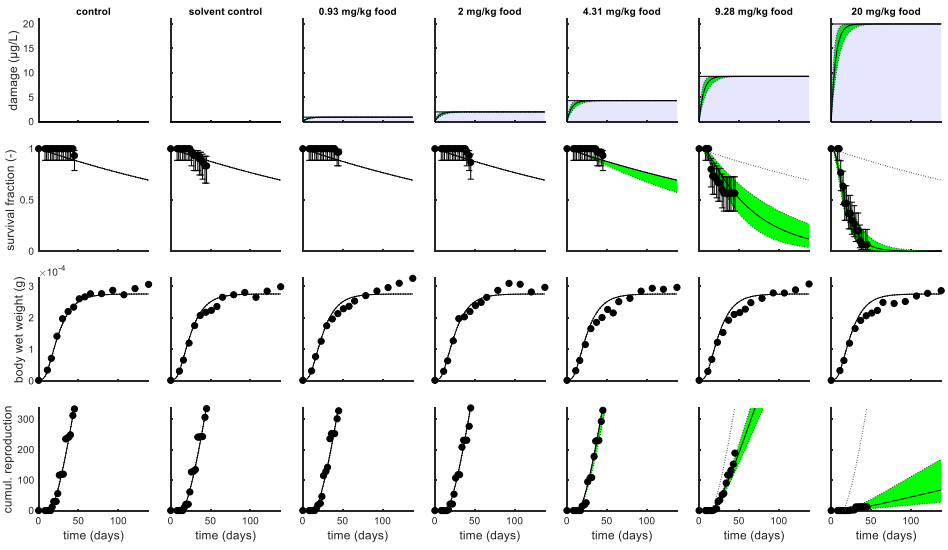

Figure 7: Fit on the complete data set, with one treatment/endpoint per panel. Green areas show the uncertainty in the model curve resulting from the parameter uncertainty in the TKTD parameters. Note that the data set was truncated for survival and reproduction data. The dotted line in the treatments indicates the control response.

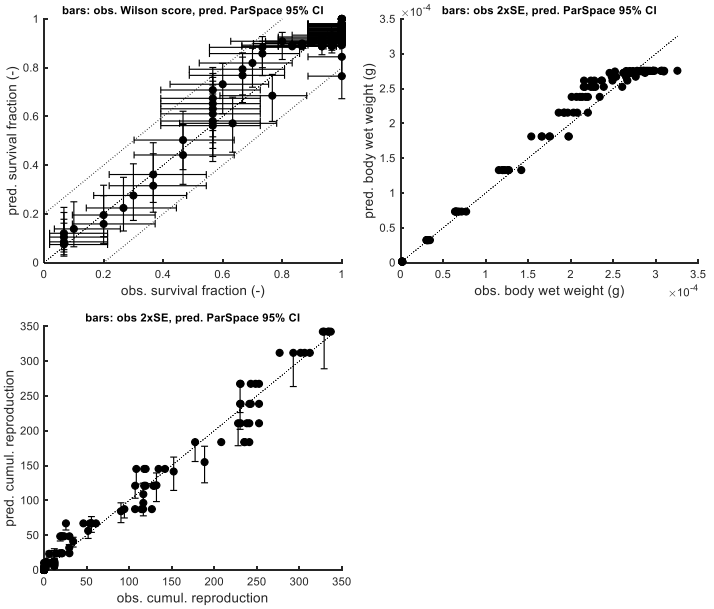

Figure 8: Predicted-observed plots for the fit in Figure 7. Uncertainty in the model prediction is shown as vertical error bars (95% CI). Uncertainty in the data is only shown for survival as the Wilson score interval on the observed survival fraction.

5.3 Local sensitivity analysis

The BYOM platform includes various methods for sensitivity analysis. The first analysis is a classic local analysis. Each parameter is changed by a small fraction, and the response on the endpoints plotted. This has to be done separately for each endpoint, each treatment, and each timepoint. Figure 9 shows the results for the damage state variable. Clearly, only parameter k_d influences this endpoint, and only for the treatments with exposure. When damage approaches steady-state, the sensitivity for k_d decreases, as this parameter only affects the speed at which steady state is achieved.

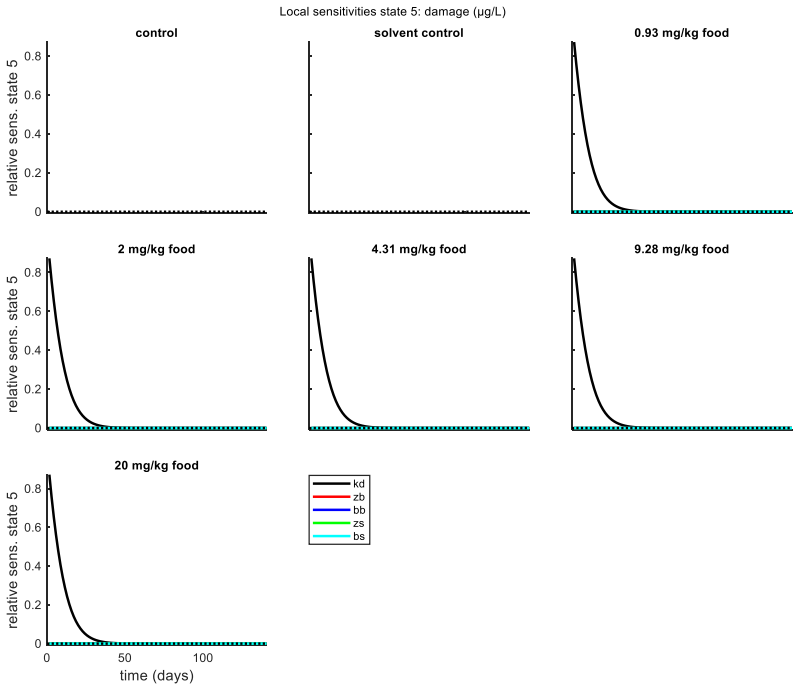

Figure 9: Local sensitivity analysis for the state variable damage.

Sensitivity for survival is shown in Figure 10. This endpoint is mainly affected by z_s and b_s, but in an opposite manner: increasing the threshold increases survival, while increasing the effect strength decreases it. Survival is not very sensitive to the value for k_d.

Figure 11 shows that reproduction is mostly sensitive to z_b and b_b, and that sensitivity is rather constant over time. Reproduction is not very sensitive to the value for k_d.

Sensitivity analysis is often mentioned as an essential aspect of modelling (e.g., [1]). However, for models that are fitted to data, it is unclear what it adds to an analysis [14]. The k_d is a rather insensitive parameter for survival and reproduction, in this case study, but this does not mean that we should remove it from the model.

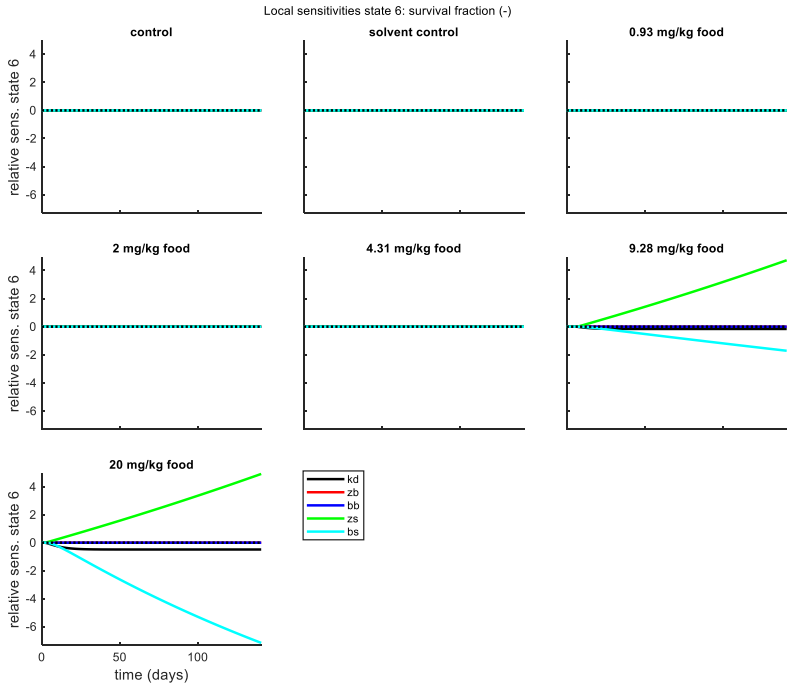

Figure 10: Local sensitivity analysis for the state variable survival.

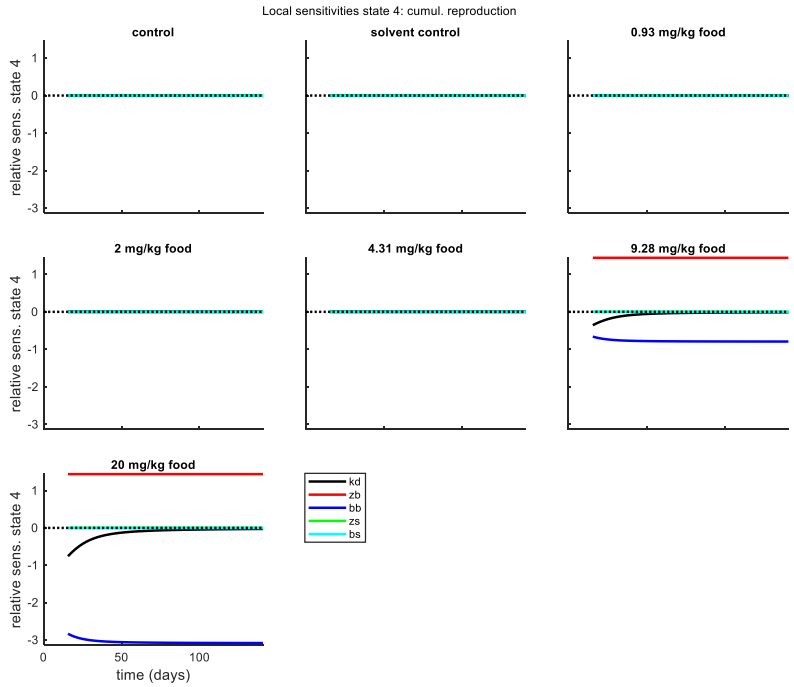

Figure 11: Local sensitivity analysis for the state variable cumulative reproduction.

5.4 Sensitivity as contribution to uncertainty in output

A different measure of sensitivity is a parameter's contribution to the overall uncertainty. We can approximate this relative contribution by calculating the correlation between a parameter's value in the sample to the value for an endpoint. Again, this metric is specific for the endpoint, the treatment, and the time point.

Figure 12 shows that k_d contributes most to the uncertainty in damage. Perhaps surprisingly, the other parameters also contribute somewhat. This relates to the (limited) correlations between the model parameters, since only k_d affects damage (this is the fit without feedbacks). The contribution to uncertainty stops at some point in time, probably because the uncertainty in damage becomes very small when damage approaches steady state. Note that for the controls the correlations are zero, as there is no uncertainty in the control fit (at least not in this step of the analysis).

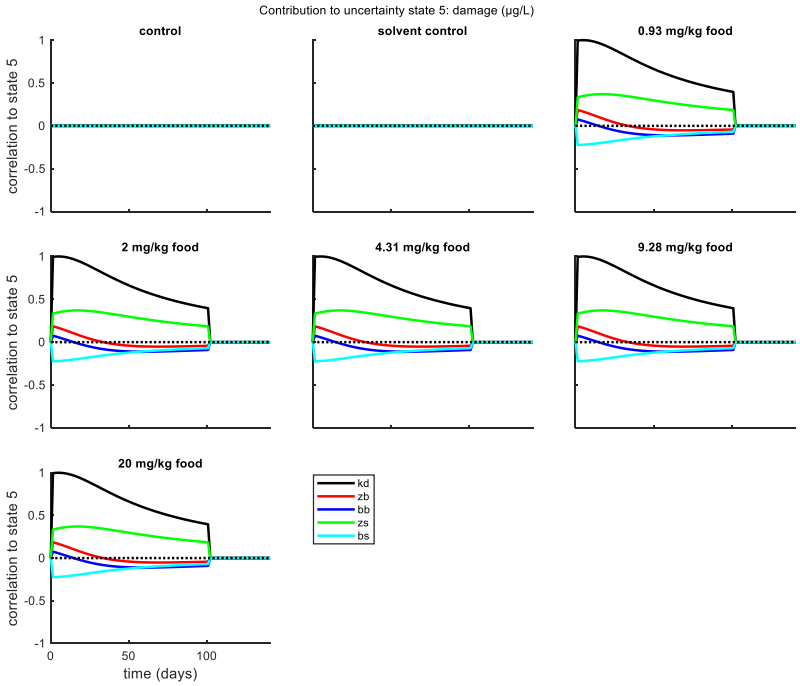

Figure 12: Sensitivity analysis, as contribution to uncertainty, for the state variable damage.

Figures 13 and 14 show the results for survival and reproduction. Again, it is not very clear what such a sensitivity analysis contributes to the analysis. In principle, it allows us to see which parameter contributes most to the uncertainty. However, it is not possible to improve the certainty for one parameter in isolation. Nevertheless, at least in theory, it may be possible to design toxicity experiments such that they stand a better chance of identifying a specific parameter. For example, testing a range of concentrations with no-to-low effects might help to get a better estimate for the threshold parameters, and using pulsed exposure may aid estimation of k_d.

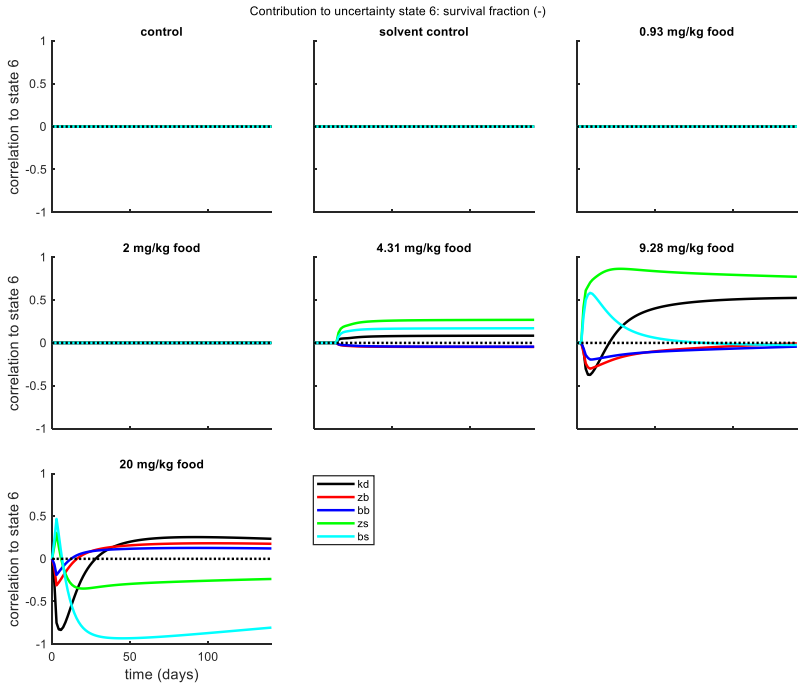

Figure 13: Sensitivity analysis, as contribution to uncertainty, for the state variable survival.

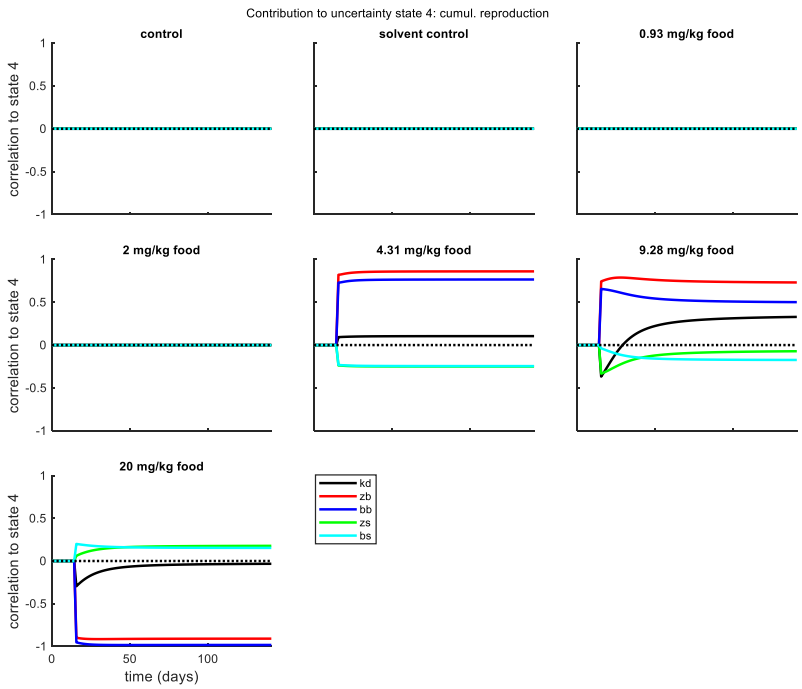

Figure 14: Sensitivity analysis, as contribution to uncertainty, for the state variable cumulative reproduction.

5.5 Alternative model configurations

In the main text, only two pMoA's and four feedback configurations were tested, and only the best of those is shown ('hazards to the embryo' without any feedback). The two pMoA's tested ('costs for reproduction' and 'hazards to the embryo') are logical choices, as there is no clear effect on body size in the toxicity test. However, we can also test the combined pMoA 'costs for growth and costs for reproduction' to see if a subtle effect on growth could be possible. For the feedback configuration, we theoretically have 16 different options. In the main text, we only showed the results for the four most obvious choices, but here we present all permutations.

Note that the feedback configurations that include 'losses with reproduction' also require a value for F_{BV}: the egg weight as fraction of the mother's weight. Following [10], we set $F_{BV} = 0.007$, which is a reasonable value for *F. candida*.

Table 8 shows that 'hazards to the embryo' almost always produces the best fits, generally closely followed by 'costs for reproduction'. The combined pMoA 'costs for growth and costs for reproduction' performs much worse and can be ignored. Interestingly, the overall best fit results from the feedback configuration [1,0,0,1]. This implies that uptake (or damage accrual) is scaled with surface:volume ratio, but not elimination (or damage repair). Furthermore, it has losses with reproduction, but not dilution by growth. The first part may be reasonable: for chlorpyrifos, it may not be such a stretch of the imagination to assume that damage accrual is dominated by uptake of the parent compound across a body surface, while damage repair is not a surface related process (e.g., because it is represents the slow repair or renewal of affected acetylcholinesterase). The second part is however unrealistic: losses with reproduction are in essence a form of growth dilution. It is only that the biomass produced does not remain attached to the mother's body. It seems unrealistic to assume that chlorpyrifos (or the damage it causes) is preferentially transported into the eggs.

At this point, it is good to take a look at this overall best fit, and see what makes the fit in Figure 15 so good. This fit does not look too different from the fit for feedbacks [0,0,0,0], as shown in the main text. However, there are two interesting differences. Firstly, for survival, the mortality in treatment 9.28 mg/kg now levels off around day 30. Secondly, the cumulative reproduction in the same treatment curves upwards, more strongly than for the fit in the main text. In both cases, the fit to treatment 9.28 mg/kg in Figure 15 provides a closer match to the observations. The data set thus indicates that toxic effects become less pronounced later in the toxicity test. The feedback configuration [1,0,0,1] implies lower body residues (or damage levels) for adults than for juveniles, and thus less effects. The uptake rate scales with surface:volume ratio, which means it is higher in small individuals. The elimination rate is not affected by size, so as a result, the steady state body residue (or damage level) is higher in juveniles. On top of that, adults obtain an extra elimination route through egg production.

It is thus understandable why configuration [1,0,0,1] should come out as the best one. However, is it reasonable to assume that this is 'true'? As already explained, losses with reproduction without associated losses due to growth dilution seems unrealistic. Furthermore, there is a reasonable possibility that the behaviour in this treatment is an artefact.

Feedbacks	repro costs	repro hazards	growth+repro costs
[0,0,0,0]	31	28	78
[0,0,0,1]	18	13	60
[0,0,1,0]	47	44	115
[0,0,1,1]	47	42	100
[0,1,0,0]	61	44	85
[0,1,0,1]	57	39	62
[0,1,1,0]	62	45	85
[0,1,1,1]	62	44	85
[1,0,0,0]	21	16	90
[1,0,0,1]	19	0	89
[1,0,1,0]	57	61	177
[1,0,1,1]	51	51	181
[1,1,0,0]	38	34	94
[1,1,0,1]	20	6.4	94
[1,1,1,0]	48	47	176
[1,1,1,1]	47	47	192

Table 8: Goodness of fit for different pMoAs and feedback configurations. Values are the difference in Akaike Information Criterion (ΔAIC), relative to the best fitting configuration.

It could be caused by random variation, or by slight differences in the actual concentration of chlorpyrifos in food (the analysis was based on nominal concentrations, since no measurements were taken). However, it could also result from a real change in sensitivity with age, e.g., an increase of the thresholds for effects over ontogeny. Another possibility is a small amount of inter-individual variation in sensitivity (e.g., the thresholds for effects). If mortality strikes the most sensitive individuals, this will produce a lower apparent severity of the sub-lethal toxic effects over time. In principle, we could check this by looking at the data for individuals over time (as the springtails were kept individually). Unfortunately, the test design was such that animals that died in the reproduction part were replaced by animals from a reserve pool kept under the same conditions. The survival data are thus based on the total of 30 animals per treatment, while reproduction is scored on 10 individuals (or less when there were no spare animals left).

All in all, these issues provide little confidence in configuration [1,0,0,1], as being more meaningful than the default configuration [0,0,0,0]. This example clearly illustrates the difficulties of identifying the most plausible feedback configuration. Applying short, pulsed, exposure at different points in the life cycle might be an experimental design better suited for this purpose.

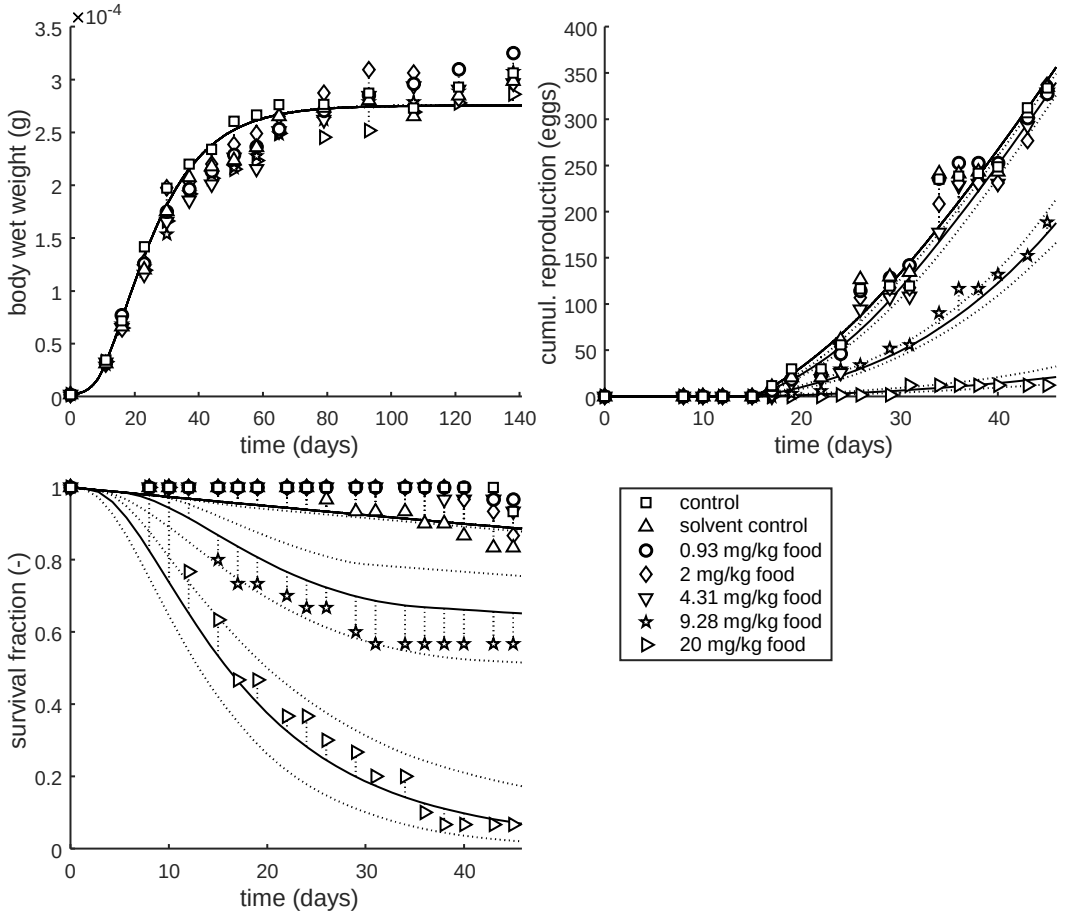

Figure 15: Overall best fit to the data, assuming the pMoA 'hazards to the embryo' and the feedback configuration [1,0,0,1]. Dotted lines around the model curves show the 95% CI on the model output, resulting from uncertainty in the toxicity parameters.

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