# *Supporting Information for:* Dynamic modeling of sub-lethal mixture toxicity in the nematode *Caenorhabditis elegans*

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7 figures, 6 tables, 17 pages.

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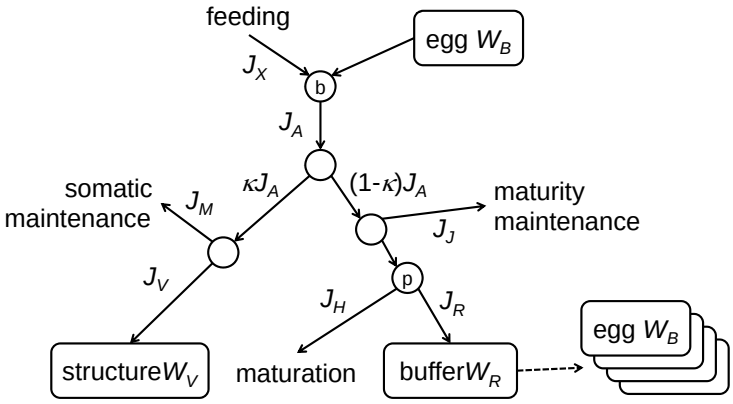


Figure S1: Schematic diagram of the mass flows in the DEBkiss model extended with maturity maintenance (and showing the maturation flux $J_H$ and the feeding flux $J_X$, which are not further specified here). The node 'b' denotes a switch at birth (switching assimilation from the egg buffer to the result of feeding), and the node 'p' denotes a switch at puberty (switching investment into maturity to the reproduction buffer). Other nodes represent a continuous split of a mass flux.

# Model description

DEBkiss is derived and presented in detail elsewhere [7]. In this study, we depart from the model for ecotoxicological analyses as presented in Barsi et al. [3] (in their supplementary materials). Here, we largely repeat that model description, including the specific adaptations for this study: initial food limitation in juveniles, and adapted toxicokinetics for cadmium. The mass flows in the model are presented schematically in Figure S1; symbols are explained in Table S1.

| Symbol | Explanation | Dimension | Sugg. value |
|---|---|---|---|
| | **Primary parameters** | | |
| $f$ | Scaled functional response (0-1) | $-$ | 1 |
| $f_B$ | Apparent $f$ for the embryo (0-1) | $-$ | 1 |
| $J^a_{Am}$ | Maximum area-specific assimilation rate | $m_a/(l^2t)$ | $-$ |
| $J^v_J$ | Volume-specific maturity maintenance costs | $m_a/(l^3t)$ | $J^v_M(1-\kappa)/\kappa$ |
| $J^v_M$ | Volume-specific somatic maintenance costs | $m_a/(l^3t)$ | $-$ |
| $W_{B0}$ | Assimilates in a single freshly-laid egg | $m_a$ | $-$ |
| $W_{Vp}$ | Structural body mass at puberty | $m$ | $-$ |
| $y_{BA}$ | Yield of egg buffer on assimilates | $m_a/m_a$ | 0.95 mg/mg (dwt) |
| $y_{VA}$ | Yield of structure on assimilates (growth) | $m/m_a$ | 0.8 mg/mg (dwt) |
| $\kappa$ | Fraction of assimilation flux for soma | $-$ | 0.8 |
| | **Conversions** | | |
| $d_V$ | Dry-weight density of structure | $m/l^3$ | 0.1 mg/mm$^3$ |
| $\delta_M$ | Shape correction coefficient | $-$ | |
| | **Fluxes, states and forcings** | | |
| $J_A$ | Mass flux for assimilation | $m_a/t$ | |
| $J_H$ | Mass flux for maturation | $m_a/t$ | |
| $J_J$ | Mass flux for maturity maintenance | $m_a/t$ | |
| $J_M$ | Mass flux for somatic maintenance | $m_a/t$ | |
| $J_R$ | Mass flux to reproduction bufeer | $m_a/t$ | |
| $J_V$ | Mass flux for structure | $m/t$ | |
| $J_X$ | Mass flux of food | $m_f/t$ | |
| $W_B$ | Mass of assimilates buffer in egg | $m_a$ | |
| $W_R$ | Mass of reproduction buffer in adult | $m_a$ | |
| $W_V$ | Mass of structural body | $m$ | |
| | **Other output and secondary parameters** | | |
| $L$ | Volumetric body length | $l$ | |
| $L_w$ | Physical body length | $l$ | |
| $R$ | Continuous reproduction rate | $\#/t$ | |
| $t_b$ | Hatching time for the egg | $t$ | |
| $W_{Vb}$ | Structural body mass at birth | $m$ | |

Table S1: Explanation of symbols for the basic model parameters, with dimensions given in mass ($m$ for body, $m_a$ for assimilates, and $m_f$ for food), length ($l_e$ for environment, $l$ for organism), numbers (#), time ($t$). Suggested values for the yields (apart from $y_{AV}$) based on the typical values in [12].

## Basic model

DEBkiss is a simplified version of the standard DEB animal model [14]. The basic model applies three state variable: the egg buffer $W_B$ (used by the embryo), structural body mass $W_V$, and reproduction buffer $W_R$ (see Fig. S1). Here, we only consider continuous reproduction (as adults of *C. elegans* produce eggs one by one), and we therefore do not use the reproduction buffer. The dynamics of the remaining state variables are given by:

$$\frac{d}{dt}W_B = -J_A \quad \text{until } W_B = 0, \text{ with } W_B(0) = W_{B0} \tag{1}$$

$$\frac{d}{dt}W_V = J_V \quad \text{with } W_V(0) \approx 0 \tag{2}$$

Note that $t = 0$ here marks the start of development in the egg. The embryo will hatch when $W_B = 0$, which thereby determines the hatching time $t_b$ and the dry weight at birth $W_{Vb}$. If we do not deal with embryos, we can start at any weight $W_{V0} > W_{Vb}$.

Apart from body weight ($W_V$), we also need the structural volume ($L^3$) of the animal. We assume a constant density for structure ($d_V$):

$$L^3 = \frac{W_V}{d_V} \tag{3}$$

We can talk about $L$ as the 'volumetric structural length' of the animal. If the structural biomass $W_V$ is compressed into a cube, this will be the length of a side of that cube. In many cases, we measure body size of an animal as some length measure (e.g., the body length of nematodes). As long as the organism does not change in shape during growth, we can translate structural length to some physical length ($L_w$) and vice versa using a constant correction factor $\delta_M$:

$$L_w = \frac{L}{\delta_M} \tag{4}$$

The assimilates obtained from feeding are directly used in metabolism, and therefore, we do not consider any storage. The assimilation flux $J_A$ is given by:

$$J_A = f J^a_{Am} L^2 \quad (\text{if } W_B > 0 \text{ then } f = 1 \text{ and } J^a_{Am} = f_B J^a_{Am}) \tag{5}$$

where $J^a_{Am}$ is the surface-area-specific maximum assimilation rate, and $f$ is the scaled functional response, which is the actual feeding rate at a certain food level divided by the maximum feeding rate for its current size. The scaled response $f$ is thus between 0 (no food) and 1 (*ad libitum* food). Here, we treat $f$ as a primary parameter, and do not consider the details of feeding, which is appropriate for constant or *ad libitum* food availability. For embryos, a different (lower) specific assimilation rate may be required, and hence the inclusion of a separate parameter $f_B$ (see [7]).

Maintenance is the lump sum of all the processes needed to maintain the body's integrity. Assimilate buffers are assumed not to require maintenance, so the total maintenance flux is proportional to the structural body volume:

$$J_M = J_M^v L^3 \tag{6}$$

where $J_M^v$ is the volume-specific maintenance rate coefficient. The assimilation flux is split with a constant fraction of $\kappa$ to the soma. Maintenance costs are paid first, and the remaining flux to the soma is used for growth:

$$J_V = y_{VA}(\kappa J_A - J_M) \tag{7}$$

where $y_{VA}$ is the yield of structural biomass on assimilates.

The maturity maintenance flux $J_J$ is also proportional to the structural body volume, but only up to puberty:

$$J_J = J_J^v L^3 \quad \text{when } W_V < W_{Vp} \tag{8}$$

$$J_J = J_J^v L_p^3 \quad \text{when } W_V \geq W_{Vp} \text{ (note: } L_p^3 = W_{Vp}/d_V) \tag{9}$$

where $J_J^v$ is the volume-specific costs for maturity maintenance. In principle, $J_J^v$ is a primary parameter that can be fitted to experimental data. However, we will set it to a 'suggested value', by assuming a link with somatic maintenance:

$$J_J^v = \frac{1-\kappa}{\kappa} J_M^v \tag{10}$$

In the standard DEB model, linking these maintenance processes in this exact way yields the situation where the cumulative investment in maturity at puberty is independent of the food availability. This is one of the assumptions underlying the 'DEBtox' simplification [9]. The maturity maintenance flux is withdrawn from the $1-\kappa$ flux first, so the reproduction flux becomes:

$$J_R = (1-\kappa)J_A - J_J \quad (\text{if } W_V < W_{Vp} \text{ then } J_R = 0) \tag{11}$$

where $W_{Vp}$ is the structural body mass where investment in reproduction starts (puberty). Note that $W_{Vp}$ can easily be translated into a corresponding physical length $L_{wp}$.

The continuous reproduction rate $R$ can be calculated as:

$$R = \frac{y_{BA} J_R}{W_{B0}} \tag{12}$$

where $y_{BA}$ is the yield for the conversion of reproduction buffer to eggs, and $W_{B0}$ is the dry weight of a single egg.

The starvation response is left out of this model description, as starvation does not occur in the current experimental setup. Model equations for starvation can be found elsewhere [7, 3].

## Deviating growth curves

Growth of nematodes regularly deviates from the von Bertalanffy pattern that is predicted by DEB models under constant conditions. For bacterivorous nematodes, the most likely cause is that juveniles cannot feed efficiently on the provided food (bacteria) [10]. Following [6], we can take a stress factor on the apparent food availability as hyperbolic function of body weight:

$$f = f_0 s_f \quad \text{with} \quad s_f = \left(1 + \frac{W_{Vf}}{W_V}\right)^{-1} \tag{13}$$

where $f_0$ is the food level experienced by the fully-grown adults.

## Adding toxicokinetics

| Symbol | Explanation | Dimension | Sugg. value |
|---|---|---|---|
| $c_A$ | Addition to the concentration in water phase | $m_q/l_e^3$ | – |
| $c_d$ | Dissolved concentration in water phase | $m_q/l_e^3$ | – |
| $c_V$ | Scaled concentration in structure | $m_q/l_e^3$ | – |
| $k_e$ | Elimination rate constant | $1/t$ | – |
| $k_e^*$ | Reference elimination rate constant | $1/t$ | – |
| $P_{RV}$ | Partition coeff. repro buffer-structure | $m/m_a$ | 1 |

Table S2: Explanation of additional symbols, 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 ($t$).

The simplest model for toxicokinetics (TK) is the first-order one-compartment model, where the entire organism is seen as a well-mixed homogeneous compartment. In the absence of a (considerable) reproduction buffer, we can use the following equation for the scaled ($c_V$) internal concentration in a growing organism (see [9]):

$$\frac{d}{dt}c_V = k_e^* \frac{L_m}{L}(c_d - c_V) - \frac{c_V}{W_V}\frac{d}{dt}W_V \tag{14}$$

where $c_d$ is the dissolved concentration in water,[1] and $k_e^*$ is the reference elimination rate constant at maximum size in the control ($L_m = \kappa J^a_{Am}/J^v_M$). The elimination rate scales with a surface:volume and thus inversely with a length measure (as long as growth is isomorphic). The last term in the equation deals with growth dilution (and increase of the concentration when shrinking). Note that $c_V(\infty) = c_d$.

We assume that chemical exchange mainly relates to the feeding process (which is supported by [5]), and therefore, we apply the same size-dependent stress factor as in Eq. 13 to the elimination rate:

[1] In practice, we replace $c_d$ with the nominal concentration in agar. This does not make a difference as long as the dissolved concentration is proportional to the nominal one. However, it should be note that all paramaters with chemical mass in their dimensions relate to nominal concentrations.

$$k_e^* = s_f k_{e0}^* \tag{15}$$

where $k_{e0}^*$ is the reference elimination rate in the absence of food limitation. This same factor was also applied in previous DEB-based analyses for nematodes, and improves the fit to effects in the initial part of the growth curve.

Chemical losses due to reproduction can easily be included in the model, when we assume that the concentration in the egg at egg laying is in equilibrium with the internal concentration of the mother. The chemical's affinity for the egg material is not necessarily the same as that for the adult tissues. Therefore, it is practical to introduce a partition coefficient between the egg material and the structure of the mother, $P_{RV}$. In practice, the value for $P_{RV}$ will be unknown (it would require measurements of residues in mother and eggs), but we can depart from an equal affinity, and thus set $P_{RV} = 1$.

When we can consider reproduction to be continuous, for adults there will be a continuous flux of chemical out of the body with eggs. This flux can be subtracted from the changes in concentration as follows:

$$\frac{d}{dt}c_V = k_e^* \frac{L_m}{L}(c_d - c_V) - \frac{c_V}{W_V}\frac{d}{dt}W_V - \frac{W_{B0}R}{W_V}P_{RV}c_V \tag{16}$$

For cadmium, the pattern of effects over the doses could not be captured by the standard toxicokinetics assumptions. Instead, we assumed an addition to the external concentration as follows:

$$c_d = c_{d0} + c_A \tag{17}$$

where $c_A$ is a constant addition to the concentration, and $c_{d0}$ is the nominal cadmium concentration.

We did not attempt to model toxicokinetics in the egg. Instead, we assumed instantaneous steady state for fluoranthene ($c_V = c_d$) and no uptake for cadmium ($c_V = 0$), which is reasonable given the estimates for the elimination rate for both compounds.

## Toxicant effects

| Symbol | Explanation | Dimension | Sugg. value |
|---|---|---|---|
| $c_0$ | Scaled no-effect concentration metabolic effects | $m_q/l_e^3$ | – |
| $c_T$ | Tolerance concentration metabolic effects | $m_q/l_e^3$ | – |
| $s$ | Stress factor for metabolic effects | $[-]$ | – |

Table S3: Explanation of additional symbols, with dimensions given in mass ($m_q$ for chemical mass), length ($l_e$ for environment), time ($t$).

The internal concentration can be linked to any of the primary parameters of the model (see [9, 8]). The affected parameter(s) is called the metabolic mode of action, or mMoA (see [2]). Following [11], we can use a linear-with threshold relationship for the dimensionless stress level on a parameter (in the control, $s = 0$):

$$s = \frac{1}{c_T} \max(0, c_V - c_0) \tag{18}$$

Here, the dose metric is the scaled internal concentration in structure $c_V$. The threshold or no-effect concentration is represented by $c_0$; below this NEC, the stress level will be zero. The proportionality $c_T$ is called the 'tolerance' concentration. Stress can increase or decrease the value of a parameter $p$ like so:

$$p \to p(1+s) \quad \text{or} \quad p \to p \max(0, 1-s) \tag{19}$$

For some parameters there is room for discussion. Take the yield coefficient for structure on assimilates $y_{VA}$. A decrease in the yield can be implemented as $y_{VA}(1-s)$. But, if we interpret the effect as an increase in the overhead costs for growth, we should take $y_{VA}/(1+s)$. It all depends on our interpretation of the affected process. In the past, effects on yields have been implemented as an increase in the overheads [11, 8, 9].

Here, we selected an effect of cadmium on the scaled functional response as follows (the superscript zero denotes the value in the control), as this stress function provided the best explanation of the effect patterns:

$$f = \frac{f_0}{1+s} \tag{20}$$

This mode of action leaves open the possibility that the decrease in assimilation is caused by a decrease of the feeding rate (e.g., an increase in the handling time of food items) or a decrease of the assimilation efficiency (e.g., an increase in the overhead costs for assimilation).

For fluoranthene, we used a combination of effects on the costs for growth and the costs for reproduction (it should be noted that the stress factor $s$ for fluoranthene is different from that of cadmium, as it depends on the specific internal concentration for each chemical):

$$y_{VA} = \frac{y_{VA}}{1+s} \quad \text{and} \quad y_{BA} = \frac{y_{BA}}{1+s} \tag{21}$$

In the mixture, all stress factors in Equations 20 and 21 are combined and are applied simultaneously in the same individual. The stress for each chemical is of course calculated from its own specific scaled internal concentration $c_V$, and its own toxicodynamic parameters $c_0$ and $c_T$.

# Combining solvent and blank for fluoranthene

For fluoranthene, we decided to combine both controls as they yield very similar data for growth and reproduction. We can compare the fit with all parameters different between the two controls (see Fig. S2) to the fit where all parameters are forced to take the same value. The resulting log-likelihood values are -141.95 and -142.65. The difference in log-likelihood is thus less than 1, whereas it would be significant only at half of the critical value for a $\chi^2$-distribution with six degrees of freedom: $12.6/2 = 6.3$ (the difference in the number of fitted parameters is the degrees of freedom). Even if we could get the same improved goodness-of-fit using one parameter difference between the two sets, it would still not be a significant improvement (with one parameter different between two fits, the critical difference in log likelihood would still need to be 1.9). Therefore, there is no reason to treat the blank and the solvent control as different.

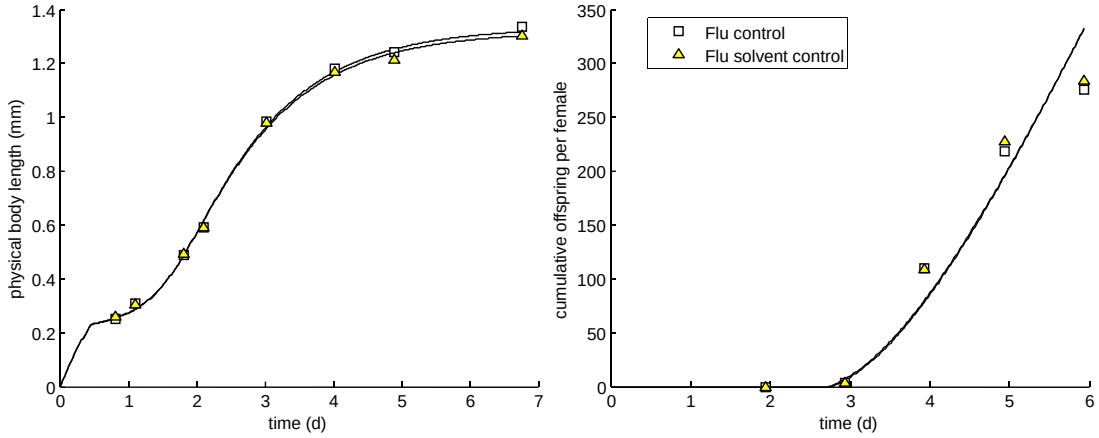


Figure S2: Fit to the blank and solvent control for fluoranthene, with all parameters different between the two data sets.

# Additional analysis for cadmium

For cadmium, we can also perform an analysis in line with the earlier work [1, 15]. This means that there is no addition to the external concentration ($c_A = 0$), but a no-effect threshold and a saturation on the uptake rate (which is included as a modification of the external concentration $c_d$):

$$c_d = \frac{c_d c_{dK}}{c_d + c_{dK}} \tag{22}$$

where $c_{dK}$ is a half-saturation constant. Furthermore, the effect on the feeding proces was implemented in a different manner as in the current study:

$$f = f_0 \max(0, 1 - s) \tag{23}$$

The fit is shown in Figure S3 and the parameter estimates in Table S4.

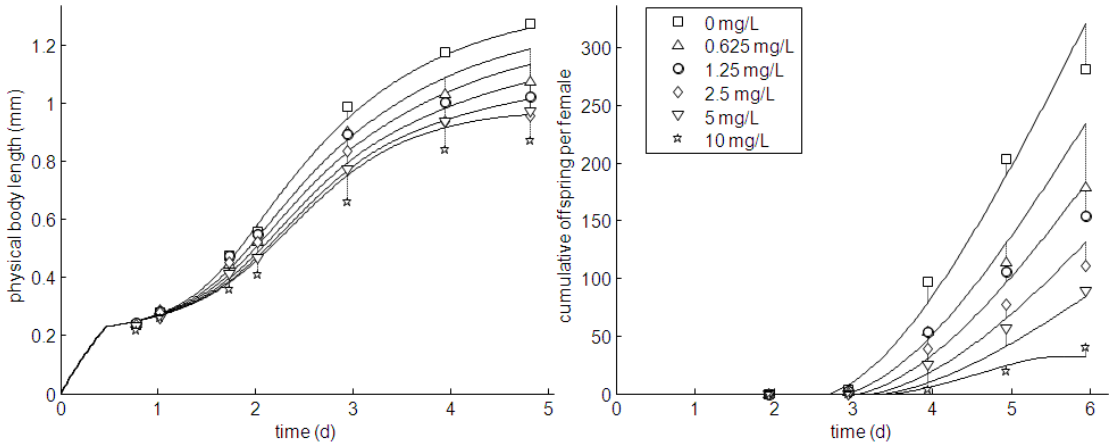


Figure S3: Fit for the effects of cadmium, assuming standard effects on assimilation with saturation.

| Symbol | Explanation | Unit | Value |
|---|---|---|---|
| $c_0$ | Scaled no-effect concentration | mg/L | 8.7 $10^{-5}$ (7.6 $10^{-5}$ - 0.0135) |
| $c_{dK}$ | Half-saturation concentration | mg/L | 0.56 (0.39 - 0.76) |
| $c_T$ | Tolerance concentration metabolic effects | mg/L | 0.0038 (0.0025 - 0.36) |
| $k_e$ | elimination rate constant | 1/d | 0.0010 (0.0010 - 0.14) |

Table S4: Parameter estimates (with 95% confidence interval) for the additional fit for cadmium. Note that the lower bound of $k_e$ is set to 0.0010 $d^{-1}$

# Profile likelihood FA

The profile likelihood for the no-effect concentration of fluoranthene (in single exposure) shows a pattern that suggests numerical problems. An interval of 0-0.006 mg/L may therefore be more realistic.

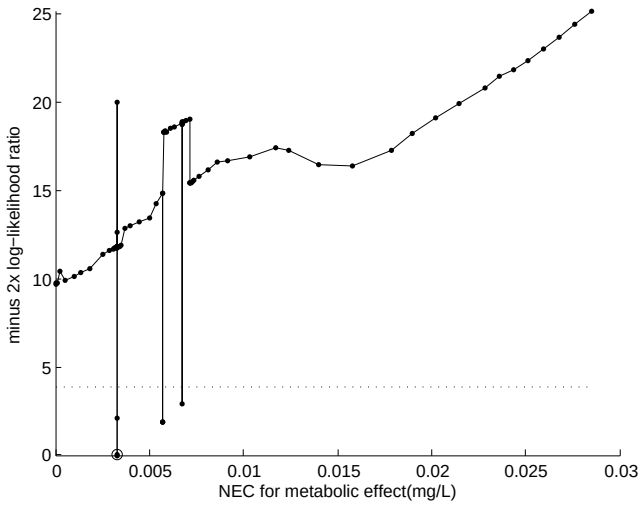


Figure S4: Profile likelihood for $c_0$ of FA. Circle indicates the best fit. Dotted horizontal line indicates the threshold for 95% confidence.

# Mixture fits with DEBkiss

We can fit the model parameters on all data simultaneously; thus both single experiments and the mixture experiment. The basic fit would be the one where each chemical has the same parameter set both in the single exposure as in the mixture (complete independence). This requires fitting 6 parameters and leads to a log-likelihood of -5793.05.

We can compare this value to a fit where we allow some parameters to differ in the mixed exposure. For example, we can assume that the elimination rates will be different in the mixture (i.e., the chemicals interact on their toxicokinetics). This requires two more parameters and yields a log-likelihood of -5742.32 (Fit 2, displayed in Fig. S5). This is a difference in log-likelihood of 51. To check its significance, we need to compare this difference to half of the critical value for a $\chi^2$-distribution with two degrees of freedom: $5.99/2 = 3.0$ (the difference in the number of fitted parameters is the degrees of freedom). Clearly, the fit is significantly improved by allowing different values for the elimination rate in the mixture than for the single exposures. In this case, assuming only the $k_e$ of FA to differ in the mixture yields a log-likelihood of -5742.65, which implies that leaving the $k_e$ for Cd free too does not add anything to the goodness of fit.

In Fit 3, a different value for the tolerance concentration $c_T$ is allowed in the mixture. This is a significant improvement compared to Fit 1, but not so spectacular as for Fit 2.

| Symbol | Fit 1 | Fit 2 | Fit 3 |
|---|---|---|---|
| $c_A$ Cd | 5.4 (5.2-5.8) | 8.0 | 7.5 |
| $c_T$ Cd | 0.18 (0.17-0.19) | 0.74 | 0.15 (0.11-0.17) |
| $k_e$ Cd | 0.0019 (0.0015-0.0023) | 0.0067 (0.0060-0.0083) | 0.0014 |
| $c_0$ FA | 0.018 (0.017-0.018) | 0.064 | 0.032 |
| $c_T$ FA | 2.5 (2.5-2.5) | 2.2 | 2.4 (2.3-2.4) |
| $k_e$ FA | 42 (13-56) | 100 (37-100) | 72 |
| $k_e$ Cd (mix) | – | 0.0065 (0.0030-0.010) | – |
| $k_e$ FA (mix) | – | 1.6 (1.2-2.6) | – |
| $c_T$ Cd (mix) | – | – | 0.18 (0.18-0.21) |
| $c_T$ FA (mix) | – | – | 2.5 (2.5-2.5) |
| loglik | -5793.05 | -5742.32 | -5780.80 |

Table S5: Parameter estimates for the fits to all data simultaneously (single and mixed exposures). Note that the lower bound of $k_e$ is set to 0.0010 $d^{-1}$ and the upper bound to 100. Values in parentheses are 95% confidence intervals (not calculated for all parameters). Fit 1 is for all toxicological parameters the same in the mixture and in the single exposures, in Fit 2, $k_e$ is allowed to differ in the mixture, and in Fit 3, $c_T$ is allowed to differ. For each fit, the log-likelihood is given; a higher (less negative) value thus means a better fit. Fit 2 and Fit 3 are significantly better than Fit 1 in a likelihood ratio test.

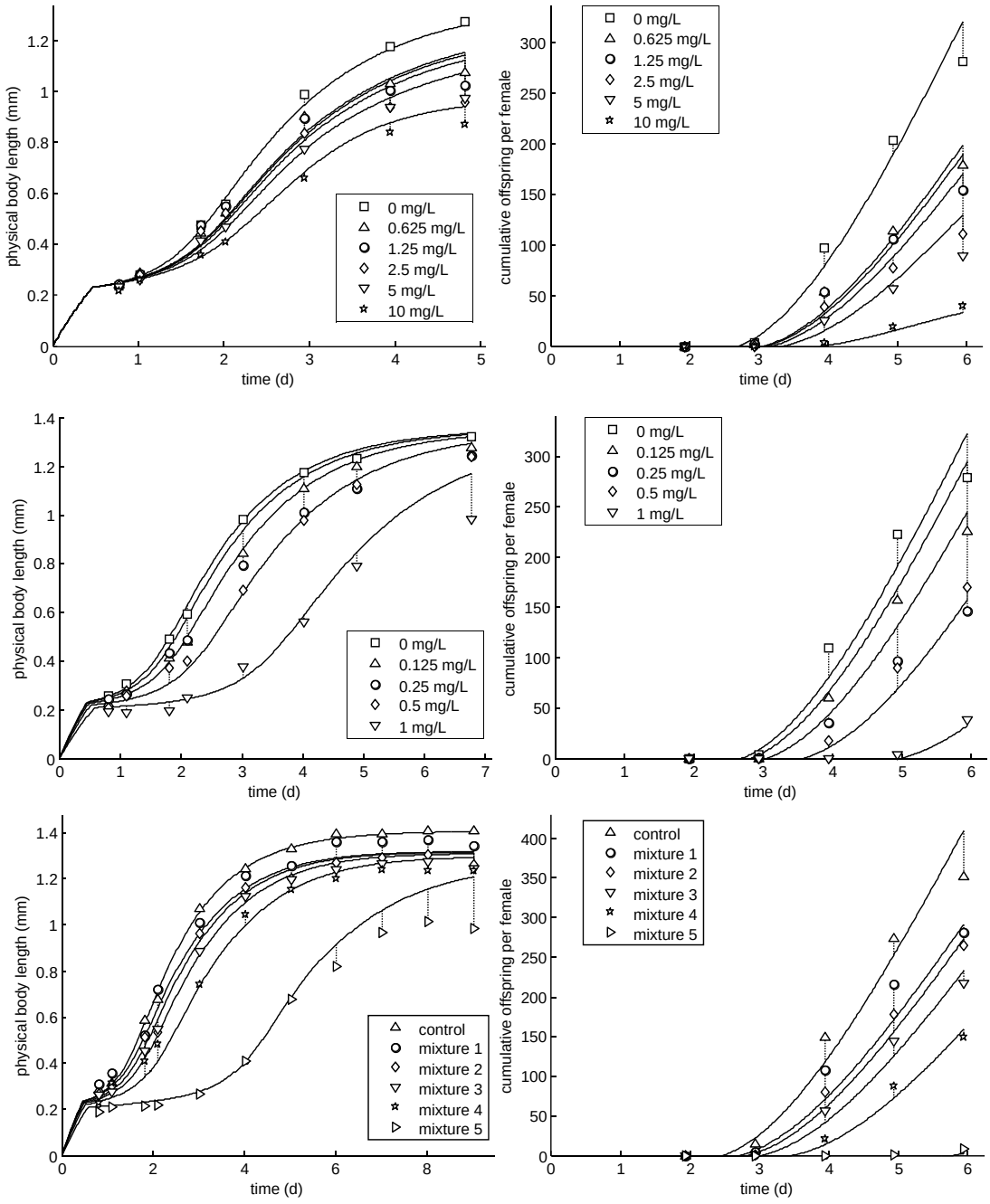


Figure S5: Fit to all data for single compounds and mixtures simultaneously, allowing a different value of the elimination rates in the mixed exposure (Fit 2).

## Mixture analysis based on dose-response curves

Data on body length and cumulative reproduction normalised to the control were described by a classical two parameter log logistic concentration-response model:

$$R = \frac{1}{1 + \left(\frac{x}{EC50}\right)^b} \tag{24}$$

Where $x$ is the concentration in agar, $EC50$ is the concentration giving a 50% decrease in the response, and $b$ is proportional to the slope of the dose-response curve around $x = EC50$. Based on the curve parameters for the individual compounds the response of the mixture was predicted under the assumption of Independent Action [13, 4]:

$$R_{mix} = R_{Cd} R_{FA} \tag{25}$$

Where $R_{mix}$ denotes the normalized response of the mixture ($0 < R_{mix} < 1$) based on the product of the normalized responses of the individual compounds at the concentrations present in the mixture. The IA prediction was then described by a concentration response curve, and significance of deviations of the observed mixture data from the predicted was tested. This was done by comparing a joint fit of all three dose-response models (Cd, FA and the mixture) with a fit of all three curves with the mixture $EC50$ and $b$ parameter fixed to those of the IA prediction with a Lack-of-fit F-test (see Table S6).

It should be noted that an IA prediction will never precisely fit a log-logistic model, as the product of two logistic models does not yield a logistic model. But since the approximation is close (see Figure S6), we chose to test for significance in this way, as it is similar to the way significance of interaction for the DEBkiss model is tested.

We performed this analysis for two time points, 4 and 6 days post-synchronisation. The size measurements for the single Cd experiment, however, did not continue for 6 days, so for Cd-growth $t = 5$ days was used as proxy (which is acceptable as the effects change little from day 4 to day 5).

| Day | Endpoint | Curve | $EC50$ | $b$ | $p$ |
|---|---|---|---|---|---|
| Day 4 | Length | Cd | 85 (-180-351) | 0.39 (0.02-0.78) | |
| | | FA | 1.35 (0.30-2.40) | 0.71 (0.25-1.18) | |
| | | CdFA | 2.01 (1.73-2.29) | 3.70 (1.98-5.42) | |
| | | IA | 1.79 | 1.69 | $6 \cdot 10^{-5}$ |
| | Repro | Cd | 1.21 (0.92-1.52) | 0.87 (0.65-1.08) | |
| | | FA | 0.20 (0.17-0.24) | 1.56 (1.17-1.95) | |
| | | CdFA | 0.41 (0.34-0.48) | 1.29 (1.01-1.57) | |
| | | IA | 0.29 | 1.46 | $6 \cdot 10^{-8}$ |
| Day 6 | Length | Cd | 131 (-31-293) | 0.31 (0.21-0.41) | |
| | | FA | 1.44 (1.15-1.73) | 1.84 (1.19-2.48) | |
| | | CdFA | 3.43 (2.87-3.99) | 1.70 (1.18-2.23) | |
| | | IA | 0.28 | 1.46 | $3 \cdot 10^{-50}$ |
| | Repro | Cd | 1.46 (1.18-1.74) | 0.77 (0.62-0.93) | |
| | | FA | 0.49 (0.43-0.56) | 1.35 (1.05-1.65) | |
| | | CdFA | 0.95 (0.82-1.07) | 1.22 (1.01-1.44) | |
| | | IA | 0.65 | 1.36 | $8 \cdot 10^{-6}$ |

Table S6: Parameter estimates from the concentration-response curves from day 4 and day 6 including their 95% confidence intervals (1.96× s.e.), and the results of the Lack-of-fit F-test comparing the free fit of the mixture results to the IA-prediction.

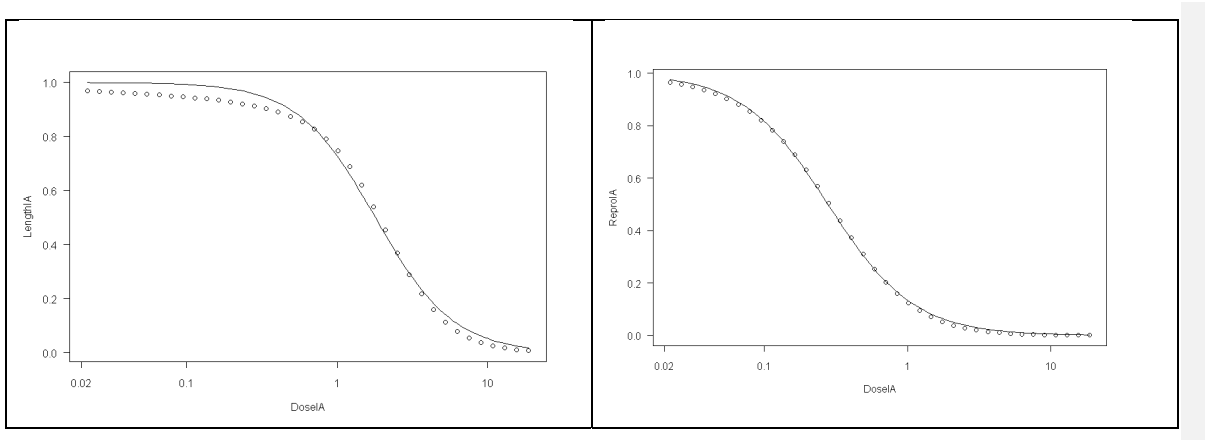


Figure S6: The IA prediction for relative nematode length and cumulative reproduction at day four based on the individual chemical concentration-response curves. The dots give the prediction values, the curve the log-logistic fit to the prediction used for the significance test.

# Size measurements for nematodes

The volume of the nematode was calculated by approximating the body as a cylinder with as width the average of the width taken at 7 points along the body length.

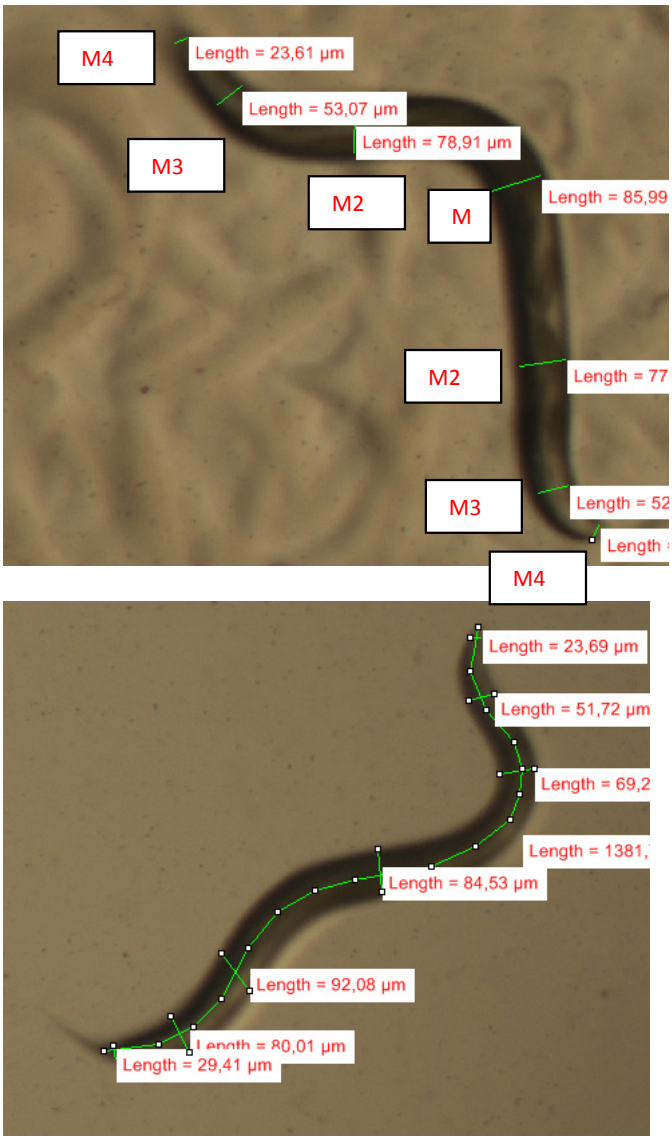


Figure S7: Measurements of length and width for nematodes.

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