# Supporting Information for "General unified threshold model of survival - a toxicokinetic-toxicodynamic framework for ecotoxicology"

*Tjalling Jager*[†], *Carlo Albert*[‡], *Thomas G. Preuss*[#], *Roman Ashauer*[‡*]

† Dept. of Theoretical Biology, Vrije Universiteit, de Boelelaan 1085, NL-1081 HV, Amsterdam, the Netherlands

‡ Eawag, Swiss Federal Institute of Aquatic Science and Technology, 8600 Dübendorf, Switzerland

# Institute for Environmental Research, RWTH Aachen University, Worringerweg 1, D-52064 Aachen, Germany

* Corresponding author, e-mail: roman.ashauer@eawag.ch

## Table of content

## Scaling the toxicokinetics and damage equations

The scaling of the equations for toxicokinetics and damage is perhaps not so intuitive, which is why we provide here the derivation in more detail.

The one-compartment model with first-order kinetics is given by:

$$\frac{dC_i(t)}{dt} = k_i C_w(t) - k_e C_i(t) \quad (1)$$

In this equation, $C_\mathrm{i}$ is the internal concentration (at the target site), $C_\mathrm{w}$ is the concentration in the exposure medium (e.g., water), $k_\mathrm{i}$ is the accumulation rate constant, $k_\mathrm{e}$ the elimination rate constant.

If internal concentrations are not measured and only toxicity data are available, then we cannot fit both rate constants ($k_\mathrm{i}$ and $k_\mathrm{e}$). The time course of survival data might still allow for estimation of the elimination rate ($k_e$), because this rate determines the time to reach equilibrium between the concentration in the organism and its environment, but not $k_\mathrm{i}$. This problem is circumvented by scaling the internal concentration. First, we will use a different parameterization of Eq. 1, by introducing the bioconcentration factor (*BCF*). The *BCF* is the ratio between $C_i$ and $C_w$ in a steady-state situation, which equals the ratio of the two rate constants ($k_i/k_e$) in first-order toxicokinetics. Eq. 1 can thus be rewritten as:

$$\frac{dC_i(t)}{dt} = k_e\left(BCF \cdot C_w(t) - C_i(t)\right). \quad (2)$$

Now, we can divide both sides of this equation by the *BCF*. The internal concentration ($C_i$) divided by *BCF* is now a scaled internal concentration, $C_i^*$. Thereby, $C_i^*$ is directly proportional to the actual (but unknown) internal concentration, and has the dimensions of an external concentration. The equation for the scaled internal concentration is then given as:

$$\frac{dC_i^*(t)}{dt} = k_e\left(C_w(t) - C_i^*(t)\right). \quad (3)$$

In equilibrium, $C_i^*$ will equal the external concentration, $C_w$. The advantage of scaling is that it enables the use of TKTD modeling without access to measured internal concentrations (although relevant measured body residues are always preferred), because the dominant rate constant $k_\mathrm{e}$ can be estimated from toxicity data.

For the buildup of damage, we can follow a completely analogous derivation. The original publications on damage models (*1-3*) applied two rate constants (for damage accrual and recovery):

$$\frac{dD(t)}{dt} = k_a C_i(t) - k_r D(t) \quad (4)$$

Here, $D$ is the damage level, $k_\mathrm{a}$ the rate constant for damage accrual, and $k_r$ is the damage recovery rate constant. We can now rewrite this equation, introducing the "damage accumulation factor" (*DAF*) as the equilibrium ratio between the damage level and the

internal concentration (analogous to the *BCF* for toxicokinetics). The *DAF* is than defined as the rate constant for damage accrual divided by the rate constant for recovery. The new equation for the change in damage is than:

$$\frac{dD(t)}{dt} = k_r\left(DAF \cdot C_i(t) - D(t)\right) \quad (5)$$

Because damage cannot be measured, the data that are available in toxicity tests cannot provide information about the absolute level of damage. The time course of survival will provide information about the recovery rate, $k_r$, but will not be able to identify *DAF*. This situation is fully equivalent to the TK model when internal concentrations are not determined. Similarly, we introduce the scaled damage level $D^*$ as $D$ divided by *DAF* (which is the same as the ratio $k_a/k_r$). Thereby, $D^*$ is directly proportional to the actual (but unknown) damage level, and has the units of an internal concentration:

$$\frac{dD^*(t)}{dt} = k_r\left(C_i(t) - D^*(t)\right) \quad (6)$$

Interestingly, these equations clarify that the scaled internal concentration $C_i^*$ and the scaled damage $D^*$ are actually very similar concepts. Both describe a physical property of the organism that has not been, or cannot be, measured. The only difference is that the concept of damage departs from measured body residues (or estimated ones), whereas the scaled internal concentration departs from information about the external concentration only. In applying the damage model, the recovery rate constant ($k_r$) needs to be estimated from the toxicity data; in the case of the scaled TK model, the elimination rate ($k_e$). In both cases, it is not entirely sure what these rate constants represent. The elimination rate in the scaled TK model might represent the elimination rate for the whole body residue, but just as well, it might represent elimination kinetics at a target site, or a TD process such as the repair of occupied receptors. For the recovery rate constant in the damage model, we can only be sure that it does *not* represent whole-body elimination. It might represent a TD process, or the slowest TD process in a chain of processes. However, it might still represent a TK process when the toxicokinetics at a target site deviate strongly from the whole body. Without further experimentation and/or information about the mechanism of action, this question cannot be answered.

If these concepts are so similar, why call them differently, and why don't we use the same symbol for both? There are two reasons; the first is that we want to keep the link with the models already published in the literature very clear. We want to show that most published TKTD approaches for survival are closely linked, and do not want to give the impression that we are presenting an entirely new model. Secondly, these concepts follow from different underlying assumptions. The scaled TK model assumes that the relevant dose metric is an internal concentration that follows one-compartment, first-order kinetics. The only problem is that we do not have measurements of the (relevant) body residues when we apply this model. The scaled internal concentration is therefore thought to represent a true internal concentration, although in practice, it might represent something else. The damage models assume that there is some kind of unspecified damage that builds up proportional to the *total* internal concentration. In practice, it might represent something else, but that does not change the concept. In short, the rate constant

estimated from the toxicity data, either $k_e$ or $k_r$, needs to be interpreted with care. It represents a dominant process, but without further information, it is unclear what it is.

## Details for the case study with *Gammarus pulex*

The model and data for this case study originate from Ashauer et al. (2010) (*4*). As diazinon is biotransformed in *Gammarus pulex* to the toxic metabolite diazoxon, the toxicokinetic equations of GUTS were modified to include biotransformation according to (*4*).

### *(i) Bayesian context*

Survival data yields information about both TK and TD parameters. If information about TK parameters from measurements of internal concentrations is available, Bayesian statistics allows for using this information as a prior distribution on the parameter space. This prior multiplied by the likelihood is then, up to a normalizing constant, the posterior distribution on parameter space, incorporating both information from TK measurements and survival data in a consistent manner.

### *(ii) Full model - explicit TK and TD:*

Starting with an external concentration of diazinon, $C_w(t)$, the full model simulates uptake and depletion of diazinon, $C_i(t)$, and bio-transformation into its toxic version diazoxon, $C_{tox}(t)$, which is then supposed to lead to the aggregation of damage, $D^*(t)$, according to the equations

$$\dot{C}_i(t) = k_i C_w(t) - (k_o + k_a) C_i(t),$$
$$\dot{C}_{tox}(t) = k_a C_i(t) - k'_o\, C_{tox}(t),$$
$$\dot{D}^*(t) = k_e (C_{tox}(t) - D^*(t)).$$

The damage $D^*(t)$ is used as dose metric, $M(t)$, and plugged into equation (5) of the main manuscript to calculate the hazard. All individuals are assumed to have the same threshold $z$. The parameters $k_o$ and $k'_o$ describe the elimination of the parent diazinon and the biotransformation product diazoxon, respectively. The parameter $k_a$ is the rate constant of biotransformation of diazinon to diazoxon (activation).

Estimates for the parameters $k_i$, $k_o$, $k_a$, and $k'_o$ are derived from independent measurements of interior concentrations of diazinon and diazoxon. Since measurements of survival time series yield information about all the parameters, we work in a *Bayesian context* and use those estimates as priors. Priors for $k_e$ as well as the parameters $k_k$ and $z$ from equation (5) of the main manuscript are set to one (no prior information).

## *(iii) Reduced model - only one dominant process modeled:*

The simpler reduced model can be derived from the full model as the limit where

$k_o = 0,$

$k'_o \longrightarrow \infty,$

$k_a \longrightarrow \infty,$

$\frac{k_i}{k_a} = const.,$

$\frac{k'_o}{k_i} = \kappa = const.$

In accordance with eq. (3) we define $C_i^*(t)=\kappa D^*(t)$, as then the reduced model is defined by the equation

$$C_i^*(t) = k_e(C_w(t) - C_i^*(t)).$$

If $k_k$ and $z$ are rescaled with $\kappa$, appropriately, $C_i^*(t)$ plays the role of the dose metric $M(t)$.

Priors for the three parameters of this model, $k_e$ and the rescaled parameters $k_k$ and $z$, are set to one (no prior information).

Survival data from 3 different exposure patterns (test duration 22d, two subsequent 24h pulses with a 2, 7 and 15 day interval between them) and 7 beakers for each exposure pattern was used to construct the posterior distribution on parameter space for both models. Each beaker contained 10 alive organisms at the beginning. Observations were made daily. For more details see Ashauer et al. (2010) (*4*).

## *(iv) Calculating the goodness of fit:*

As a quantitative measure for the goodness of fit we calculate

$$\chi^2 = \sum_{i,\alpha,a} \frac{(z_i^{\alpha,a} - \hat{z}_i^{\alpha})^2}{\hat{z}_i^{\alpha}},$$

where $z_i^{\alpha,a}$ denotes the measured and

$$\hat{z}_i^{\alpha} = y_0(S_{i-1}^{\alpha}(\boldsymbol{\theta}) - S_i^{\alpha}(\boldsymbol{\theta}))$$

the predicted number of deaths occurring between $t_{i-1}$ and $t_i$ in beaker $a$ treated with treatment α. For $z_i^{\alpha,a}$ distributed according to the multinomial distribution (9), $\chi^2$ is known to converge to a chi-square distribution for large numbers of individuals. However, as the number of individuals per beaker (=10) is too small compared to the dimension of the multinomial distribution (=23) we use a Monte Carlo simulation to determine the theoretical distribution of $\chi^2$ under the hypothesis that our model is correct. As the

number of summands in $\chi^2$ (23x3x7=483) is large compared to the effective number of parameters (3), the reduction in degrees of freedom due to parameter estimation is negligible.

## Details for the case study with *fathead minnows*

For both the stochastic death and individual tolerance model, the scaled internal concentration is used as a dose metric (and thus $M=C_i^*$):

$$\frac{dC_i^*(t)}{dt} = k_e\left(C_w(t) - C_i^*(t)\right)$$

For the stochastic death model, the hazard rate is calculated as follows (ignoring background hazard):

$$h(t) = k_k \max\left(M(t) - z, 0\right)$$

The cumulated hazard is found by integration:

$$H(t) = \int_{\tau=0}^{t} h(\tau)d\tau$$

which in turns gives the survival probability as a function of time:

$$S(t) = \exp(-H(t))$$

For the individual tolerance model, the survival probability is given by:

$$S(t) = \left(1 - F\left(\max_{0<\tau<t} M(\tau)\right)\right)$$

where $F$ is the cumulative log-normal distribution for the threshold $z$. It is specified by two parameters: the median of the distribution and its spread (as the factor by which the median needs to be divided and multiplied to cover 95% of the total distribution).

For both models, optimization was performed by maximizing the log-likelihood function as given in the main text. The log-likelihoods for each exposure concentration were added to yield an overall log-likelihood value for the entire data set.

## References

(1) Ankley, G. T.; Erickson, R. J.; Phipps, G. L.; Mattson, V. R.; Kosian, P. A.; Sheedy, B. R.; Cox, J. S., Effects of light-intensity on the phototoxicity of fluoranthene to a benthic macroinvertebrate. *Environ. Sci. Technol.* **1995,** *29*, (11), 2828-2833.

(2) Ashauer, R.; Boxall, A. B. A.; Brown, C. D., New ecotoxicological model to simulate survival of aquatic invertebrates after exposure to fluctuating and sequential pulses of pesticides. *Environ. Sci. Technol.* **2007,** *41*, (4), 1480-1486.
(3) Lee, J. H.; Landrum, P. F.; Koh, C. H., Prediction of time-dependent PAH toxicity in *Hyalella azteca* using a damage assessment model. *Environ. Sci. Technol.* **2002,** *36*, (14), 3131-3138.
(4) Ashauer, R.; Hintermeister, A.; Caravatti, I.; Kretschmann, A.; Escher, B. I., Toxicokinetic-toxicodynamic modeling explains carry-over toxicity from exposure to diazinon by slow organism recovery. *Environ. Sci. Technol.* **2010,** *44*, (10), 3963-3971.