General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology

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S *Supporting Information*

ABSTRACT: Toxicokinetic-toxicodynamic models (TKTD models) simulate the time-course of processes leading to toxic effects on organisms. Even for an apparently simple endpoint as survival, a large number of very different TKTD approaches exist. These differ in their underlying hypotheses and assumptions, although often the assumptions are not explicitly stated. Thus, our first objective was to illuminate the underlying assumptions (individual tolerance or stochastic death, speed of toxicodynamic damage recovery, threshold distribution) of various existing modeling approaches for survival and show how they relate to each other (e.g., critical body residue, critical target occupation, damage assessment, DEBtox survival, threshold damage). Our second objective was to develop a general unified threshold model for survival (GUTS), from which a large range of existing models can be derived as special cases. Specific assumptions to arrive at these special cases are made and explained. Finally, we illustrate how special cases of GUTS can be fitted to survival data. We envision that GUTS will help increase the application of TKTD models in ecotoxicological research as well as environmental risk assessment of chemicals. It unifies a wide range of previously unrelated approaches, clarifies their underlying assumptions, and facilitates further improvement in the modeling of survival under chemical stress.

INTRODUCTION

A major challenge in ecotoxicology is the comparison and extrapolation of toxic effects between different substances, species, exposure conditions, exposure durations, and levels of biological organization. It is impossible to test every combination experimentally, nevertheless a quantification of toxic effects is needed for the environmental assessment of chemicals. Mechanistic modeling has been suggested as an essential tool to address these challenges.[1−6] In mechanistic models for toxic effects, explicit assumptions are made about the dynamic processes underlying the toxic response, which then allows extrapolation beyond test conditions. Such models have been developed for different levels of biological organization, for example, individuals,[6,7] populations,[8−10] and ecosystems.[11,12]

Toxicokinetic-toxicodynamic models (TKTD models) simulate the time-course of processes leading to toxic effects on organisms (Figure 1). As a first step, toxicokinetics translate an external concentration of a toxicant to an internal concentration over time. In their simplest form, toxicokinetics include the processes of uptake and elimination, but refined toxicokinetic models may also include further processes that modify the concentration of the toxicant at the target site, such as biotransformation or internal distribution. As a second step, toxicodynamics quantitatively link the internal concentration to the effect at the level of the individual organism over time (e.g., mortality).

TKTD models provide a range of advantages over more descriptive methods to analyze toxicity test data.[5,6,13,14] For example, these models make better use of the available data as all effects observations over time are used to estimate model parameters. Many standard test protocols require that effects are determined at intermediate time-points (e.g., OECD acute tests), although these are not used in the derivation of summary statistics such as the LC50. Furthermore, TKTD models do not require constant exposure concentrations in the toxicity test,[7,15−17] which means that toxicity tests can be used that otherwise would have to be discarded. Moreover, TKTD models have been used successfully to extrapolate toxic effects between different exposure scenarios,[7,15,18] in particular fluctuating or repeated pulsed exposures, and to explain effects of mixtures over time.[18−21] Finally, TKTD models facilitate a mechanism-based comparison of effects from different substances,[22] species,[23] life stages,[24] environmental conditions,[25,26] and endpoints.[27,28]

Even for an apparently simple endpoint as survival, a large number of very different TKTD approaches exist in the literature.[7,29−42] These approaches differ in their underlying hypotheses and assumptions, although the exact difference is often difficult to establish when the assumptions are not explicitly stated. This diversity of models and assumptions has led to confusion among scientists and has hindered the assimilation of TKTD models within ecotoxicological research and environmental risk assessment. Thus, our first objective was to illuminate the underlying assumptions of various existing modeling approaches for survival and show how they relate to each other. Our
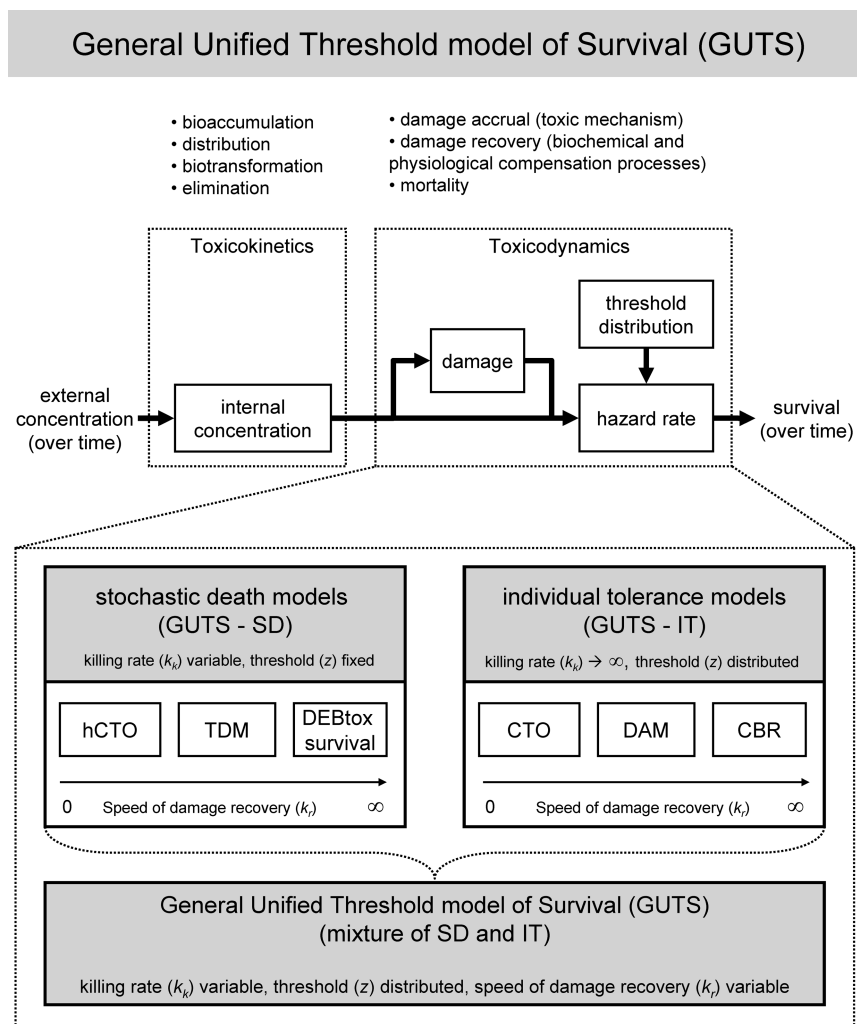
Figure 1. Schematic structure of the general unified threshold model of survival (GUTS), a toxicokinetic-toxicodynamic (TKTD) model, consisting of four modules: internal concentration, damage (optional), hazard rate, and threshold distribution. Various existing TKTD models can be derived as special cases within GUTS by making specific toxicodynamic assumptions about the killing rate and the threshold. These special cases consist of stochastic death models (SD) and individual tolerance models (IT), whereas GUTS is a more general, mixed model, comprising both SD and IT. hCTO: hazard (SD) versions of the Critical Target Occupation model; TDM: Threshold Damage Model; DEBtox survival: survival part in the Dynamic Energy Budget models for toxic stress; CTO: Critical Target Occupation model; DAM: Damage Assessment Model; CBR: Critical Body Residue concept.

second objective was to develop a unified theory for survival, from which a large range of existing models can be derived as special cases. Finally, we illustrate how these special cases can be fitted to survival data of aquatic amphipods and fathead minnows.

■ BIOLOGICAL ASSUMPTIONS OF TKTD MODELS FOR SURVIVAL

Individual Tolerance and Stochastic Death. The first published TKTD models that we are aware of were based on the dynamic Critical Body Residue (CBR) approach.[29−31,34] These models follow from the assumption that an animal dies (or is immobilized) immediately upon reaching a certain internal concentration (the CBR). Not all individuals die at the same time because they are assumed to have different sensitivities.[43] This sensitivity threshold follows a frequency distribution in the exposed population. Consequently, there is 50% mortality in the test population when the internal concentration reaches the

median of the distribution. The assumption of a distributed threshold is also known as "individual tolerance" (IT) and is generally the only assumption that is mentioned in ecotoxicological textbooks (e.g., refs 44 and 45) to explain sigmoid dose response curves (see also ref 46). However, this view has been challenged,[46−48] and an alternative assumption, stochastic death (SD), was used in TKTD models based on hazard modeling.[35,36] Instead of assuming differences in sensitivity, all individuals are taken identical but mortality is treated as a stochastic process at the level of the individual. This implies that the individuals that die are not the most sensitive ones (but simply the unlucky ones).

These two views lead to different survival predictions for prolonged constant as well as for time-varying exposure to toxicants. For prolonged constant exposure, the IT assumption predicts that a certain fraction of the population can survive indefinitely (ignoring background mortality), whereas the SD assumption implies that, given enough time, any exposure level that leads to some mortality will eventually wipe out the test population.[48] For pulsed exposure patterns, the IT assumption

predicts that the survivors of the first pulse will be the less sensitive ones, and therefore that subsequent pulses will have less and less impact (given enough time in between pulses for full organism recovery), or even no impact if the pulses were of the same concentration and duration. This prediction of IT is inherent to the assumption of different sensitivities between individuals. In contrast, SD will predict the same impact for each pulse (again given full organism recovery).[47,49]

Toxicodynamic Damage. Not all TKTD approaches link internal concentrations directly to the mortality process; alternative approaches assume that the internal concentration leads to damage (which can be repaired at a certain rate), and that this damage in turn leads to mortality (Figure 1). This toxicodynamic concept of damage was originally introduced for phototoxicity of PAHs[50] and widely applied in various TKTD models based on the assumption of IT [38,51,52] as well as in some TKTD models based on the assumption of SD.[7,15] Models with explicit consideration of receptor kinetics such as the Critical Target Occupation (CTO) model [37,53] or the hazard-based receptor kinetics model[39] can be considered a variation on the damage theme (for an overview see ref 40). The concept of damage can explain why, at least in some cases, the kinetics of the body residues (time course of internal concentration) can not explain the time course of mortality.[15,22,30]

Current Obstacles and Confusion when Comparing TKTD Models for Survival. To date, imprecise use of terminology has led to confusion, obscured the relationships between existing models and prevented the comparison of model parameter values from different studies. For example, TKTD models that include a damage state have used the assumption of an IT distribution [38,51] or the assumption of SD.[7,40,42] As both cases differ in their assumptions, also their model results and interpretation differ, but the use of the word "hazard" in both cases (wrongly suggesting the same meaning) has consequently led to confusion among readers. Further confusion may arise from the use of the same symbols for different model parameters or state variables (e.g., killing rate or recovery rate constants in refs 38 and 7) in different models, or the use of the term "elimination rate constant" for a fitted parameter in SD models for acute toxicity,[35] which may also reflect a toxicodynamic recovery processes instead of toxicokinetics alone. And furthermore, some models apply a threshold for effects (e.g., ref 35), whereas others (otherwise very similar ones) do not.[36]

In the following we propose a framework that should overcome these causes of confusion by the use of a consistent terminology within a unifying framework that merges different existing model concepts.

■ GENERAL UNIFIED THRESHOLD MODEL OF SURVIVAL (GUTS)

Background. In the following we outline a mathematical framework to quantify the time-course of survival in a population of organisms (we use "population" in a general statistical sense, not necessarily the biological sense; the organisms in a laboratory test therefore also constitute a population). This framework is generally applicable to chemical stress (toxicity), but, with modifications, it could be applicable to other types of stressors that cause mortality. Here, we will focus on mortality as a result of chemical stress only. The general structure of the framework is outlined in Figure 1, which shows the subdivision in toxicokinetic and toxicodynamic models. We will first present the full model and then clarify how a range of previously published models can

Table 1. Parameters and Symbols Used[a]

parameter	symbol	dimensions	example units
internal concentration in organism	C_i	$\# \, m_o^{-1}$	mol kg_{wwt}^{-1}
scaled internal concentration	C_i^*	$\# \, m_e^{-1}$	mol L^{-1}
external concentration in medium	C_w	$\# \, m_e^{-1}$	mol L^{-1}
damage level	D	$[D]$	"damage"
scaled damage level	D^*	$\# \, m_o^{-1}$	mol kg_{wwt}^{-1}
background hazard rate	h_b	t^{-1}	d^{-1}
hazard rate for individual with threshold z	h_z	t^{-1}	d^{-1}
cumulative hazard for individual with threshold z	H_z	$[-]$	$[-]$
elimination rate constant, (also "dominant rate constant")	k_e	t^{-1}	d^{-1}
accumulation rate constant	k_i	$m_e \, m_o^{-1} \, t^{-1}$	L $kg_{wwt}^{-1} \, d^{-1}$
killing rate	k_k	$[M]^{-1} \, t^{-1}$	see Table 2
damage recovery rate constant	k_r	t^{-1}	d^{-1}
selected dose metric	M	$[M]$	see Table 2
survival probability	S	$[-]$	$[-]$
survival probability for individual with threshold z	S_z	$[-]$	$[-]$
time	t	t	d
time	τ	t	d
threshold for effects	z	$[M]$	see Table 2

[a] For the dimensions: m_o is mass or volume of organism, m_e is mass or volume of the external medium, $\#$ is number of molecules or mass of the toxicant, t is time. Dimensions for damage are not specified explicitly.

be derived from it as special cases, under specific, limiting assumptions. The parameters and symbols used in the model description are explained in Table 1.

Toxicokinetics. Chemicals generally need to be taken up and transported to a target site before they can exert a toxic effect. The first model in the chain is therefore a toxicokinetics model, with the role of translating external concentrations (which could be time-varying) to internal concentrations (at a target site) as a function of time. In its simplest form, a one-compartment model with first-order kinetics can be used:

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

where C_i is the internal concentration, C_w is the concentration in the exposure medium (e.g., water), k_i is the accumulation rate constant, k_e the elimination rate constant. For many exposure patterns, this equation can be solved analytically. Even though this model is really simple, it generally captures the kinetics of the whole-body residue well.[54−57] The assumptions behind this equation are that the uptake flux into the organism is proportional to the external concentration, and that the elimination flux is proportional to the internal concentration. If needed, this model can be extended to include multiple compartments, biotransformation, saturating uptake kinetics or dilution by growth.

If internal concentrations are not measured, as is most common in ecotoxicity tests, the time course of survival data might still allow for estimation of an elimination rate, because this rate determines the time to reach equilibrium between the concentration in the organism and its environment. However, it is

not guaranteed that this estimated elimination rate still represents whole-body elimination of the compound. The slowest compensating process (which can be a toxicokinetic or toxicodynamic recovery process) will dominate the overall dynamics of toxicity (details in chapter selecting a dose metric). Hence, we will refer to that rate constant in that situation as the "dominant rate constant" and continue to use the symbol k_e. Survival data can provide information about elimination, but can never identify the uptake rate constant k_i (which determines, together with k_e, the absolute level of C_i). This problem is circumvented by rescaling the internal concentration. We divide both sides of eq 1 by the ratio of the two rate constants; k_i/k_e (this ratio is the bioconcentration factor). The internal concentration (C_i) divided by k_i/k_e 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.[22,35] The equation for the scaled internal concentration is

$$\frac{dC_i^*(t)}{dt} = k_e(C_w(t) - C_i^*(t)) \qquad (2)$$

In equilibrium, C_i* will equal the external concentration, C_w. This scaling enables TKTD modeling without access to internal concentrations, with the sole requirement that the time course of the internal concentration can be described by first-order one-compartment kinetics.

Damage. It is possible to link the internal (scaled) concentration directly to the survival model, but in cases where the time course of the internal (scaled) concentration does not describe or explain the time course of survival (e.g., ref 15), it is necessary to introduce an additional toxicodynamic stage of "damage". The concept of damage is rather abstract, as it can not (generally) be measured directly, but it is an integrative state variable that lumps all kind of biochemical and physiological processes that are involved in toxicity. The generic definition of damage makes GUTS applicable for different types of compounds and modes of action, and accounts for physiological disturbances (even when the mode of action is unknown). If it is demonstrated that the time course of damage reflects the time course of a specific descriptor of a known mode of action, then damage could be interpreted more specifically.

We assume that damage accumulates proportional to the internal concentration, and is repaired proportional to the actual damage level. In contrast to earlier publications with two rate constants (for damage accrual and recovery),[7,15,38,50−52] we propose here a slightly different formulation. Because damage can not be measured, the data that are available in toxicity tests can not 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 the rate constant for damage accrual. This situation is equivalent to the TK model when internal concentrations are not determined. Analogous to the rescaling of the internal concentration in eq 2, we introduce a scaled damage D^*, which is proportional to the actual (but unknown) damage level, and has the units of an internal concentration (see Supporting Information (SI)):

$$\frac{dD^*(t)}{dt} = k_r(C_i(t) - D^*(t)) \qquad (3)$$

It is recommended to use a damage stage only where a relevant elimination rate constant k_e can be estimated from body residue

Table 2. Units and Model Parameters for Various Dose Metrics[a]

	dose metric	example units z	example units k_k	model parameters
external concentration	$M = C_w$	mol L^{-1}	L mol^{-1} d^{-1}	k_k, z
internal concentration	$M = C_i$	mol kg$_{wwt}$$^{-1}$	kg$_{wwt}$ mol^{-1} d^{-1}	k_i, k_e, k_k, z
scaled internal concentration	$M = C_i*$	mol L^{-1}	L mol^{-1} d^{-1}	k_e, k_k, z
scaled damage	$M = D^*$	mol kg$_{wwt}$$^{-1}$	kg$_{wwt}$ mol^{-1} d^{-1}	k_i, k_e, k_r, k_k, z

[a] The choice of dose metric affects the units of z and k_k only.

data or by other means (e.g., read-across from related chemicals or quantitative structure activity relationships (QSARs)). Using the scaled internal concentration C_i* (instead of C_i) in eq 3 is not recommended (see next section).

Selecting a Dose Metric. In the survival model, we will link the most appropriate dose metric to the survival probability. As most appropriate dose metric we can select the scaled damage (D^*), the internal concentration (C_i), the scaled internal concentration (C_i^*), or even the external concentration (C_w). We will use the symbol M for the selected dose metric (Table 2). In general, the choice will depend on the available data and hypotheses regarding the mechanism of action of the chemical of interest. This choice will affect the interpretation of the toxicodynamic parameters and their dimensions (Table 2). Here we assume that the value of the dose metric is the same for all individuals within a treatment.

The distinction between toxicokinetics and damage kinetics is difficult in practice, because we generally lack appropriate information to differentiate between these processes. For toxicokinetics, we might know the value of whole-body residues, but this does not necessarily reflect concentrations at the target site. As for damage, it can not be measured because its nature is not precisely specified. In the following, we assume that whole-body residues are the relevant TK endpoint, either for linking the toxic effect directly to survival, or indirectly through TD damage production.

When measured body residues are available (or the TK parameters can be estimated otherwise, for example, read-across or QSARs), we can use C_i or D^* as dose metric. When the dynamics of the internal concentration can explain the temporal patterns of effects i.e., time course of survival, either survival is directly linked to body residues, or damage repair is much faster than elimination of the toxicant from the body ($k_r \gg k_e$). When damage repair is much slower than elimination ($k_r \ll k_e$) then the time course of internal concentration may not explain the temporal patterns of effect and scaled damage (D^*) should be used.

Without measured (or estimated) body residues, it is generally not advisable to use a state of damage. Rather, one would use scaled internal concentrations (C_i^*) as dose metric and carefully interpret the resulting dominant rate constant k_e. Although eqs 2 and 3 specify a two-compartment system, with two rate constants (k_e and k_r), the resulting dynamics for D^* will usually be quite similar to one-compartment behavior. The slowest compensating process (elimination or damage repair) will dominate the overall dynamics of toxicity, making it impossible to estimate

both k_e and k_r independently; rather the dominant rate constant will reflect this slowest compensating process.

Survival Model. For the unified survival model, we combine the approaches for IT and SD. We assume that there is a threshold (z) for effects. This implies that there is some level of the dose metric (M) that has no effect on survival, other than background mortality, even after prolonged exposure. The value for this threshold can be taken as zero when the toxicity data or theoretical considerations point to the absence of a no-effect threshold. The threshold value z does not have to be the same for each individual, but follows a probability distribution in the test population.

When the selected dose metric M exceeds the threshold value z, the individual has an increased probability to die (compared to the controls). The "instantaneous probability to die", the so-called hazard rate (h_z), of an individual with threshold z is defined as

$$h_z(t) = -\frac{1}{S_z(t)}\frac{dS_z(t)}{dt} \tag{4}$$

where $S_z(t)$ is the probability of the individual to survive until time t. The hazard rate of an individual is assumed to increase linearly with the dose metric above the threshold:

$$h_z(t) = k_k \max(0, M(t) - z) + h_b(t) \tag{5}$$

where the proportionality constant k_k is called the killing rate and the max function selects the maximum of 0 and $(M(t)-z)$. A linear relationship is taken for simplicity (it only requires one parameter next to the threshold), and because it is reasonable to assume that every molecule above the threshold has the same effect on the hazard rate. We assume that mortality in the control is a stochastic event (SD), independent from the mortality caused by the toxicant (therefore, we can add the hazard rates). For short-term laboratory experiments, the background hazard rate can be taken as a constant. For long-term experiments, or experiments where organisms are not fed, it is common that the background hazard rate increases with time, and an appropriate function (or submodel) will be needed.

Equation 4 can be solved to an explicit expression for the survival probability of the individual with threshold value z as follows:

$$S_z(t) = \exp(-H_z(t)) \tag{6}$$

where $H_z(t)$ is the individual's cumulative hazard at time t. The cumulative hazard is obtained by integration:

$$H_z(t) = \int_0^t h_z(\tau)d\tau \tag{7}$$

The variable τ represents time, which runs from 0 to t (we can not use the symbol t again, as this parameter is already present in the upper bound of the integral). Equations 4, 6, and 7 represent the standard method to deal with chance events over time in biology, engineering and economics (http://en.wikipedia.org/wiki/Survival_analysis).

The probability of an arbitrary member of a population to survive until time t, $S(t)$, is given by

$$S(t) = \int_0^\infty S_z(t)f(z)dz \tag{8}$$

where f denotes the probability distribution that the individuals draw their threshold value z from. Function $S_z(t)$ is the

probability for a member of the population to survive until time t, *conditioned* that it has threshold z. Multiplying with the probability for an individual to have threshold z and integrating over z yields the *unconditional* probability for an arbitrary member of the population to survive until time t. For $f(z)$, the frequency distribution of the threshold z in the test population, any probability density function on the positive real axis can be used, but popular ones are the log-normal, log-logistic, Weibull and exponential (there are no strong theoretical reasons to prefer one distribution over the other).

This model represents a mixture of IT and SD. A pure IT model can be derived by taking the killing rate (k_k) infinitely large; this implies certain death when the threshold of an individual is exceeded. A pure SD model can be derived by taking a Dirac delta distribution at z for f; which implies that the integral in eq 8 is trivial, and $S(t)$ equals $S_z(t)$ as there is only one value for z for all individuals.

Likelihood Function. To estimate the model parameters from a survival data set, we require the likelihood function, which in turn requires a closer look at the error structure. Survival data are quantal data, which means that we record the number of individuals out of a population responding to a treatment over time. For this reason, the popular assumption of normal independent errors (and thus least-squares regression) is inappropriate. Instead, the outcome of a survival experiment can be seen as a trial from a multinomial distribution.[35] We measure a time series of numbers of survivors $\mathbf{y} = (y_0, ..., y_n)$, with $y_i = y(t_i)$, for subsequent times $0 = t_0 < t_1 < ... < t_n$. Every individual will die in one of the intervals between the observation times (remaining individuals after the test will die in the interval between the last observation time and infinity). The probability for a randomly chosen individual to die between t_{i-1} and t_i is given by $S_{i-1}(\boldsymbol{\theta}) - S_i(\boldsymbol{\theta})$, where $S_i(\boldsymbol{\theta}) = S(t_i)$ is defined in eq 8 (these probabilities for every time interval will sum up to one). The parameter k_k, the parameters that describe the distribution f and the parameters involved in the calculation of $M(t)$ are collectively denoted by $\boldsymbol{\theta}$. Hence, the likelihood function for the observation $\mathbf{y}$ given the vector of parameters $\boldsymbol{\theta}$ is expressed as the multinomial distribution

$$l(\mathbf{y}|\boldsymbol{\theta}) = y_0! \prod_{i=1}^{n+1} \frac{(S_{i-1}(\boldsymbol{\theta}) - S_i(\boldsymbol{\theta}))^{y_{i-1}-y_i}}{(y_{i-1}-y_i)!} \tag{9}$$

where we have set $S_{n+1}(\boldsymbol{\theta})=0$ and $y_{n+1}=0$. As $l(\mathbf{y}|\boldsymbol{\theta})$ is typically extremely small, we often work with its logarithm. Taking the natural logarithm for both sides of eq 9 yields:

$$\ln l(\mathbf{y}|\boldsymbol{\theta}) = \sum_{i=1}^{n+1}(y_{i-1}-y_i)\ln(S_{i-1}(\boldsymbol{\theta})-S_i(\boldsymbol{\theta}))$$

$$+ \ln\left(\frac{y_0!}{\prod_{i=1}^{n+1}(y_{i-1}-y_i)!}\right) \tag{10}$$

The last term is independent of $\boldsymbol{\theta}$ and not needed to find the parameter set $\boldsymbol{\theta}$ that maximizes the likelihood, or to derive confidence intervals, and can therefore be ignored (which simplifies the equation considerably).

If more than one treatment is available (e.g., a range of exposure concentrations), then the likelihoods for each treatment can be multiplied (or the log-likelihoods added) to yield an overall likelihood for a data set. Similarly, the likelihood allows combining multiple data sets that share common parameters

Table 3. Overview of how various published TKTD Models can be derived from the standard GUTS (the list is not Exhaustive)

individual tolerance models ($k_k = \infty$)				

references	dose metric	$f(z)$	other assumptions	acronym in Figure 1
Zitko (1979) [30]	$M = C_i$	not specified (only 50% effect)	$k_r = \infty$	CBR
Kooijman (1981) [31]	$M = C_i^*$	log-logistic	$k_r = \infty$	CBR
Breck (1988) [33]	$M = D^*$	log-normal	no TK, damage accrual is a power function of C_w	
Mackay et al. (1992) [34]	$M = C_i$	Weibull	$k_r = \infty$	CBR
Legierse et al. (1999) [37]	$M = D^*$	not specified (only 50% effect)	$k_r = 0$, ad hoc addition of LC50(∞)	CTO
Lee et al. (2002), [38] Schuler et al. (2004), [51] Lee et al. (2006) [21]	$M = D^*$	exponential		DAM
Ashauer et al. (2006) [5]	$M = D^*$	exponential	restriction $dS/dt \geq 0$	

stochastic death models ($f = \delta$)			

references	dose metric	$f(z)$	other assumptions	acronym in Figure 1
Bedaux and Kooijman (1984) [35]	$M = C_i^*$	Dirac delta	$k_r = \infty$	DEBtox survival
Widianarko and Van Straalen (1996) [36]	$M = C_i$	Dirac delta	$z = 0, k_r = \infty$	
Jager and Kooijman (2005) [39]	$M = D^*$	Dirac delta	saturating receptor kinetics instead of first-order damage	
Ashauer et al. (2007), [7] (2010) [15]	$M = D^*$	Dirac delta		TDM
Ashauer and Brown (2008) [40]	$M = D^*$	Dirac delta	$k_r = 0$	hCTO
Lee et al. (2009) [42]	$M = C_i$	Dirac delta	h is a logistic function in C_i, instead of linear, $k_r = \infty$	

mixed models			

references	dose metric	$f(z)$	other assumptions
Baas et al. (2009) [41]	$M = C_i^*$	log-normal	$k_r = \infty$

(e.g., toxicity data for the same compound and species at a different temperature). The best fitting parameter set $\boldsymbol{\theta}$ can be found by maximizing the (log-)likelihood function of either eqs 9 or 10. Confidence intervals can be generated by applying Wald statistics or by profiling the likelihood function.[58] Alternatively, the likelihood function can be used in a Bayesian statistical framework, as it is the probability of obtaining these data, given the parameter set (see SI).

Special Cases: Individual Tolerance Distribution Models. The special case where k_k approaches infinity corresponds to the IT models. Here, death is deterministic for an individual, and stochasticity is assumed solely at the level of the population. The assumption of infinite k_k has the consequence that those individuals whose threshold is exceeded by M die immediately ($S_z = 0$) and the others do not die at all ($S_z = 1$, if we have zero background mortality), as can be seen from eq 5 to eq 7. The probability to draw an individual from the population that survives until time t is then given by rewriting eq 8 (setting h_b to zero to simplify notation):

$$S(t) = \int_{\max_{0 < \tau < t} M(\tau)}^{\infty} f(z)\mathrm{d}z = \left(1 - F\left(\max_{0 < \tau < t} M(\tau)\right)\right) \quad (11)$$

where F denotes the cumulative distribution function of f and τ is a variable for time, running from zero to t. The lower bound of

the integral in eq 11 is the logical consequence of eqs 5–8 for an infinite killing rate. Taking the maximum of $M(\tau)$ for $0 < \tau < t$ rather than just $M(t)$ is required in cases where the dose metric M decreases in time (nonconstant exposure), to ensure that $S(t)$ does not increase in time (dead individuals can not be revived). This requirement is not widely recognized, perhaps because most applications focus on constant exposure.

Lee and co-workers[38] chose the individual thresholds z to be distributed exponentially, which yields for f:

$$f(z) = \beta e^{-\beta z} \quad (12)$$

where β determines the width of the distribution. When we use the scaled damage as the dose metric we get the population survival probability:

$$S(t) = \exp\left(-\beta \max_{0 < \tau < t} D^*(\tau)\right) \quad (13)$$

It should be noted that the original authors did not use a scaled version of damage, but combined two rate constants (D_L/k_a in ref 38) to avoid the problem that k_e and k_r cannot be estimated independently (see dose metric section). Furthermore, the original authors did not mention the problem for time-varying concentrations (which was discussed and addressed by ref 5). Equation 13 looks somewhat similar to eq 14, although the underlying model assumptions are completely different; the model of Lee et al.[38] assumes IT. This confusion is enhanced by the use of the term
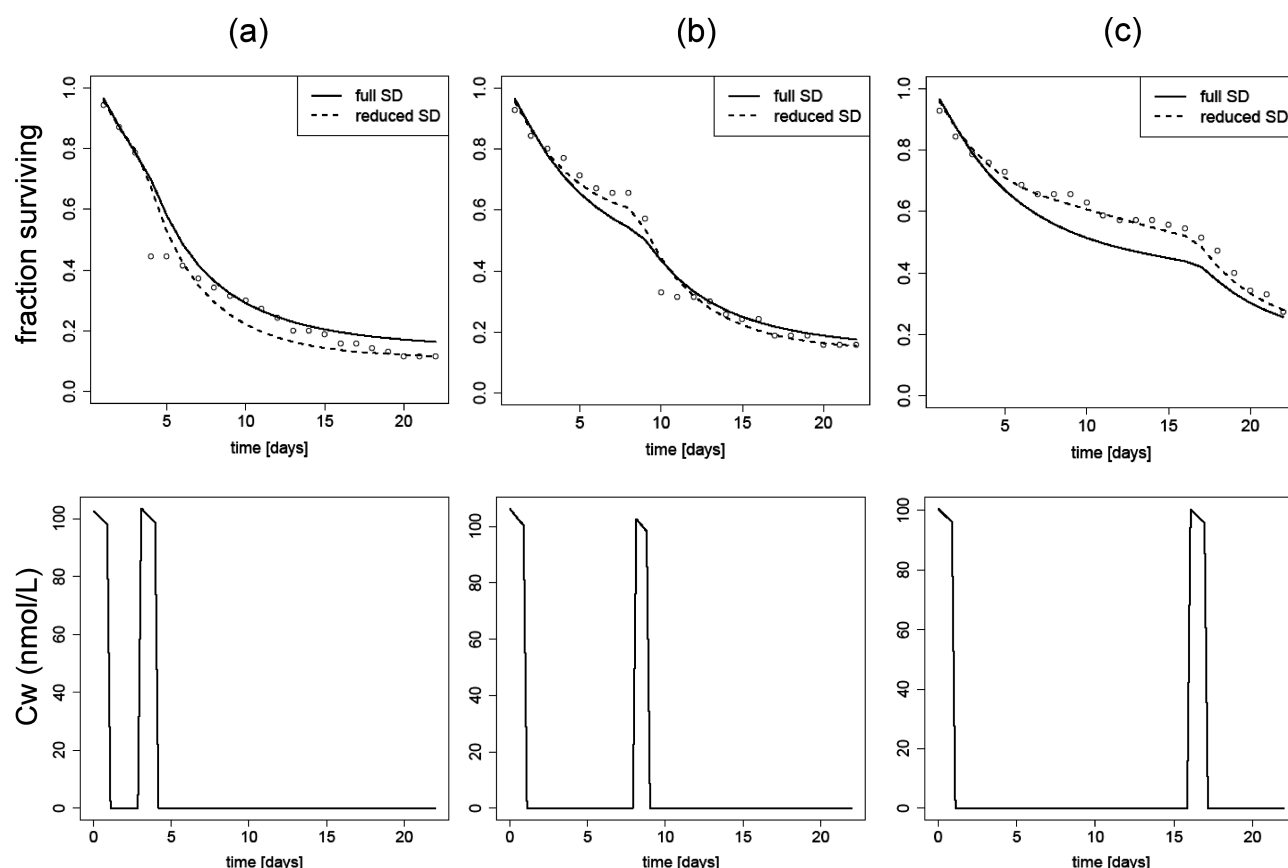
Figure 2. Full and reduced stochastic death (SD) model fitted to the same survival data in three different exposure patterns (treatments, shown in the lower panels). Parameter estimates are in Table 4.

"hazard rate" in the original publication.[38] The derivation of particular IT models from the unified framework is shown in Table 3.

Special Cases: Stochastic Death Models. The so-called hazard models assume that death is stochastic at the level of the individual, and that all individuals have the same value for the threshold z. No a priori assumption about the killing rate parameter k_k is made. SD models constitute another special case of the general unified framework, which can be derived from the general model by taking a Dirac delta distribution for the threshold z. For this distribution, all of the probability density is concentrated in a single value, the integral over z in eq 8 becomes trivial and can be carried out $S(t) = \int S_{z'}(t)\delta(z-z')dz' = S_z(t)$. The survival probability for models assuming SD is then:

$$S(t) = \exp(-H_z(t)) \tag{14}$$

where H_z is a function of time only (as there is only a single threshold value z). The derivation of particular SD models is shown in Table 3.

■ CASE STUDIES

Fitting SD with and without Experimentally Derived Toxicokinetics. GUTS can be used with and without experimentally (or otherwise, e.g., read-across or QSARs) derived toxicokinetics. For the first example we restrict ourselves to SD models and demonstrate how to fit TD parameters. We analyze data of the freshwater arthropod *Gammarus pulex* exposed to the organophosphate insecticide diazinon, where measured internal

concentrations of diazinon and diazoxon as well as survival time series are available. The model and data for this case study originate from ref 15.

We compare two nested SD models:

(i) The full model simulates toxicokinetics and damage dynamics. As diazinon is biotransformed in *G. pulex* to the toxic metabolite diazoxon, the toxicokinetic equations of GUTS were modified to include biotransformation according to ref 15 (see SI). The dose metric is the scaled damage ($M = D^*$) and the TK parameters are based on separate experiments[15] and are used as prior information in a Bayesian context (see SI for further explanation).

(ii) In the reduced model, toxicokinetics and damage dynamics are not modeled as separate processes, rather, both are lumped and the dominant rate constant k_e corresponds to the slower, that is, rate limiting, of the two processes. The dose metric is in that case the scaled internal concentration ($M = C_i^*$) (see SI).

Both models are fitted to the same survival data from three different exposure patterns (initially 70 organisms in each treatment, test duration 22d), which consisted of two subsequent 24 h pulses with a 2, 7, and 15 day interval between them (Figure 2a, b, c).

The simultaneous best fit (maximum posterior) for the three exposure patterns is shown in Figure 2 and the corresponding parameter values in Table 4. The χ^2-values indicate that the reduced model performs slightly better than the full model. This is due to interassay variability between the TK experiment where the TK

Table 4. Parameter Estimates for the Model Fits in the Two Case Studies As Shown in Figures 2 and 3[a]

symbol	G. pulex, diazinon		P. promelas, naphthalene		P. promelas, 1,2,4-trimethylbenzene	
	full model (stochastic death)	reduced model (stochastic death)	stochastic death	individual tolerance	stochastic death	individual tolerance
k_e	$3.22\ \mathrm{d}^{-1}$	$0.082\ \mathrm{d}^{-1}$	$5.53\ \mathrm{d}^{-1}$	$1.48\ \mathrm{d}^{-1}$	$9.95\ \mathrm{d}^{-1}$	$9.11\ \mathrm{d}^{-1}$
	(2.27–5.46)	(0.063–0.17)	(3.78–17.2)	(1.26–1.75)	(9.23–27.7)	(9.11–9.28)
k_r	$0.13\ \mathrm{d}^{-1}$	$\infty\ \mathrm{d}^{-1}$	$\infty\ \mathrm{d}^{-1}$	$\infty\ \mathrm{d}^{-1}$	$\infty\ \mathrm{d}^{-1}$	$\infty\ \mathrm{d}^{-1}$
	(0.076–0.33)	ne[b]	ne	ne	ne	ne
k_k	$4.07\ \mathrm{g_{wwt}\,nmol^{-1}\,d^{-1}}$	$24\cdot 10^{-3}\ \mathrm{nM^{-1}\,d^{-1}}$	$0.102\ \mu\mathrm{M^{-1}\,d^{-1}}$	$\infty\ \mu\mathrm{M^{-1}\,d^{-1}}$	$972\ \mu\mathrm{M^{-1}\,d^{-1}}$	$\infty\ \mu\mathrm{M^{-1}\,d^{-1}}$
	(1.57–11.7)	$(9\text{–}33)\cdot 10^{-3}$	(0.0652–0.152)	ne	(9.49–62400)	ne
z	$6.4\times 10^{-3}\ \mathrm{nmol\ g_{wwt}^{-1}}$	$4.53\ \mathrm{nM}$	$44.0\ \mu\mathrm{M}$	median $46.4\ \mu\mathrm{M}$	$67.3\ \mu\mathrm{M}$	median $67.3\ \mu\mathrm{M}$
	$(0.92\text{–}17.8)\cdot 10^{-3}$	(2.56–5.76)	(42.0–45.3)	(43.9–49.0)	(67.3–67.3)	(67.3–67.3)
				spread 1.40 [−] (1.32–1.50)		spread 1 [−] (1–1)

[a] For the G. pulex study, values are maximum posterior parameter values with 95% credibility intervals; for P. promelas, values are maximum likelihood estimates with 95% likelihood-based confidence intervals. Threshold values z are single values for the SD models, but follow log-normal distribution for the IT model, specified as the median and spread (the factor by which the median must be divided or multiplied to cover 95% of the distribution).
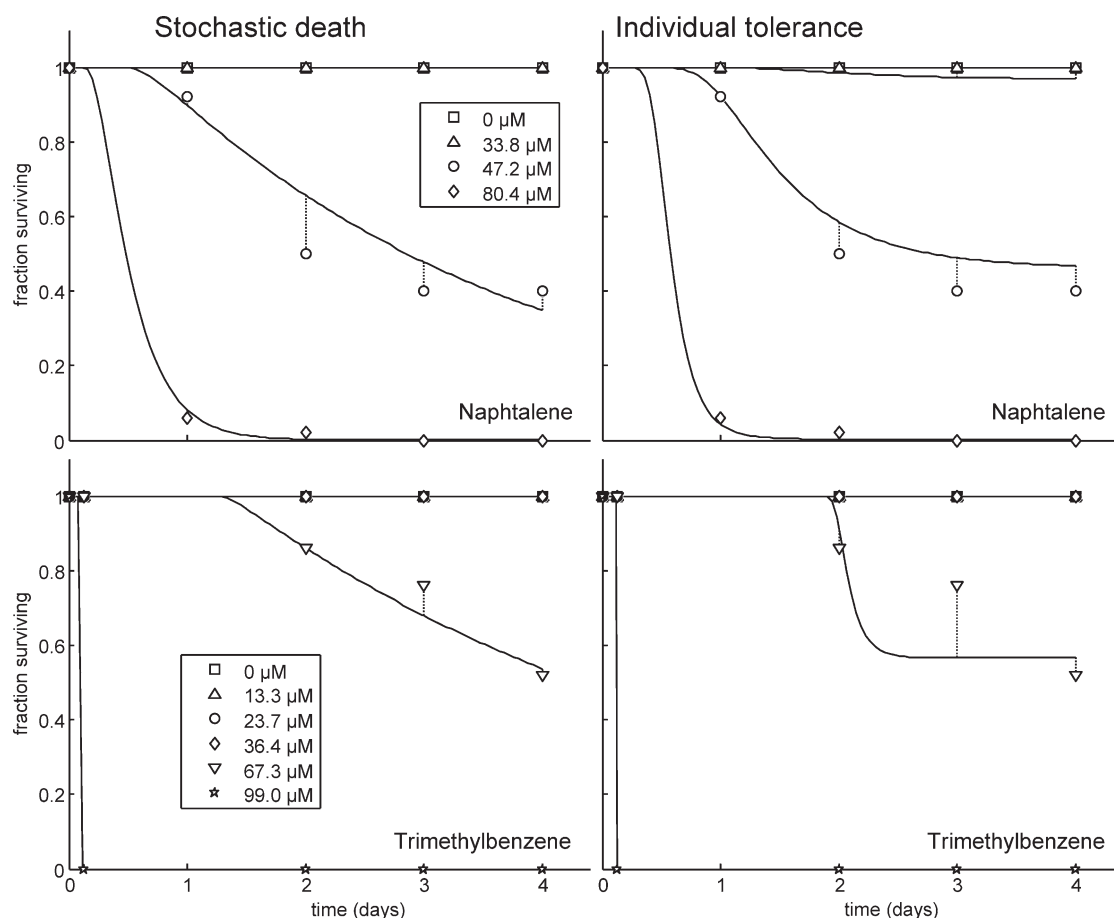[b] ne = Not estimated.

Figure 3. Two data sets for fathead minnows, analyzed with two simplified models applying stochastic death or individual tolerance on the basis of scaled internal concentration. Parameter estimates are in Table 4.

parameters originate from and the TD experiment which was simulated using those TK parameters (Figure 2). In Bayesian language, there is a conflict between the prior derived from TK experiments and the TD data. We also estimated the theoretical distribution of χ^2 (see SI) and found that the probability of seeing at least as large χ^2-values as measured is about 20%, for both the reduced and full model. This means that for popular choices of significance

levels (such as 5% or 10%) the hypothesis of our model being correct does not have to be rejected based on a χ^2 test. In other words, SD explains the observed variability reasonably well. However, allowing some (or all) parameters to vary over the population would presumably lead to a better explanation of the observed variability.

Although the full model is parametrized with additional data (TK), those data do not completely determine the additional

parameters of the full model. Hence, the posteriors are sharper for the reduced model as there is no uncertainty about TK parameters.

The estimates of (the slowest rate) k_r in the full and k_e in the reduced model agree very well with each other, in the sense that the estimate for k_r lies in the range of k_e and vice versa. We conclude that damage repair dominates the organism recovery of *G. pulex* after exposure to diazinon. This conclusion is further supported by the known fast elimination of diazinon and its biotransformation products from *G. pulex*.[15]

Fitting the Special Cases of IT and SD. In the second case study, we analyzed data sets for juvenile fathead minnows (*Pimephales promelas*, 50 individuals per concentration, test duration 4 days) for two compounds: naphthalene[59] and 1,2,4-trimethylbenzene.[60] The purpose is 2-fold: first we show how typical acute toxicity data (constant exposure, no measured body residues, few survival observations with partial mortality in the test population) can be used, and second, we show how the GUTS special cases of IT and SD explain the same sets of survival data. Thus we fitted SD and IT (see SI) to the survival data (four time points) using the average measured toxicant concentration in water (in μM), as model input.

Figure 3 shows the analysis for the case of SD (left), and IT (right, assuming a log-normal distribution of the threshold). Both models apply the scaled internal concentration and do not account for damage accrual ($M = C_i^*$), so k_e represents the dominant rate constant for both compounds (Table 4). The data for napthalene (Figure 3, top) is best explained by the IT model because mortality stops after some time (between 3 and 4d). This marks the point where, in the IT model, the scaled body residue reaches equilibrium with the external concentration. For tri-methylbenzene (Figure 3, bottom), a continuous decline in survivors at one concentration, in combination with the rapid death at the highest concentration, can not be captured by the IT model. In contrast, the SD model can explain this pattern nicely. The estimated median threshold value for both approaches, IT and SD, is very similar, but the estimates for the dominant rate constant differ substantially. Systematic analysis of larger data sets is needed to confirm whether this is a general pattern. These case studies are meant as illustration; they are certainly insufficient to support statements about the appropriateness of either SD or IT for these data.

■ DISCUSSION

Data Needs. To apply TKTD models, one would generally need observations on survival over time. Within standard tests, daily observation of mortality is already conducted, so these can directly be used in the simplest derivations of GUTS. Unfortunately, most scientific publications and ecotoxicological databases currently only store summary statistics such as the LC50 after standard test durations, which is of little use (see ref 22). We suggest that the raw survival data from toxicity experiments are also stored and made available. To test and further develop TKTD models, measured time series of internal concentrations or independently derived toxicokinetic parameters can greatly increase the understanding of temporal aspects of toxicity[15] and questions related to the appropriate dose metric (Table 2).

Assumptions in the Special Cases IT and SD. Probably the most fundamental toxicodynamic assumption in a TKTD model for survival is the hypothesis for why one organism dies and another survives. At this moment, it is unclear which of the extreme views of SD or IT is closer to the truth. On the basis of survival data it is often difficult to distinguish between the two extreme views; both seem to be able to explain survival data, although the resulting rate constants can be very different (Table 4). It is very likely that both assumptions play a role, and that the contribution of each of these mechanisms depends on the toxicant and the population of interest. The framework we present here allows testing these extreme views on a particular data set, but also mixed models can be tried. The simulations of ref 41 show that it may be possible to estimate both the killing rate and the parameters of the threshold distribution from survival data.

The survival probability has to be interpreted in different ways for the different assumptions. For SD models, the survival probability equals the probability of an individual to survive until a specified time. IT models calculate the probability to select an individual from a population, which survives until a specified time. Assuming SD or IT has different consequences for risk assessment of chemicals[47,49] as outlined above (section IT and SD).

Variability of Model Parameters. We only considered inter-individual differences in the value of the threshold. However, the reason to focus on the threshold alone is purely inspired by tradition and practicality. The earliest TKTD model attempts for survival were based on the assumptions of variation in a threshold.[29−32] In reality, it is likely that all parameters in a TKTD model will vary between individuals. For example, the development of resistance toward toxicants could be explained by selection of individuals with particular parameter values. The consequences of variable TKTD model parameters (see, e.g., ref 49), the extent to which such a level of detail is needed, the extent and interpretation of the contribution of stochasticity and the challenge of quantifying the distributions of these TKTD parameters, remain to be investigated.

Different Dose Metrics. Within the GUTS, several different dose metrics can be used: external concentration, internal concentration, scaled internal concentration or scaled damage level. The GUTS enables rigorous testing of the consequences of the various dose metrics within one consistent framework. The most relevant metric obviously depends on the question at hand, as well as on the mechanism of action of the compound in the species of interest. Furthermore, this choice is also limited by the available data. Toxicokinetic parameters can be derived from measured internal concentrations or they can be estimated from prediction models for such parameters (e.g., ref 61). If toxicokinetic parameters or measured internal concentrations are not available, the only option is to use the external or scaled internal concentration as dose metric. The resulting rate constant (the dominant rate constant) does not necessarily represent whole-body toxicokinetics anymore; it represents the slowest process, which may be a TK or a TD damage repair process. For example, our first case study illustrates a case of damage repair dominating organism recovery. The question, for which compounds and organisms TK or damage repair are the slowest process, can be investigated by fitting the full and reduced GUTS to further data sets. Having said that, even if kinetics of the toxicant concentration in a homogenized individual (whole-body residues) are measured, they may not be representative of the kinetics (of the relevant metabolite) at the target site.[22,30] Thus, k_r in damage models should also be interpreted with care, in relation to what is known about the toxicant's mechanism of action in the species of interest.

If measured concentrations of the toxicant inside the organism are available, we can test whether the kinetics of the body residue are able to explain the temporal patterns in survival. If slower kinetics are needed to explain survival, the toxicodynamic section in Figure 1 can be extended with a damage model (note that

damage can only slow down the kinetics of the dose metric; the upper limit is set by the elimination rate from the measured body residues). Even though damage can not be directly observed, separating toxicokinetics from damage dynamics might allow for a better understanding of the mechanisms governing toxicity. It is known that toxicokinetics depend on physicochemical properties of the toxicants (e.g., hydrophobicity) and we expect that damage dynamics depend on the mechanism of action of the compound. Additionally, both, TK and TD are hypothesized to depend on different traits of the species.[23] Thus, a TKTD modeling approach can ultimately increase the possibilities to extrapolate between different species and different substances.

The models we have presented here to calculate dose metrics are simple one-compartment models that may serve as general reference models. As we demonstrated with our case studies, such models can explain temporal patterns of survival under constant and time-varying exposure. If more information about the toxicokinetics and/or mechanism of action of a particular compound is available, these models can be extended or modified to include such information.[15,39]

Relation of GUTS to Previously Published Models. Within GUTS a range of previously published models for survival are integrated as special cases and their specific assumptions are made transparent (Figure 1, Table 3). Besides SD or IT as causes for death, many models differ in their assumptions about the existence of a threshold, and/or the speed of damage recovery (k_r) (see ref 40 for SD models). Within GUTS, nonthreshold models are a special case ($z = 0$), although this special case only makes sense in combination with SD. The models of critical body residues (CBR, e.g., ref [34]) and critical target occupation (CTO, [37]) can be derived by assuming IT in conjunction with irreversible damage ($k_r = 0$) to arrive at the CTO model, or instantaneous recovery (k_r = infinite) to arrive at the CBR model. Similarly on the side of SD models, one can assume irreversible damage ($k_r = 0$) to arrive at a SD version of the CTO model (hCTO in ref 40), whereas the DEBtox survival model for acute toxicity[35] represents a SD model with the assumption of instantaneous damage recovery (refer to ref 36 for a corresponding model with internal concentrations instead of scaled internal concentrations). For both SD and IT, there is a model that does not make any a priori assumption about the speed of damage recovery, at the cost of (at least) one additional parameter; the TDM[7] and DAM[38], respectively.

Basically, GUTS integrates all previously published TKTD models for survival that we are aware of (i.e., models that include aspects of both TK and TD). Models that do not follow a TKTD concept, such as time-to-event models[62] or classical dose-response models are not included. Note however, that any TKTD model also yields dose-response curves. The majority of the models in Table 3 can be derived from GUTS by simply choosing different parameter values. However, several cases[33,37,39,42] require a slight modification of the structure of one of the submodules. As they still follow the basic GUTS scheme of Figure 1, we consider them to be special cases. GUTS can be expanded to TK with multiple compartments (e.g., physiologically based pharmacokinetic (PBPK) models[63]) or more elaborate TD (e.g., receptor kinetics[39]) without sacrificing the unification.

Relevance for Environmental Risk Assessment of Chemicals. We hope that GUTS will help increase the application of TKTD models in ecotoxicological research as well as environmental risk assessment of chemicals. It unifies a wide range of previously unrelated approaches, clarifies their underlying assumptions, and

facilitates further improvement in modeling of survival under chemical stress. The case study for fathead minnows demonstrates that standard acute toxicity tests can already be analyzed in this framework. GUTS delivers summary statistics for risk assessment (e.g., the threshold for effects) and GUTS can be used to calculate effect levels corresponding to any exposure pattern.

However, we have only dealt with one quantal endpoint, survival. A similar approach may be used for other quantal endpoints that can be viewed as the result of a chance process (e.g., immobilization), but not for continuous endpoints such as growth and reproduction. For such endpoints, we do not follow the *fraction* of individuals that are responding, but rather the *average response* of the individuals. Clearly, a 50% effect on reproduction does not imply that half of the individuals have stopped reproducing. Therefore, the mechanism of chance events or differences in sensitivity as used in GUTS can not be applied to continuous end points. The TK and damage modules of GUTS may still be used for these endpoints, but a different TD link between the dose metric and the toxic effect is required (e.g., ref 6).

TKTD models are crucial to understand toxic effects over time, interpret apparent differences in toxicity between chemicals, understand differences in sensitivity between species and life stages and understand the interaction with other stressors. If we interpret toxicity data with TKTD models, this may also offer the possibility to extrapolate toxic effects to untested species or chemicals, or to time-varying exposure. This extrapolation capability based on mechanisms is a unique property of TKTD models, but their underlying assumptions and their interpretation need to be absolutely clear for a wider acceptance. GUTS is an essential element in this discussion.

■ ASSOCIATED CONTENT

ⓢ Supporting Information. An extended derivation of scaled dose metrics and additional information on the case studies are available online. This material is available free of charge via the Internet at http://pubs.acs.org.

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