Recommendations to address uncertainties in environmental risk assessment using toxicokinetics-toxicodynamics models

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Abstract

Providing reliable environmental quality standards (~~EQS~~EQSs) is a challenging issue ~~for~~ in environmental risk assessment (ERA). These ~~EQS~~ EQSs are derived from toxicity endpoints estimated from dose-response models to identify and characterize the environmental hazard of chemical compounds such as those released by human activities. ~~The classical toxicity endpoints are the~~ These toxicity endpoints include the classical x% effect/lethal concentrations at a specific time t ($EC/LC(x,t)$) ~~,~~ ~~or the~~ and the new multiplication factors applied to environmental exposure profiles leading to x% ~~of~~ effect reduction at a specific time t ($MF(x,t)$, or denoted $LP(x,t)$ by the EFSA). However, classical dose-response models used to estimate ~~the~~ toxicity endpoints have some weaknesses, such as their dependency on observation ~~time-points~~ time points, which are likely to differ between species ~~.~~ (e.g., experiment duration). ~~Also, real~~ Furthermore, real-world exposure profiles are ~~hardly ever~~ rarely constant over time, ~~what makes impossible~~ which makes the use of classical dose-response models difficult and compromises the derivation of $MF(x,t)$~~, actually designed to tackle time-variable exposure profiles~~. When dealing with survival or immobility toxicity test data, these issues can be overcome with the use of the ~~General Unified Threshold model of Survival~~ general unified threshold model of survival (GUTS), a toxicokinetics-toxicodynamics (TKTD) model ~~, providing~~ that provides an explicit framework to analyse both ~~time~~ time- and concentration-dependent data sets ~~,~~as well as obtain a mechanistic derivation of $EC/LC(x,t)$ and $MF(x,t)$ ~~whatever~~ regardless of x and at any time t of interest. In addition, the assessment of a risk is inherently built upon probability distributions, ~~so~~ such that the next critical step for ERA is to characterize the uncertainties of toxicity endpoints ~~, andsequentially of EQS~~and, consequently, those of EQSs. ~~The innovative approach investigated in our paper is~~ With this perspective, we investigated the use of ~~the~~ a Bayesian framework to ~~deal with uncertainties raising in~~ obtain the uncertainties from the calibration process and ~~propagated all along the successive prediction steps until the~~ to propagate them to model predictions, including $LC(x,t)$ and $MF(x,t)$ derivations. We also explored the mathematical properties of $LC(x,t)$ and $MF(x,t)$ as well as the impact of different experimental designs ~~in order~~ to provide some recommendations for a robust derivation of toxicity endpoints leading to reliable ~~EQS.~~ EQSs: avoid computing $LC(x,t)$ and $MF(x,t)$ for extreme x values (0 or 100%), where uncertainty is maximal; compute $MF(x,t)$ after a

long period of time to take depuration time into account and test survival under few correlated and uncorrelated pulses of the contaminant in terms of depuration.

Keywords. Survival models; ~~Dose-Response~~ Dose Response; GUTS; Lethal Concentration; Multiplication Factor; ~~Margin of safety~~ Lethal Profile; Margin of Safety; Environmental Risk Assessment

1. Introduction

Assessing the environmental risk of chemical compounds requires environmental quality standards (~~EQS)such as PNECs, RACs and MAC-EQS under the ECHA, EFSA PPR and WFP regulatory frameworks respectively (EFSA PPR, 2013; ECHA, 2017)~~EQSs), which are based on several calculations depednging on the context and insitutions such as predicted-no-effect concentrations (PNECs) (EFSA PPR, 2013) and specific concentration limits (SCLs) (ECHA, 2017). ~~Derivation of EQS results from the~~ Specifically, the derivation of EQSs results from a combination of assessment factors with toxicity endpoints mainly ~~derivated from estimated or measured exposure response~~ estimated from measured exposure responses of a set of target species to ~~that~~ a certain chemical compound (EFSA PPR, 2013; Isigonis et al., 2015; Syberg and Hansen, 2016; ECHA, 2017). ~~Deriving~~ Estimating reliable toxicity endpoints is challenging and ~~the subject matter is~~ very controversial (Laskowski, 1995; Jager, 2011). ~~Today, Environmental Risk Assessment~~ Currently, the first step of environmental risk assessment (ERA) ~~rests on~~ is the hazard identification of acute effects, which consists of fitting classical dose-response models to quantitative toxicity test data. For acute effect assessment, such data are collected from standard toxicity tests, from which the 50% lethal or effective concentration (LC_{50} or EC_{50}, respectively) is generally estimated at the end of the exposure ~~duration~~period, meaning that ~~the monitoring of observations over time is not fully exploited~~not all observations are used. In addition, classical dose-response models implicitly assume that the exposure concentration remains constant ~~all along~~ throughout the experiment, ~~what makes~~ which makes it difficult to extrapolate the results to more realistic scenarios with time-variable exposure profiles combining different heights, widths and frequencies of contaminant pulses (Reinert et al., 2002; Brock, 2009; Jager, 2011; Ashauer et al., 2013).

To overcome this ~~gap~~ limitation at the organism level, the use of mechanistic models, such as toxicokinetics-toxicodynamics (TKTD) models, is now promoted ~~in order~~ to describe the effects of a substance of interest by integrating the dynamics of the exposure (Jager et al., 2011; EFSA PPR, 2013; Hommen et al., 2016). Indeed, TKTD models appear highly advantageous in terms of gaining a mechanistic understanding of the chemical mode of action, ~~of~~ deriving time-independent parameters, ~~of~~ interpreting time-varying exposure and ~~of~~ making predictions under untested ~~situations~~ conditions (Jager et al., 2011; Ashauer et al., 2013). Another ~~of their advantages~~ advantage of TKTD models for ERA is the possible calculation of lethal concentrations for any x% ~~lethal $LC(x,t)$ or effective $EC(x,t)$ whatever x and~~ of the population at any given exposure duration t, denoted

$LC(x,t)$. ~~Also~~Furthermore, from time-variable concentration profiles ~~as~~ observed in the environment, it is possible to estimate a margin of safety such as the exposure multiplication factor ~~,~~ $MF(x,t)$, leading to any x% ~~of~~ effect reduction due to the contaminant at any time t ~~(Ashauer et al., 2013).~~ (Ashauer et al., 2013; EFSA PPR Scientific Opinion, 2018) (also called the lethal profile and denoted $LP(x,t)$ by EFSA PPR Scientific Opinion (2018)).

When focusing on the survival rate of individuals, ~~a General Unified Threshold model of Survival~~ the general unified threshold model of survival (GUTS) has been proposed to unify the majority of TKTD survival models (Jager et al., 2011). In the present paper, we consider the two most used derivations~~named Stochastic Death~~ , namely, the stochastic death (GUTS-RED-SD) and ~~Individual Tolerance~~ individual tolerance (GUTS-RED-IT) models. The GUTS-RED-SD model assumes that all individuals are identically sensitive to the chemical substance by sharing a common internal threshold concentration and that mortality is a stochastic process once this threshold is reached. ~~On the contrary,~~ In contrast, the GUTS-RED-IT model is based on the ~~Critical Body Residues~~ critical body residue (CBR) approach, which assumes that individuals differ in their ~~threshold~~thresholds, following a probability distribution, and die as soon as the internal concentration reaches the individual-specific threshold (Jager et al., 2011). The robustness of GUTS models ~~for~~ in calibration and prediction has been widely demonstrated~~in previous studies~~, with little ~~differences between both~~ difference between GUTS-RED-SD and GUTS-RED-IT models ~~in terms of calibration and prediction~~ (Ashauer et al., 2013; Baudrot et al., 2018c; Jager and Ashauer, 2018). Sensitivity analysis of toxicity endpoints ~~derivated from GUTS~~ derived from GUTS models, such as $LC(x,t)$ and $MF(x,t)$, ~~have~~ has also been investigated (Ashauer et al., 2013; Baudrot et al., 2018c), but the question of how uncertainties are propagated is still under-studied.

Quantifying uncertainties or ~~level~~ levels of confidence associated with toxicity endpoints is undoubtedly a way to improve trust in risk predictors and to avoid ~~decision~~ decisions that could increase ~~,~~ rather than decrease ~~, the risk (Gray and Cohen, 2012; Beck et al., 2016)~~the risk (Dale et al., 2008; Gray and Cohen, 2012; Beck . The Bayesian framework has many advantages ~~to deal~~ for dealing with uncertainties since the distribution of parameters ~~, and so their uncertainties ,~~ and thus their uncertainties is embedded in the inference process (Siu and Kelly, 1998). While the construction of priors on model parameters can be seen as ~~a carrier of subjectivity (Ferson, 2005), there is a proved added-value~~ subjective (Ferson, 2005), it provides added value by taking advantage of information from the experimental design (Delignette-Muller et al., 2017; Baudrot et al., 2018c). Consequently, coupling TKTD models with Bayesian inference allows one to estimate the probability distribution of toxicity endpoints ~~,~~ and any other predictions coming from the mechanistic (TKTD) model ~~,~~ by taking into account all the constraints resulting from the experimental design. Moreover, Bayesian inference, which ~~revealed~~ is particularly efficient with GUTS models (Delignette-Muller et al., 2017; Baudrot et al., 2018c), can also be used

to optimize the experimental design by quantifying the gain ~~of~~ in knowledge from priors to posteriors (Albert et al., 2012). ~~At last~~Finally, Bayesian inference is ~~also~~ tailored for decision making as it ~~confronts the~~ provides assessors with a range of values ~~, rather than just a~~ rather than a single point, which is particularly valuable ~~for~~ in risk assessment (Ferson, 2005; Gray and Cohen, 2012).

In the present study, we explore how scrutinizing uncertainties helps ~~to provide recommendations on the~~ provide recommendations for experimental design and the characteristics of toxicity endpoints used ~~for EQS,~~ in EQSs while maximizing their reliability. We first give an overview of TKTD models, with a focus on ~~GUTS (Jager et al., 2011)~~the GUTS (Jager et al., 2011) to derive EQS explicite equations. ~~Handling~~ We then illustrate how to handle GUTS models within the ~~R-package~~ R package *morse* (Baudrot et al., 2018a) ~~is then illustrated~~ with five example data sets. Then, we explore how a variety of experimental designs influence the uncertainties in derived $LC(x,t)$ and $MF(x,t)$. Finally, we provide a set of recommendations on the use of TKTD models for ERA ~~,~~based on their ~~added-value~~ added value and the way the uncertainty may be handled under ~~the~~ a Bayesian framework.

2. Material and methods

2.1. Data from experimental toxicity tests

We used experimental toxicity data sets ~~, detailled in (Ashauer et al., 2011; Nyman et al., 2012; Ashauer et al., 2016) testing all together~~ described in Ashauer et al. (2011) and Nyman et al. (2012) testing the effect of five chemical compounds (carbendazim, cypermethrin, dimethoate, malathion and propiconazole) on the survival rate of the amphipod crustacean *Gammarus pulex*. Two experiments were performed for each compound, one exposing *G. pulex* to constant concentrations ~~,~~ and the other exposing *G. pulex* to time-variable concentrations (see Table 1). In the constant exposure experiments, *G. pulex* was exposed to eight concentrations for four days. In the time-variable exposure experiments, *G. pulex* was exposed to two different pulse profiles ~~, consisting in~~ consisting of two one-day exposure pulses with ~~short and longer~~ either a short or long interval between them.

2.2. GUTS modelling

In ~~the following~~this section, we detail the mathematical equations of GUTS models describing the survival rate over time ~~for~~ of organisms exposed to a profile of concentrations of a single chemical product. All other possible derivations of GUTS models are fully described in (Jager et al., 2011; Jager and Ashauer, 2018). ~~We provide here~~ Here, we provide a summary of GUTS-RED-SD and GUTS-RED-IT reduced models ~~in order~~ to introduce notations and equations relevant for mathematical derivation of explicit formulations of the x% ~~Lethal Concentration~~ lethal concentration at time t, denoted $LC(x,t)$, and of the ~~Multiplication Factor~~ multiplication factor leading to x% mortality at time t, denoted $MF(x,t)$.

Table 1: Characteristics of data sets used in the manuscript. ~~The~~ "Profile type" is the type of exposure ~~profiles~~ profile (constant or time-variable), "Data points" refers to the number of data points in the data set, "Nbr profiles" is the number of profiles in the ~~dataset~~ data set, "N_{init}" is the initial number of individuals in the profile, "Nbr days" is the number of days for each experiment, and "Time points per profile" is the number of observation time points for each time series (each constant ~~profiles~~ profile consisted ~~in~~ of 5 ~~time-points~~time points).

Product	Profile type	Data points	Nbr profiles	N_{init}	Nbr days	Time points per profile
~~Carbendazim~~ carbendazim	constant	40	8	20	4	5
~~Cypermethrin~~ cypermethrin	constant	40	8	20	4	5
~~Dimethoate~~ dimethoate	constant	40	8	20	4	5
~~Malathion~~ malathion	constant	40	8	20	4	5
~~Propiconazole~~propiconazole	constant	40	8	20	4	5
~~Carbendazim~~ carbendazim	variable	51	4	80	10	[8, 14, 16, 13]
~~Cypermethrin~~ cypermethrin	variable	61	4	80	10	[10, 18, 18, 15]
~~Dimethoate~~ dimethoate	variable	58	4	80	10	[10, 16, 17, 15]
~~Malathion~~ malathion	variable	70	2	70	22	[35, 35]
~~Propiconazole~~propiconazole	variable	74	4	70	10	[11, 21, 21, 21]

2.2.1. Toxicokinetics

We ~~denote~~ define $C_w(t)$ as the external concentration of a chemical product, which can be variable over time. As there is no measure of internal concentration, we use the scaled internal concentration, denoted $D_w(t)$, which is therefore a latent variable ~~as~~ described by the toxicokinetics part of the model as follows:

$$\frac{dD_w(t)}{dt} = k_d(C_w(t) - D_w(t)) \tag{1}$$

where k_d $[time^{-1}]$ is the dominant rate constant, corresponding to the slowest compensating process dominating the overall dynamics of toxicity.

As we assume that the internal concentration ~~equal~~ equals 0 at $t = 0$, the explicit formulation for constant concentration profiles is given by ~~:~~

$$D_w(t) = C_w\left(1 - e^{-k_d t}\right) \tag{2}$$

An explicit expression for time-variable exposure profiles is provided in the Supplementary Material as it can be ~~usefull for implementation ,~~ useful for implementation but not for ~~mathematical calculus~~ the mathematical calculus presented below. The GUTS-RED-SD and GUTS-RED-IT models are based on the same model for the scaled internal concentration. ~~They~~ These models do not differ in the TK part ~~, but~~ but do differ in the TD part describing the death mechanism.

From the toxicokinetics equation (2), we can easily compute the x% depuration time DRT_x, that is, the period of time after a pulse leading to an x% ~~of~~ reduction in the scaled internal concentration:

$$DRT_x = \frac{-\log(x\%)}{k_d} \tag{3}$$

While GUTS-RED-SD and GUTS-RED-IT models have the same toxicokinetic equation (1), the DRT_x likely differs between them since the meaning of damage depends on the toxicodynamic equations, which are different.

2.2.2. Toxicodynamics

~~*Model GUTS-RED-SD:*~~. The GUTS-RED-SD model supposes that all the organisms have the same internal threshold concentration, denoted z $[mol.L^{-1}]$, and that ~~, once~~ once this concentration threshold is exceeded, the instantaneous probability ~~to die, named~~ of death, denoted $h(t)$, increases linearly with the internal concentration. The mathematical equation is ~~:~~

$$h(t) = b_w \max_{0 \leq \tau \leq t} (D_w(\tau) - z, 0) + h_b \tag{4}$$

where b_w $[L.mol.time^{-1}]$ is the killing rate and h_b $[time^{-1}]$ is the background mortality rate.

Then, the survival probability ~~along time under~~ over time under the GUTS-RED-SD model is given by ~~:~~

$$S_{SD}(t) = \exp\left(-\int_0^t h(\tau)\, d\tau\right) \tag{5}$$

~~*Model GUTS-RED-IT:*~~. The GUTS-RED-IT model supposes that the threshold concentration is distributed among organisms ~~, and that the~~ and that death is immediate as soon as this threshold is reached. The probability ~~to die~~ of death at the maximal internal concentration with background mortality h_b is given by ~~:~~

$$S_{IT}(t) = \exp(-h_b t)(1 - F(\max_{0 < \tau < t}(D_w(\tau)))) \tag{6}$$

Assuming a log-logistic function, we get $F(x) = \dfrac{1}{1 + (x/m_w)^{-\beta}}$, with the median m_w ~~$[mol.L^{-1}]$~~ ~~the median and~~ $[mol.L^{-1}]$ and shape β ~~the shape~~ of the threshold distribution, ~~what gives :~~ which gives

$$S_{IT}(t) = \exp(-h_b t)\left(1 - 1/\left(1 + \left(\frac{\max_{0 \leq \tau \leq t}(D_w(\tau))}{m_w}\right)^{-\beta}\right)\right) \tag{7}$$

2.3. Implementation and Bayesian inference

GUTS models were implemented within a Bayesian framework ~~through~~ with *JAGS* (Plummer, 2016) by using the ~~R package~~ R package *morse* (Baudrot et al., 2018a). The Bayesian inference methods, choice of priors and parameterisation of the MCMC process have previously been fully explained (Delignette-Muller et al., 2017; Baudrot et al., 2018c,a). The joint posterior distribution of parameters was used to predict survival ~~curve~~ curves under tested and untested exposure profiles, ~~for~~

the calculation of to calculate $LC(x,t)$ and $MF(x,t)$, and for the computing of to compute goodness-of-fit measures (see hereinafter). The use of the joint posterior distribution allow allowed us to quantify the uncertainty around all these predictions, and therefore the computing of their median and their ; therefore, their medians and 95% credible intervals as follow were computed as follows: under a specific exposure profile, we simulated the survival rate over time for every joint posterior parameter set; then, at each time point of the time series, we computed 0.5, 0.025 and 0.975 quantiles, thus providing median medians and 95% limits.

2.4. Measures of model robustness

Modelling is always associated with testing its robustness: robustness: not only the robustness in fitting data used for calibration , but also the robustness for predictions on in generating predictions with new data (Grimm and Berger, 2016). To evaluate the robustness of estimations and predictions with the two GUTS models, we calculated their statistical properties by means of the Normalized Root Mean Square Error normalized root mean square error (NRMSE), the Posterior Predictive Check posterior predictive check (PPC), the Watanabe-Akaike Information Criterion and the Leave-One-Out Cross-Validation information criterion and leave-one-out cross-validation (LOO-CV) (Gelman et al., 2013).

2.4.1. Normalized Root Mean Square Error root mean square error

The Root Mean Square Error root mean square error (RMSE) allows to charaterize one to characterize the difference between observations and predictions from the posterior distribution. With N observations and $y_{i,obs}$ the observed number of observed individuals ($i \in \{1,\dots,N\}$), then for each estimation $y_{.,j}$ of the markov Markov chain of size M ($j \in \{1,\dots,M\}$) resulting from the Bayesian inference, we can define the $RMSE_j$ such as : as

$$RMSE_j = \sqrt{\frac{1}{N}\sum_{i}^{N}(y_{i,j} - y_{i,obs})^2} \quad \Rightarrow \quad NRMSE_j = \frac{RMSE_j}{\overline{y_{obs}}} \tag{8}$$

Where Normalized where the normalized RMSE (NRMSE) is given by dividing RMSE with by the mean of the observations, denoted $\overline{y_{obs}}$. We then have the distribution of the NRMSE, from which we returned can obtain the median and the 95% credible interval, as presented in Table 2.

2.4.2. Posterior Predictive Check predictive check (PPC)

The Posterior Predictive Check consists in posterior predictive check consists of comparing replicated data drawn from the joint posterior predictive distribution to observed data. A measure of goodness-of-fit is the percentage of observed data lying falling within the 95% predicted credible intervals (Gelman et al., 2013).

2.4.3. WAIC and LOO-CV

Information criteria ~~as~~ such as the WAIC and LOO-CV are common measures of predictive precision also used to compare models. The WAIC is the sum of the log predictive density computed for every ~~points~~point, to which a bias is added to take into account the number of parameters. The LOO-CV ~~use~~ method uses the log predictive density estimated from a training subset and ~~applied it on~~ applies it to another one (Gelman et al., 2013). Both the WAIC and LOO-CV criteria were computed with the ~~R package~~ R package *bayesplot* (Gabry and Mahr, 2017).

2.5. Mathematical definition and properties of $LC(x,t)$

The $LC(x,t)$ makes sense only ~~in the situation~~ under conditions of constant exposure profiles (i.e., ~~whatever~~ for any time t, $C_w(t)$ is constant). In such situations, we can provide an explicit formulation of the survival rate over time ~~considering both models~~ by considering both the GUTS-RED-SD and GUTS-RED-IT models. Many software ~~provides~~ provide an implementation of GUTS models ~~what facilitate the possibility~~ that make it possible to compute the $LC(x,t)$ at any time and ~~any x%~~ for any x% (Jager and Ashauer, 2018). Our Bayesian implementation of GUTS models using the R ~~language is one of them (Baudrot et al., 2018a).~~ environment is one example (Baudrot et al., 2018a).

Let $LC(x,t)$ be the lethal concentration for ~~x%~~ x% of organisms at any time t ~~,~~and $S(C,t)$ be the survival rate at the constant concentration C and time t. Then, the $LC(x,t)$ is defined as ~~:~~

$$S(LC(x,t),t) = S(0,t)\left(1 - \frac{x}{100}\right) \tag{9}$$

where $S(0,t)$ is the survival rate at time t when there is no contaminant, which reflects the background mortality.

2.5.1. GUTS-RED-SD model

The lethal concentration $LC_{SD}(x,t)$ is given by ~~:~~

$$LC_{SD}(x,t) = \frac{-k_d \ln\left(1 - \frac{x}{100}\right)}{b_w\left(k_d(t-t_z) - e^{-k_d t_z} + e^{-k_d t}\right)} + \frac{k_d z(t-t_z)}{k_d(t-t_z) - e^{-k_d t_z} + e^{-k_d t}} \tag{10}$$

As ~~mention in~~ mentioned in the Supplementary Material, under time-variable exposure, t_z ~~is also variable with~~ also varies over time, while in the case of constant exposure, t_z is exactly $-1/k_d \ln(1 - z/C_w)$. ~~When time~~increase~~When time~~This expression of t_z prevents an explicit formulation of $LC_{SD}(x,t)$. For increasing time, the $LC_{SD}(x,t)$ curve ~~become~~ becomes a vertical line at ~~point~~ concentration z~~, and~~ . We assume that the threshold concentration z is reached in a finite amount of time, which means that ~~$\lim_{t\to+\infty}$~~ $t - t_z = +\infty$. Therefore, when time tends to infinity, the convergence is ~~:~~

$$\lim_{t\to+\infty} LC_{SD}(x,t) = z \quad , \quad \text{with} \quad t_z = \frac{-1}{k_d}\ln\left(1 - \frac{z}{LC_{SD}(x,t)}\right) \tag{11}$$

2.5.2. GUTS-RED-IT model

The lethal concentration $LC_{IT}(x,t)$ is given by ~~:~~

$$LC_{IT}(x,t) = \frac{m_w}{(1-e^{-k_d t})} \sqrt[\beta]{\frac{x}{100-x}} \tag{12}$$

It is then ~~straightfoward to see that when~~ clear that as t increases, the $LC_{IT}(x,t)$ converges to ~~:~~

$$\lim_{t\to+\infty} LC_{IT}(x,t) = m_w \sqrt[\beta]{\frac{x}{100-x}} \tag{13}$$

In the specific case of ~~$x = 50\%$~~$x = 50\%$, we get ~~:~~ $\lim_{t\to+\infty} LC(50,t) = m_w$.

2.5.3. Calculation of the density distribution of $LC(x,t)$

The calculation of $LC(x,t)$ is based on equation (9). ~~Then, using~~ Using the GUTS models and the estimates of parameters from the calibration processes, we compute the survival rate without contamination (i.e., the background mortality, denoted $S(0,t)$) and a set of ~~prediction~~ predictions of the survival rate over a range of concentrations (i.e., $S(C,t)$). ~~This process provides the distribution of the $LC(x,t)$ using equation .~~

2.6. Mathematical definition and properties of the multiplication factor $MF(x,t)$

Contrary to the lethal concentration $LC(x,t)$ used ~~in situations~~ under conditions of constant exposure profiles, the multiplication factor ~~,~~$MF(x,t)$ can be computed for both constant and time-variable exposure profiles.

With the exposure profile $C_w(\tau)$, with τ ~~running~~ ranging from 0 to t, the $MF(x,t)$ is defined as ~~:~~

$$S\left(MF(x,t) \times C_w(\tau), t\right) = S\left(0,t\right)\left(1 - \frac{x}{100}\right) \tag{14}$$

In the Supplementary Material, we show that the internal damage $D_w(t)$ is linearly related to the multiplication factor since ~~whatever~~ regardless of the exposure profile (constant or time-variable), we get the following ~~relation~~relationship:

$$D_w^{MF}(t) = MF(x,t) \times D_w(t) \tag{15}$$

where $D_w^{MF}(t)$ is the internal damage when the exposure profile is ~~multipied~~ multiplied by $MF(x,t)$.

2.6.1. GUTS-RED-SD model

The multiplication factor $MF_{SD}(x,t)$ is given by ~~:~~

$$MF_{SD}(x,t) = \frac{-\frac{1}{b_w}\ln\left(1-\frac{x}{100}\right) + \int_0^t \max_{0<\tau<t}\left(D_w(\tau) - z\,,\,0\right)d\tau}{\int_0^t \max_{0<\tau<t}\left(D_w(\tau) - \frac{z}{MF(x,t)}\,,\,0\right)d\tau} \tag{16}$$

~~When the external concentration is constant, we can use the explicit expression of $D_w(t)$ for $C_w(t) = C_w$, and get:~~

$$MF_{SD}(x,t) = \frac{-\frac{1}{b_w}\ln\left(1-\frac{x}{100}\right) + \frac{C_w}{k_d}\left(e^{-k_d t} - e^{-k_d t_z}\right) + (C_w - z)(t - t_z)}{\frac{C_w}{k_d}\left(e^{-k_d t} - e^{-k_d t_{z,MF}}\right) + \left(C_w - \frac{z}{MF(x,t)}\right)(t - t_{z,MF})}$$

~~where t_z has been previously defined and $t_{z,MF} = \frac{-1}{k_d}\ln\left(1 - \frac{z}{MF(x,t)C_w}\right)$. As for the $LC_{SD}(x,t)$, the expression of $t_{z,MF}$ prevents to have a whole explicit formulation of $MF_{SD}(x,t)$.~~

2.6.2. GUTS-RED-IT model

The multiplication factor $MF_{IT}(x,t)$ is given by ~~:~~

$$MF_{IT}(x,t) = \sqrt[\beta]{\frac{100 + x\left(\frac{\max_{0<\tau<t}(D_w(\tau))}{m_w}\right)^{-\beta}}{100 - x}} \tag{17}$$

Therefore, from a GUTS-RED-IT model, solving the toxicokinetics part~~giving~~, which gives $\max_{0<\tau<t}(D_w(\tau))$, is enough to find any multiplication factor for any x at any t. When the external concentration is constant, this maximum is $C_w\left(1 - e^{-k_d t}\right)$.

3. Results

3.1. Goodness-of-fit of GUTS-RED-SD and GUTS-RED-IT models

For all compounds, ~~Table 2 shows that fitting on~~ fitting observed survival with test data obtained under constant exposure profiles ~~provide better fit than for~~ provides better fits than using data from testing under time-variable exposure profiles (~~see also graphics of Posterior Predictive Check~~ Table 2, see also posterior predictive check graphics in Supplementary Material), ~~whatever~~ regardless of the measure of goodness-of-fit (except ~~with~~ for the NRMSE measure ~~of GUTS-RED-IT on~~ used on the GUTS-RED-IT model of dimethoate). This result ~~could be expected~~ is unsurprisingly since, as ~~pointed by~~ shown in Table 1, there are always more time series in data sets with constant exposure profiles. ~~But also~~However, since there are explicit solutions of differential equations with constant exposure profiles

for both ~~models~~ the GUTS-RED-SD and GUTS-RED-IT ~~, the computing process is easier contrary to~~ models, the computational process for constant exposure profiles is easier than that for time-variable exposure ~~profile requiring~~ profiles, which requires the use of a numerical integrator.

For validation, ~~whatever the~~ we calibrated the model on a data set A to then predict another data set B. As a result, regardless of the measure of goodness-of-fit, the predictions are always better when ~~parameters are calibrated on data sets with variable~~ the calibration is carried out using data of time-variable exposure profiles to then predict ~~on data set under~~ data from constant exposure profiles ~~, than the other way round~~than when the inverse was carried out, that is, calibration using data from testing under constant exposure profiles to then predict data from testing under time-variable exposure profiles.

~~Based on~~ Table 2 ~~, it is hard to differentiate~~ shows that the GUTS-RED-SD ~~from~~ and GUTS-RED-IT ~~with~~ models are similar in the quality of their fits. ~~At least, we can notice that~~ However, the GUTS-RED-IT model ~~is particularly bad for Carbendazim and Dimethoate~~ particularly underperforms for carbendazim and dimethoate under time-variable exposure profiles. ~~Still under variable exposure profiles , for Malathion and Propiconazole~~ Nonetheless, under time-variable exposure profiles for the malathion and propiconazole data sets, ~~we can observed a large~~ the 95% credible interval for the GUTS-RED-IT model is large (see figures in the Supplementary Material). ~~While NRMSE and % PPC tend to better qualified GUTS-RED-IT, the uncertainty is penalized with~~ However, when uncertainties are large, the 95% credible interval around predictions used for the PPC tends to cover all the observations regardless of the fitting accuracy. The Bayesian measures WAIC and LOO-CV are better for penalizing excessively large uncertainties. ~~In fact, the percentage of recovery extracted from a PPC is totally blind to point large credible interval, since it increases when the credible interval increases.~~

Table 2: Results of calibration and validation of ~~models~~ the GUTS-RED-SD and GUTS-RED-IT models for the five chemical compounds: ~~Carbendazim~~ carbendazim (car), ~~Cypermethrin~~ cypermethrin (cyp), ~~Dimethoate~~ dimethoate (~~Dim~~dim), ~~Malathion~~ malathion (mal) and ~~Propiconazole~~ propiconazole (prz). Profiles of exposure ~~concentration~~ concentrations are either constant, denoted *cst*, or variable, denoted *var*. The notation *cst* → *var* ~~means~~ indicates that calibration was ~~done on~~ carried out with a data set of constant exposure and that validation was ~~done on~~ carried out with a data set ~~oftime-variable~~ of time-variable exposure ~~profile~~ (see data set in Table 1). The measures NRMSE, %PPC, WAIC and LOO-CV assess the goodness-of-fit and are fully explained in section 2.4.

		GUTS SD				**GUTS IT**			
Product	Profile	NRMSE	%PPC	WAIC	LOO-CV	NRMSE	%PPC	WAIC	LOO-CV
Calibration									
car	cst	0.112	100	402.41	403.27	0.124	100	420.11	422.09
cyp	cst	0.095	100	196.37	206.78	0.092	100	188.07	189.09
dim	cst	0.122	97.5	308.94	309.41	0.171	90.0	357.38	358.74
mal	cst	0.090	100	248.87	249.59	0.112	92.5	273.01	273.54
prz	cst	0.102	100	282.03	285.57	0.118	80.0	308.03	314.93
car	var	0.159	82.1	1006.0	1012.1	0.499	32.1	1222.4	1216.4
cyp	var	0.196	91.7	829.04	833.48	0.116	97.2	793.95	801.23
dim	var	0.129	83.3	1224.8	1226.8	0.161	55.6	1357.2	1344.7
mal	var	0.196	97.8	762.58	766.76	0.148	100	908.56	934.80
prz	var	0.164	95.5	951.10	894.02	0.038	97.7	3262.8	1436.2
Validation: data used for parameter calibration → data for prediction and goodness-of-fit									
car	cst → var	0.159	42.9	17709	4578.4	0.148	50.0	12800	4541.0
cyp	cst → var	0.196	91.7	1760.5	1423.5	0.183	88.9	1283.4	1141.0
dim	cst → var	0.129	83.3	1845.7	1685.3	0.199	63.9	1708.7	1628.9
mal	cst → var	0.196	67.4	10162	2610.7	0.169	63.0	1258.5	1286.1
prz	cst → var	0.164	95.5	940.54	900.90	0.176	90.9	894.41	940.74
car	var → cst	0.164	67.5	537.14	537.79	0.228	90.0	437.01	437.01
cyp	var → cst	0.071	82.5	537.62	488.90	0.051	87.5	453.65	378.89
dim	var → cst	0.013	97.5	302.24	302.30	0.157	87.5	389.32	393.68
mal	var → cst	0.053	80.0	470.28	512.86	0.049	90.0	869.45	732.94
prz	var → cst	0.040	77.5	797.60	660.09	0.041	80.0	1661.3	1107.8

3.2. Comparison of $LC(x,t)$ ~~with~~ between GUTS-RED-SD and GUTS-RED-IT models

There is no obvious difference between the GUTS-RED-SD and GUTS-RED-IT models in their goodness-of-fit nor in the calculation of $LC(x,t)$ ~~along~~ over time t or ~~percentage of affected population~~ ~~,~~ for different percentages of the population affected (x).

3.2.1. $LC(x,t)$ as a function of time t

As expected, ~~from~~ Figures 1-(A,B) and ~~Supplementary Material, we see~~ the Supplementary Material show that $LC(x,t)$ decreases with time. ~~Rarely pointed is the~~ The shape of this decrease, which is exponential and converges toward different values according to the model, is rarely analysed. This asymptotic behavior is known as the incipient $LC(x,t)$ (Jager et al., 2006). A direct consequence for risk assessors is that the evaluation of $LC(x,t)$ at an early time induces higher sensitivity to time t than that at a later time (with the specific time being relative to the species and the compound). In other words, the sensitivity of $LC(x,t)$ to time t decreases as long as t increases. For instance, ~~we see on~~ Figures 1-(A,B) ~~that a little~~ reveal that a small amount of change in time around day 2 leads to a greater change in the estimation of $LC(x,t)$ than does a small amount around day 4. However,

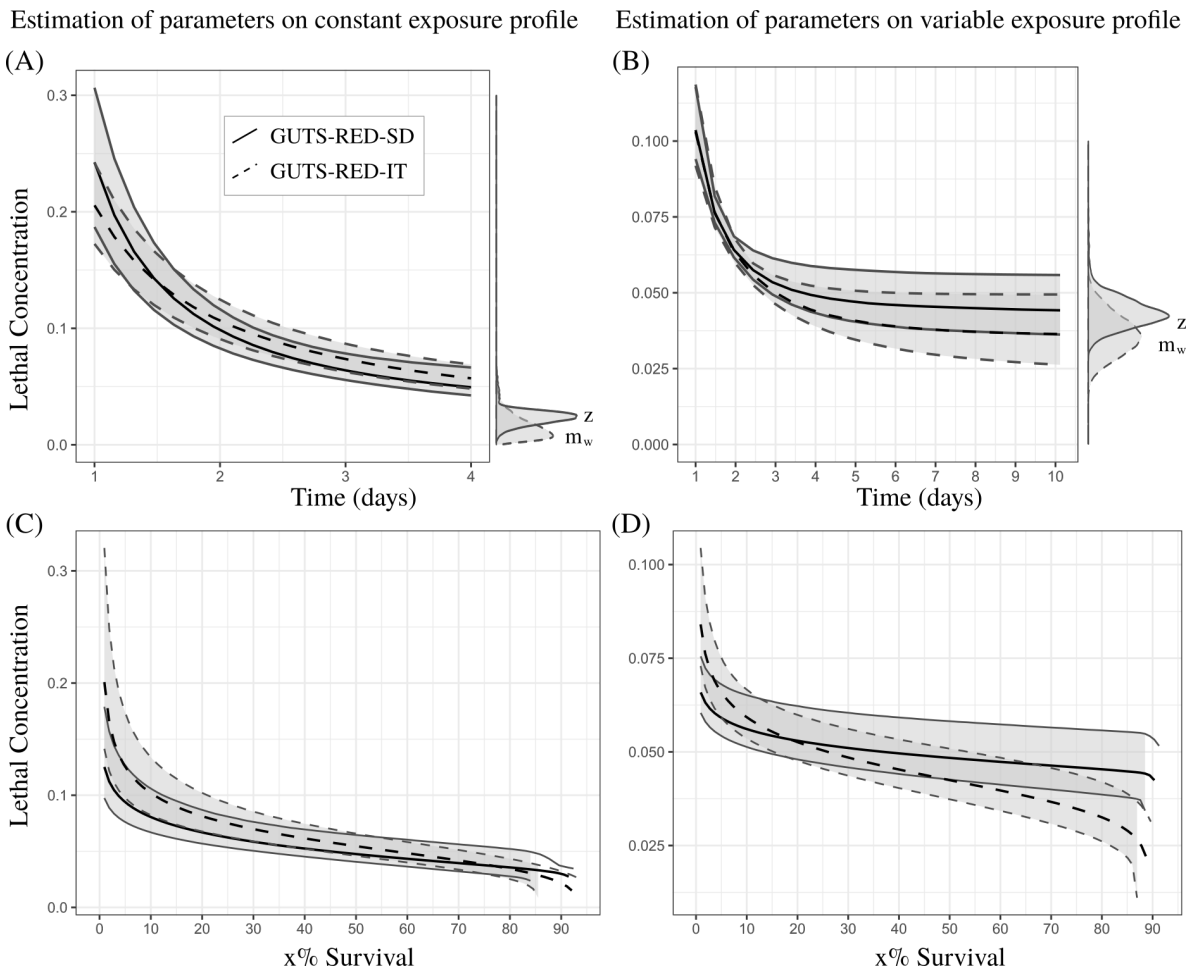

Figure 1: Comparison of $LC(x,t)$ ~~for~~ between GUTS-RED-SD, solid lines, and GUTS-RED-IT models, dashed lines, for ~~Cypermethrin~~ cypermethrin (see Supplementary Material for other compounds). Parameters are estimated ~~on~~ with data collected under constant (A, C) and variable (B, D) concentration profiles. Black lines are ~~median~~ medians, and grey zones are 95% credible bands. (A, B) Lethal concentration for 50% of the organisms ($LC(50,t)$) from day 1 to the end of the experiment. (C, D) Lethal concentration at the end of experiment (4 and 10 days~~respectivelly~~, respectively) ~~along~~ against the percentage of the population affected.

~~we have to~~ note that the uncertainty of $LC(x,t)$ does not always decreases when time increases. For instance, as shown in Figure 1-(B), the uncertainty at day 6 and ~~afterwards~~ afterward is greater than that around day 3.

When t increases to infinity, ~~the~~ $LC(x,t)$ ~~convergences~~ converges towards the distribution of parameter z for the GUTS-RED-SD model (see equation (11)) and $m_w \sqrt[\beta]{\frac{x}{100-x}}$ for the GUTS-RED-IT model (see equation (13)). The specific $LC_{50,t}$ tends to z for the GUTS-RED-SD model and to m_w for the GUTS-RED-IT model (see equations (11) and (13)). ~~The recommendation for risk assessors would be to use the advantages of TKTD models in order to extrapolate the $LC(x,t)$ on a longer period than the duration of the experiment in order to visualize the uncertainties around the incipient $LC(x,t)$ defined by the asymptote. At least, we recommend to look at the $LC(x,t)$ at the last time of the experiment, what is in line with the common procedure in ERA.~~

3.2.2. $LC(x,t)$ as a function of percentage of ~~affected~~ the population affected, x

~~From~~ As shown in Figure 1-(C,D), ~~we can see that~~ the uncertainty of $LC(x,t)$ is greater at low values of x, that is, when the effect of the contaminant is weak. ~~While~~ Although computing $LC(x,t)$ at $x > 50\%$ is never used for ERA, ~~we can also see that~~ the uncertainty of $LC(x,t)$ increases when x tends to 100%. As a consequence, while the uncertainty is not always minimal at the standard value of $x = 50\%$, it seems to ~~be always~~ always be smaller around this value than around $x = 10\%$, another classical value used in ERA. Consequently, ~~for risk assessors,~~ while TKTD models allow risk assessors to compute the $LC(x,t)$ ~~whatever~~ for any value of x, if only one value has to be chosen, we recommend ~~to keep the standard of~~ that the standard $x = 50\%$ ~~. On the other hand, the risk assessor has to keep in mind that 50% is not the optimal threshold in term of reduction of uncertainty, depending on the data set, the model (GUTS-RED-SD or GUTS-RED-IT) and the parameter estimates.~~ be chosen.

3.3. Comparison of $MF(x,t)$ ~~with~~ between GUTS-RED-SD and GUTS-RED-IT models

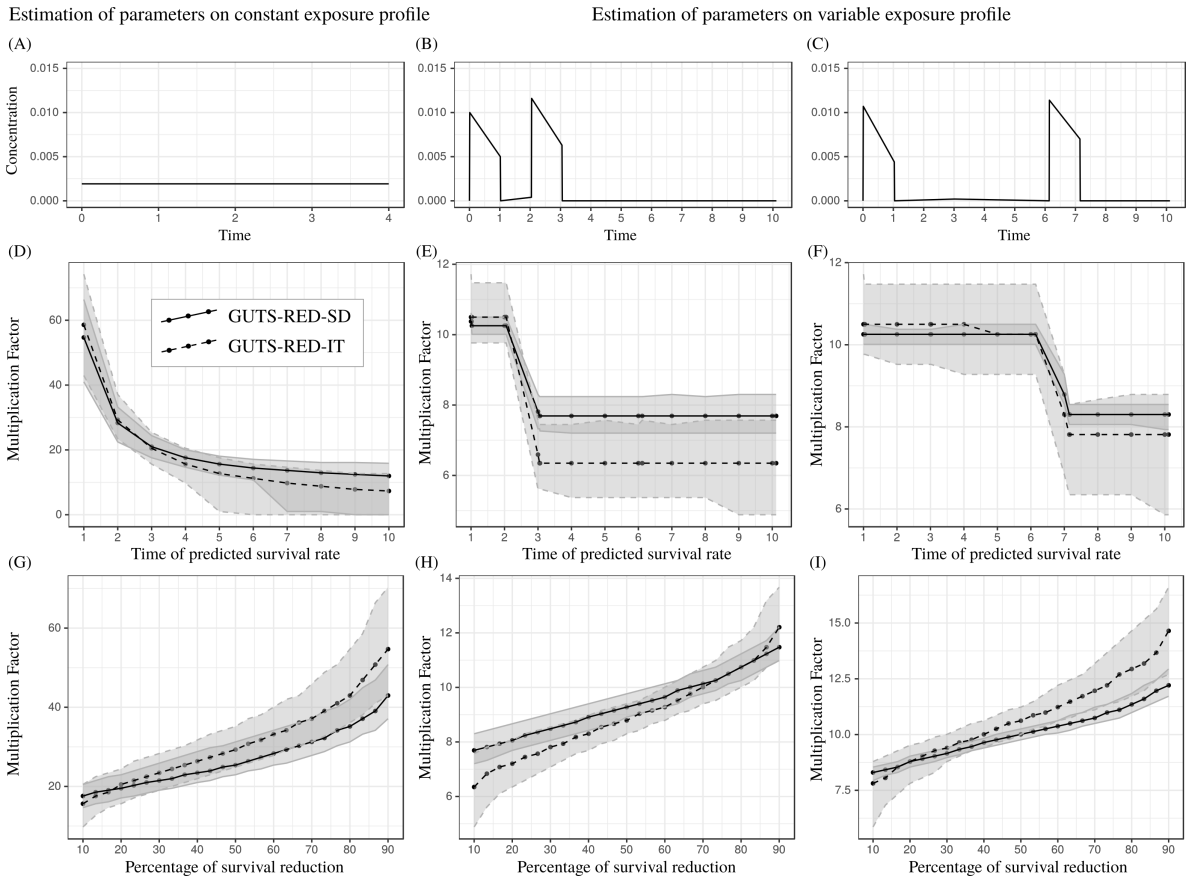

Figure 2: Comparison of $MF(x,t)$ ~~for~~ between GUTS-RED-SD, solid lines, and GUTS-RED-IT models, dashed lines, for ~~Cypermethrin~~ cypermethrin (see Supplementary Material for other compounds). Parameters are estimated ~~on~~ with data collected under constant (A, D, G) and variable (B, C, E, F, H, I) concentration profiles. (A-C) Exposure profiles, (D-F) Multiplication factors estimated for a 10% reduction ~~of~~ in survival (i.e., $MF(x = 10, t)$) ~~along~~ over time. (G-I) Multiplication factors estimated at the end of experiments (time=4 for (G) and 10 for (H, I)) ~~along~~ against the ~~percentage of~~ percent survival reduction.

3.3.1. $MF(x,t)$ as a function of time t

As expected, Figures 2-(D-F) show that the multiplication factor ~~is decreasing~~ decreases, or stay constant, when the time at which the survival rate is checked increases. In other words, the later the survival rate is assessed, the lower ~~is~~ the multiplication factor is. ~~Also~~In addition, these graphics reveal that there is no typical pattern ~~of~~ in the curves of multiplication factors over time t of exposure. Under a constant exposure profile, the curve shows an exponential decreasing pattern, while under pulsed exposure, ~~we observe~~ it shows a constant phase and, ~~surrounding peaks, an sudden decrease of~~ at the time when exposure peaks, a sudden decrease in the multiplication factor. The multiplication factor is ~~obviously~~ clearly highly variable around a ~~pulse in the concentration~~ concentration pulse of the chemical product. ~~Therefore, a recommendation would be to wait for some times (e.g., several days) after a peak before computing a multiplication factor. More generally, the multiplication factor is designed to be compared with the assessment factor (AF) classically used in concert with the effect/lethal concentration value based on realistic time-variable exposure profiles to derive an EQS. As a consequence, when using $MF(x,t)$ based on real exposure profiles, it is important to pay close attention to the amplitudes and frequencies of pulses, as well as to the times at which multiplication factors are computed. As for the $LC(x,t)$, taking advantage of TKTD capabilities to predict at any time is of real interest to described the survival response under pulsed exposure.~~

3.3.2. $MF(x,t)$ as a function of ~~percentage of~~ percent survival reduction x

~~Logically~~Unsurprisingly, Figures 2-(G-I) show that the multiplication factor increases with ~~the increase of the percentage of reduction of~~ an increase in the percent reduction in the survival rate. An interesting ~~results~~ result is the non-linearity of this increase. As observed for the $LC(x,t)$, the uncertainty is greater at low and high percentages ~~compared to what happens in the middle around~~ than for intermediate values near a 50% ~~of~~ survival reduction. As a consequence, it would be relevant to ~~fix~~ set 50% as a standard for ERA.

3.4. Effect of the depuration time on the predicted survival rate

3.4.1. Patterns of internal scaled ~~concentration~~concentrations

The dominant rate constant ~~,~~ k_d, ~~regulating~~ which regulates the kinetics of the toxicant, is always greater for the GUTS-RED-SD ~~compared to~~ model than for the GUTS-RED-IT ~~, so~~ model, such that the depuration time for the GUTS-RED-SD model is always smaller than ~~for~~ that for the GUTS-RED-IT model (see Figure 3 and Supplementary Material). As a consequence, under a time-variable exposure concentration, the internal scaled concentration with the GUTS-RED-SD ~~has~~ model has a greater amplitude than ~~with~~ that with the GUTS-RED-IT model (Figures 4, 5 and Supplementary Material). In other words, ~~toxicokinetics with~~ the toxicokinetics with the GUTS-RED-IT ~~is more smooth than with~~ model are smoother than those with the GUTS-RED-SD model. ~~The compensation~~

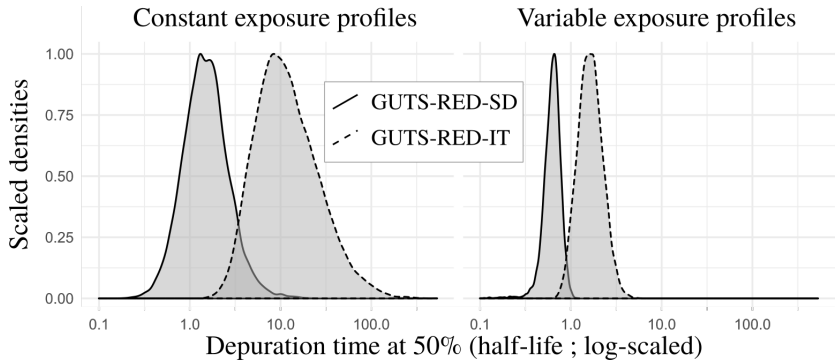

Figure 3: Distribution of estimated ~~half-life~~ depuration time (see equation (3)) for cypermethrin in GUTS-RED-SD and GUTS-RED-IT models for data sets collected under constant (left) and ~~variables~~ variable (right) exposure profiles. Median and 95% ~~Credible~~ credible interval of the 50% depuration time are 1.48 [0.502, 5.00] under constant exposure profiles ~~1.48 [0.502, 5.00]~~ for the GUTS-RED-SD model and 10.8 [3.21, 68.5] under constant exposure profiles for the GUTS-RED-IT model, and those under variable exposure profiles are 0.633 [0.386, 0.890] for the GUTS-RED-SD model and 1.62 [0.917, 3.06] for the GUTS-RED-IT model.

~~of the difference~~ Compensation for differences in k_d ~~,~~ and therefore in the scaled internal ~~concentration~~ concentrations comes from the other ~~paramaters:~~ parameters: the threshold z and ~~killing~~ the mortality rate k_k for the GUTS-RED-SD ~~, and~~ model and the median threshold m_w and shape β for the GUTS-RED-IT model. However, when the calibration of ~~models being~~ the models is based on the same observed number of survivors, the threshold parameter z for the GUTS-RED-SD model and the median ~~of~~ threshold m_w for the GUTS-RED-IT model are shifted.

3.4.2. Variation in the number of pulses in exposure profiles

~~A~~ The first step has been to explore the effect of the number of pulses (9, 6 and 3 pulses of one day each) ~~along~~ over a period of 20 days with the same cumulative amount of contaminant in the external concentration after the 20 days (Figure 4 and Supplementary Material). ~~From~~ For a conservative approach for ERA, ~~whatever the model,~~ regardless of whether the GUTS-RED-SD or GUTS-RED-IT model is used, it seems better to have few pulses of high amplitude than ~~frequent~~ many pulses of low amplitude. Indeed, the survival rate over time with only 3 high pulses is lower than the survival rate under frequent lower exposure. This difference is confirmed in the Supplementary Material for ~~Malathion and Propiconazole~~ the malathion and propiconazole data sets. ~~With GUTS mechanistic models, the higher is the pulse, the higher is the scaled internal concentration and so is the damage. Thus, from these simulations,~~ Since the cumulative amount of contaminant is not changed, we do not see ~~the effect of the depuration time~~ any effect of contaminant depuration (equation (3) and Figure 3), which could help ~~individual to recover when reducing the~~ individuals recover under a lower frequency of peaks.

The comparison between constant and time-variable exposure profiles (Figure 4 and Supplementary Material) suggests that uncertainty is smaller when calibration ~~has been done on data under~~ is performed with data collected under a time-variable exposure profile. ~~The result is counterintuitive~~This

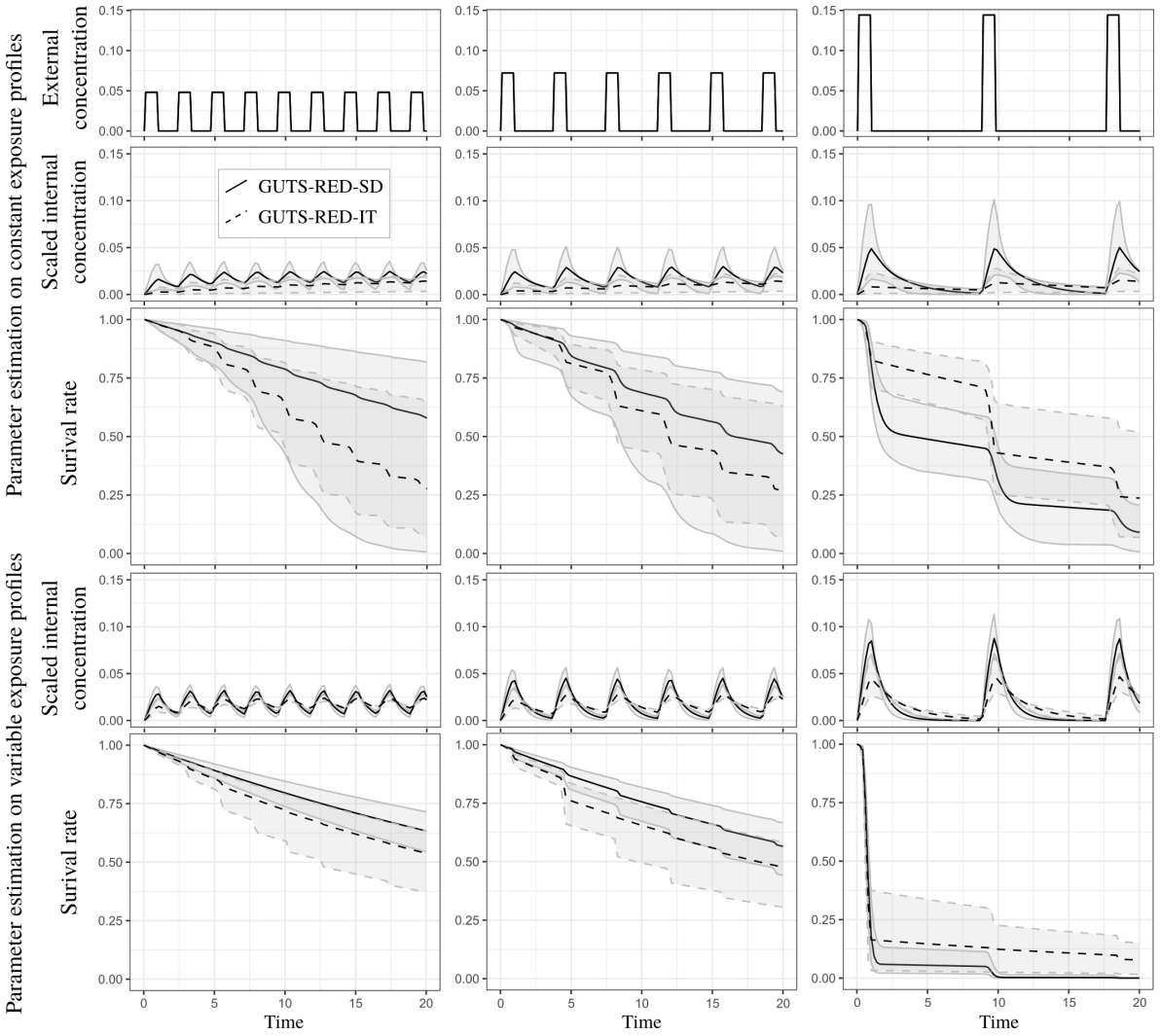

Figure 4: Survival rate over time with GUTS-RED-SD and GUTS-RED-IT models (~~respectively~~ solid and dashed lines, respectively) under different exposure profiles with the same area under the curve (with differences ~~are~~ in the ~~duration~~ time after pulses and ~~in~~ the maximal concentration of pulses). Parameters were estimated from the ~~Cypermethrin~~ cypermethrin data set, ~~either~~ under either constant (upper panel of the figure) or time-variable (lower panel of the figure) exposure.

result is counter-intuitive, especially since the number of time series was higher ~~with~~ for the constant exposure profiles~~what would reduce~~, which would reduce the uncertainties of parameter estimates. If this result is confirmed, then it would be better to predict variable exposure profiles with parameters calibrated from time-variable exposure data sets.

3.4.3. Variation in the period between two pulses

~~In order to~~ To explore the effect of ~~the~~ depuration time, we simulated exposure profiles under two pulses with different ~~time period~~ periods of time between them (i.e., 1/2, 2 ~~and~~ or 7 days). The cumulative amount of contaminant ~~remains~~ remained the same for the three simulations. Figure 5 shows that increasing the period between two pulses may increase the survival rate of individuals, ~~whatever the model,~~ regardless of whether the GUTS-RED-SD or GUTS-RED-IT model is used.

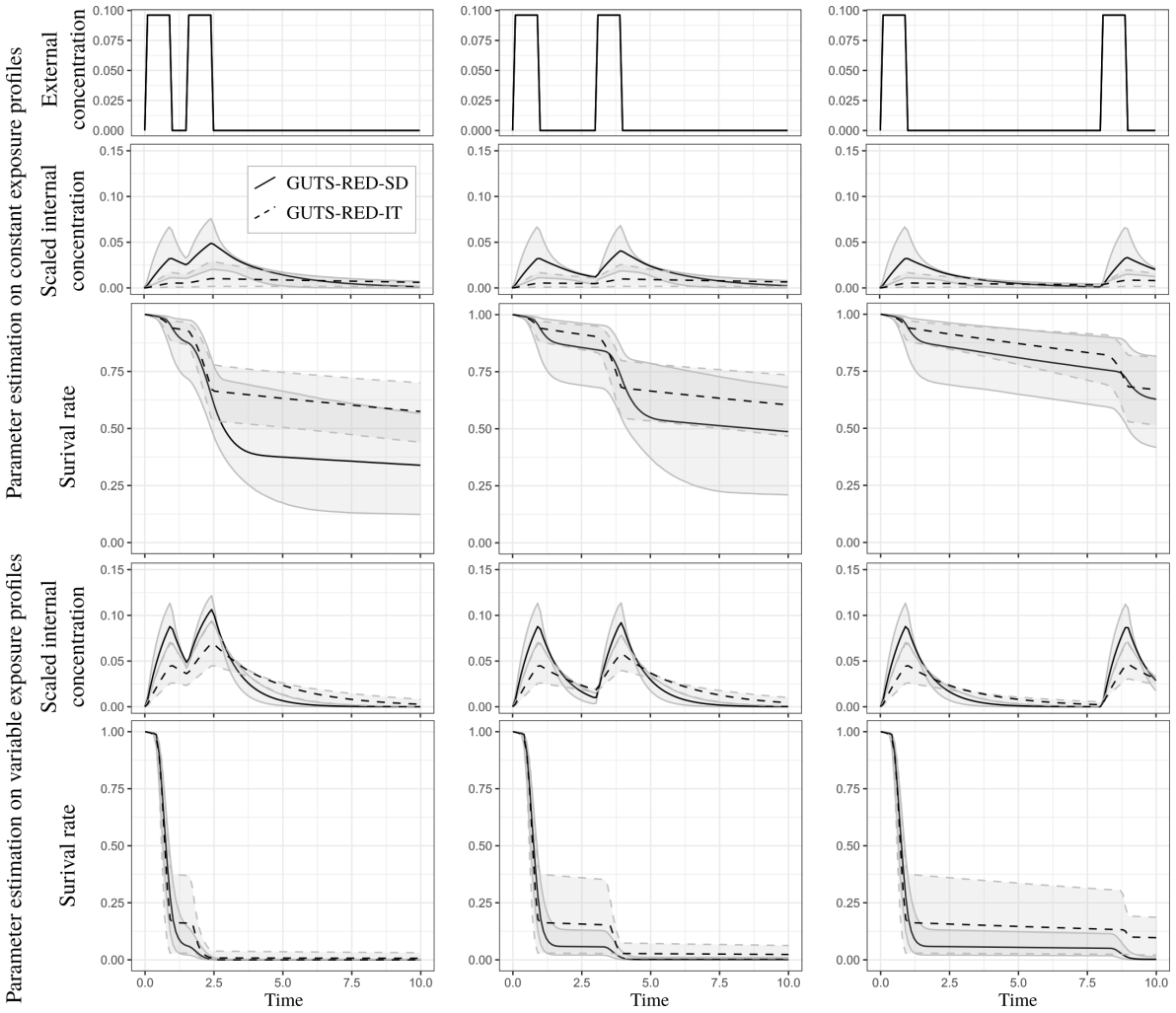

Figure 5: Survival rate over time with GUTS-RED-SD and GUTS-RED-IT models (~~respectively~~ solid and dashed lines, respectively) under ~~two pulsed~~ a two-pulse exposure profile with the same area under the curve (with differences ~~are~~ in the ~~duration~~ time between the two pulses). Parameters were estimated from ~~Cypermethrin~~ the cypermethrin data set, ~~either~~ under either constant (upper panel of the figure) or time-variable (lower panel of the figure) exposure.

This is a typical result of extending the depuration period~~which reduce~~, which reduces the level of scaled internal concentration ~~,~~ and therefore reduces the damage. We can easily see that the highest scaled internal concentration is reached when the pulse interval is the smallest. In this ~~situation, we clearly observe~~ scenario, the addition of damages from the two pulses is clear. Again, ~~depuration time being different with~~ because of the different depuration times of the two GUTS models, ~~results are also~~ the results are different. ~~For ERA, having two close pulses being the most conservative, we recommend to perform such an experiment. However, the depuration time being the differentiating parameter of GUTS-RED-SD and GUTS-RED-IT, it is also relevant to add an experiment with two pulses separated by a long enough period in order to decorrelate their effect. Thus, having both correlated and uncorrelated experiments, we can better assess the influence of GUTS-RED-SD and GUTS-RED-IT hypothesis on the simulation outputs.~~

4. Discussion

4.1. Tracking uncertainties for environmental quality standards

~~Whatever~~ Regardless of the scientific field, risk assessment is by definition linked to the notion of probability, ~~holding~~ characterized by different uncertainties such as the variability ~~between organisms and noises~~ among organisms and noise in observations. In this sense, tracking how ~~the~~ uncertainty propagates into models ~~,~~ from collected data to model calculations of toxicity endpoints that are finally used for ~~EQS~~ EQSs derivation is of fundamental interest for ERA (Dale et al., 2008). For ERA, ~~having good fits over~~ achieving good fits of experimental data is not enough. ~~Indeed~~Instead, the key objective is the application of these fits to predict adverse effects under real environmental exposure profiles ~~,~~ and to derive robust ~~EQS~~ EQSs (Laskowski, 1995; Jager, 2011; Gray and Cohen, 2012; EFSA PPR, 2013; EFSA PPR Scientific Opinion, 2018). In this context, as we ~~show~~ have shown in this paper, TKTD models calibrated under a Bayesian framework ~~combines~~ have two great advantages: on the one hand, TKTD models ~~, such as the GUTS models,~~ allow predictions of regulatory toxicity endpoints under any type of exposure ~~profiles~~ profile; on the other hand, the Bayesian approach provides the marginal distribution of each parameter, and in this way, allows one to track the uncertainty of any prediction of interest.

Previous studies investigating goodness-of-fit did not find typical differences between GUTS-RED-SD and GUTS-RED-IT models (Ashauer et al., 2013; Baudrot et al., 2018c). ~~Here again, from the~~ Our study confirms that under the specific consideration of uncertainties in regulatory toxicity endpoints, ~~we do not show evidence to choose~~ there is no evidence to support choosing either the GUTS-RED-SD ~~compared to~~ or GUTS-RED-IT model over the other. A simple recommendation is therefore to use both and then~~to~~ , if they are successfully validated, take the most conservative scenario in terms of the ERA. With the 10 data sets we used and the 20 fittings we ~~did~~performed, the four measures of goodness-of-fit ~~show~~ showed similar outputs for ~~both~~ the GUTS-RED-SD and GUTS-RED-IT ~~,~~ models under both constant and ~~variables~~ time-variable exposure profiles. The percentage of observed data ~~lying in~~ falling within the 95% predicted credible interval, ~~denoted~~ $\%PPC$, has the advantage of being linked to visual graphics, i.e., PPC plots, and is therefore easier ~~to interpret~~ for risk assessors and ~~stackholders~~ stakeholders to interpret than the Bayesian WAIC and LOO-CV measures (Beck et al., 2016). However, ~~it may hide a very largeuncertainty due to its limitation to 100 % of covering~~when the uncertainty is very large, predictions with their 95% credible intervals are likely to cover all of the observations, even in cases of low model accuracy. We showed that the WAIC and LOO-CV criteria are more robust probability measures for penalizing fits with large uncertainties (Gelman et al., 2013). Since the NRMSE is easy to calculate ~~whatever the inference methods~~ for any inference method (e.g., ~~Maximum Likelihood Estimation~~maximum likelihood estimation), it ~~could be~~ is also a relevant measure ~~to check~~ for checking the goodness-of-fit of models, as recently recommended by

EFSA PPR Scientific Opinion (2018).

4.2. What about the use and abuse of the lethal concentration?

After checking the quality of model parameter calibration, the next question is about the uncertainty ~~in toxicity endpoints to derive EQS~~of toxicity endpoints used to derive EQSs. Lethal concentrations are ~~nowadays~~ currently a standard for hazard characterization at ~~levels of~~ the levels of a 10, 20 and 50% effect on the population. We show that the uncertainty of lethal concentrations differs according to the percentage x under consideration (Figure 1). It appears that this uncertainty is maximal at the extremes (toward 0 and 100%) and limited around 50%. Since the point of minimal uncertainty may drastically change depending on the experimental design, it could be relevant to extrapolate the lethal concentration for a continuous range of x (e.g., 10 to 50%), as we did for Figures 1-(C,D).

Many criticisms have ~~been addressed to~~ targeted the lethal and effective concentrations for x% of the population and other related measures (Jager, 2011). For instance, the classical way to compute the lethal concentration, at the final ~~point, removes~~ time point, ignores information provided by the observations made ~~all along the experiment , and~~ throughout the experiment and thus hides the time dependency. For the lethal effect, a classical approach to limit the variability ~~of time duration,~~ in the period of time is to consider a long enough exposure duration ~~in order~~ to obtain the incipient lethal concentration (i.e., $LC(x, t \to +\infty)$) (Jager et al., 2006), that is~~when the $LC(x,t)$~~ , when the lethal concentration reaches its asymptote and ~~does not change with~~ no longer changes with an increasing duration of exposure~~as observed on~~ , as observed in Figure 1. We provide mathematical expression of ~~$LC(x,t)$ convergence ,~~ the lethal concentration convergence and explicit results when $x = 50\%$ for both GUTS models. We can therefore use the joint posterior parameter distribution provided by ~~the~~ Bayesian inference to compute the distribution ~~the incipient LC~~of the incipient lethal concentration.

A consequence of the exponential ~~decay of $LC(x,t)$~~ decrease in the lethal concentration with increasing time ~~t,~~ is that the sensitivity to time ~~t is greater at early time where~~ is greater early on, when a small change in time ~~t~~ induces a great change in the ~~$LC(x,t)$ whatever~~ lethal concentration regardless of x. ~~For this reason, classical measures of LC are done at the latest time of experiments~~Our analysis confirms that the classical evaluation of lethal concentration at the last time point of an experiment is supported by theoretical considerations. Hence, ~~to compare $LC(x,t)$~~ when comparing the lethal concentrations of different compounds or species that may require different ~~duration of experiments~~experiment durations, using TKTD to extrapolate ~~at~~ to other time points is ~~of great advantage~~highly advantageous. ~~Also, in order to reduce the uncertainty, extrapolation to greater time would be a preferable choice.~~

~~We show in this study that the uncertainty of $LC(x,t)$ is different according to percentage x under consideration (Figure 1). It appears this uncertainty is limited around 50%, while not specifically at 50%, what is in favor of the classical approach to return the LC_{50}. However, it is still of real importance~~

~~to report the uncertainty of the toxicity endpoints since we show it can drastically change depending on the experimental design, the combination product-species.~~

4.3. What does it mean to use a margin of safety?

Among the criticisms of the ~~$LC(x,t)$~~lethal concentration, one is that it is meaningful only under a set of constant environmental conditions, including a constant exposure profile (Jager et al., 2006; Jager, 2011). When the concentration of chemical compounds in the environment is highly variable over time~~in the environment~~, the use of toxicity endpoints based on toxicity data for constant exposure profiles may hide some processes, such as the response to pulses of exposure. This inadequacy is the reason underlying the interest ~~of multiplication factor for ERA (Ashauer et al., 2013)~~in multiplication factors for ERA (Ashauer et al., 2013; EFSA PPR Scientific Opinion, 2018).

4.4. ~~What does it mean to take a margin of safety?~~

~~The deduction of a~~ A margin of safety deduced from a multiplication factor ~~, $MF(x,t)$,~~ quantifies how far the exposure profile is below toxic concentrations (Ashauer et al., 2013). Then, a key ~~question~~ objective for risk assessors is to target the safest exposure duration ~~, t, and percentage of~~ and percentage effect on survival, x. Our study ~~show~~ reveals a lower uncertainty around an x value of 50%. Thus, to reduce the uncertainty of the ~~$MF(x,t)$ estimationwe recommend to select~~ multiplication factor estimation, we recommend that 50% be selected, at least for ~~comparison~~ comparisons between studies. We also show that under constant exposure profiles, ~~there is~~ the multiplication factor exhibits an asymptotic shape ~~of the $MF(x,t)$ as it is for $LC(x,t)$~~similar to that of the lethal concentration. There is an incipient value of the multiplication factor for any x ~~when t goes to a long time~~as time goes to infinity. Therefore, under constant profiles, we ~~could recommend to use~~ recommend that the latest time ~~of~~ point in the exposure profile ~~for toxicity endpoints in order~~ be used to determine toxicity endpoints to reduce the ~~uncertainty of the $MF(x,t)$ estimation .~~ sensitivity of the multiplication factor estimation to time.

However, the ~~$MF(x,t)$~~ multiplication factor is meaningful when applied ~~on~~ to realistic exposure profiles, which are rarely constant, and our study shows that there is no asymptotic shape ~~in such situations~~under such conditions. In addition, we observed ~~a~~ great sensitivity of the multiplication factor to time around peaks in the exposure profiles, that is~~a high variation of the $MF(x,t)$ with a little~~ , high variation in the multiplication factor with a small amount of change in time~~t~~. Therefore, it is recommended that multiplication factors be computed only some time (e.g., several days) after a peak. More generally, the multiplication factor is designed to be compared to the assessment factor (AF) classically used with the effect/lethal concentration value to derive EQSs based on real-world exposure profiles. As a consequence, ~~the assessors has to~~ assessors must be very careful ~~about~~ in examining the characteristics of pulses in the exposure profiles ~~in order~~ (e.g., frequencies and amplitudes) to

understand how they drive changes in the multiplication factor. ~~To do so, we recommend to compute the multiplication factor all along the period of the exposure profile, rather than choosing a single distribution at a specific time~~ For such exploration, taking advantage of TKTD capabilities to generate predictions at any time is valuable.

4.3.1. ~~Depuration time~~

4.4. Effect of depuration in time-variable exposure profiles

The survival response to pulses depends on the depuration time driven by the toxicokinetics part of the TKTD model. The kinetics of assimilation and elimination of compounds integrated within the toxicokinetic module ~~is~~ are a fundamental part of ecotoxicological models (Wang and Fisher, 1999). In reduced GUTS models, namely, GUTS-RED-SD and GUTS-RED-IT models, we assume no measure of the internal concentration, which is therefore calibrated at the same time as other parameters included in the toxicodynamics part. The resulting ~~"scaled internal concentration" is linked to a level of damage~~ scaled damage is defined by the ~~toxicodynamic which has~~ toxicodynamics, for which there are two different hypotheses ~~on the death mechanism~~ regarding the mechanism of mortality for GUTS-RED-SD and GUTS-RED-IT models. ~~The mechanistic construction of the model, reflecting biological processes, may be misleading since the toxicokinetic is defined independently of the toxicodynamic part which is chosen afterwards. What is true in the mechanism is not in the inference process where the model parameters, from TK and TD parts, are calibrated all together.~~ As a consequence, ~~as illustrated with our results , the scaled internal concentration~~ our results illustrate that the scaled damage does not have the same ~~biological~~ meaning in GUTS-RED-SD and GUTS-RED-IT ~~,~~ models and therefore cannot be directly compared between ~~both models~~them.

In both models~~of course~~, from the underlying mechanism, we know that damage is positively correlated with pulse amplitude: ~~lower amplitude, lower damage, as we observed from~~ the lower the amplitude is, the lower the damage is, as shown in Figure 4. ~~A resultis that, with~~ As a result, for the same cumulative amount of contaminant ~~along~~ in an experiment, using fewer pulses reduces final survival rates. ~~So~~Therefore, the most conservative experimental design is ~~the~~ one with fewer pulses of relatively high ~~amplitudes~~amplitude.

Furthermore, ~~from~~ in Figure 5, we bring to light the effect of ~~the~~ depuration time. When pulses are close together, the organisms do not have time to depurate~~and thereforethere is an addition of the damage and finally~~ ; therefore, the damage accumulates and thus has a cumulative effect on survival. As a consequence, ~~on~~ in a long enough experiment, when pulses become less correlated in terms of cumulative damage, then the final survival rate increases. Because of this phenomenon, we recommend an experimental design with two close pulses, as it is the more conservative in terms of ERA. However, to ~~have a~~ achieve better calibration of the toxicokinetic parameter, which would potentially differentiate the GUTS-RED-SD model from the GUTS-RED-IT one, it is important to

also ~~have two~~ include uncorrelated pulses in the experimental design.

Finally, our study reveals that the uncertainty ~~for prediction~~ of predictions under time-variable exposure profiles seems to be smaller when calibration ~~was~~ is performed with data sets under time-variable rather than ~~under~~ constant exposure profiles. While this observation makes theoretical sense, since predictions are made ~~on~~ with the same type of profile ~~than calibration of~~ as that used for calibration of the parameters, further empirical studies ~~have to~~ must be performed to confirm this point.

The environmental dynamics of chemical compounds can be highly variable depending not only on the whole environmental context (e.g., ~~anthropogenics~~ anthropogenic activities, geochemical kinetics, and ecosystem processes) but also on the chemical and ~~bio-transformation~~ biological transformation of the compound under study. Therefore, as a general recommendation, we would like to point out the relevancy of experimenting with several type of exposure profiles. ~~Basically, the~~ Generally, a control and both constant and time-variable exposure profiles including toxicologically dependent and independent pulses seem to be the minimum ~~requirement~~requirements.

4.5. Practical use of GUTS models

4.5.1. Optimization and exploration of experimental ~~design~~designs

The complexity of environmental systems combined with ~~the thousand~~ thousands of compounds produced by human activities implies the need to assess environmental risk for a ~~huge set of species-compounds combination~~ very large set of species-compound combinations (Ashauer and Jager, 2018). As a direct consequence, optimizing experimental design ~~in order~~ to maximize the gain ~~of~~ in high-quality information from experiments is a challenging requisite ~~, where~~ for which mechanism-based models combined with ~~the~~ a Bayesian approach offer several tools (Albert et al., 2012). ~~A next step~~ An extension of the present study ~~is~~ would be to use the joint posterior distribution of ~~parameter,~~ parameters and the distribution of toxicity endpoints ~~in order~~ to quantify the gain ~~of~~ in knowledge of several potential experiments ~~in order~~ to select the most informative. The next objective is thus to develop a framework that could help in the construction of new experimental designs ~~in order~~ to minimize their complexity and ~~their~~ number while maximizing the robustness of toxicity endpoint estimates.

4.5.2. ~~Implementation~~

~~Although~~ Despite their many advantages, TKTD models ~~,~~and therefore GUTS models ~~, still~~ remain little used. This lack of use is due to ~~their mathematical complexity~~ the mathematical complexity of such models based on differential equations that need to be numerically integrated when fitted to data (Albert et al., 2016). ~~Associated to their promotion~~ By promoting GUTS models within regulatory documents associated ~~to ERA, the use of GUTS~~ with ERAs, the models could be further extended when available within a software environment allowing their ~~handling without immersing into~~ implementation

without the need to engage with technicalities. ~~Nowadays~~Currently, several software allow ~~to overpass these difficulties~~ these difficulties to be circumvented (Jager and Ashauer, 2018; Albert and Vogel, 2017; Baudrot et al., 2018a), and a ~~web-platform is also~~ web platform has been proposed (Baudrot et al., 2018d).

4.5.2. Limitations

Survival is the most often ~~observed response of a chemical toxic effect~~ measured response to chemical toxins in the environment, but ~~sub-lethal effects~~ it may be more relevant to manage ~~for ERA ,~~ sub-lethal effects in ERA to prevent community collapse (Baudrot et al., 2018b). While the lethal concentration decreases ~~when~~ as time increases, other sub-lethal effects (e.g., reproduction ~~, growth) does~~ and growth) do not always follow this pattern (Álvarez et al., 2006; Jager, 2011). The ~~levels of concentration~~ concentration levels in acute toxicity tests are higher than those classically observed in the environment. Therefore, under real environmental ~~condition~~conditions, sub-lethal effects may have more direct impacts on ~~the~~ population dynamics than ~~effects~~ on survival. Thus, it would be of real interest to encompass different effects in a global TKTD approach ~~, in order to better predict when scaling up at~~ to generate better predictions when scaling up to the population and community levels (Jager, 2011) and at multi-generationnal scales (Dale et al., 2008).

Another well-known limitation is the derivation of ~~EQS~~ EQSs from specific species-compound ~~combination~~combinations. ~~In order to~~ To extrapolate ecotoxicological information from a set of single species tests to a community, ERA uses ~~Species Sensitivity (Weighted) Distribution,~~ a species sensitivity (weighted) distribution (SS(W)D~~,~~) which can be used to derive ~~EQS~~ EQSs covering a set of taxonomically different species (Duboudin et al., 2004). This calculation is classically applied ~~on~~ to $LC(x,t)$ and could ~~be easily done~~ easily be performed with $MF(x,t)$ with the benefit ~~to be applied on~~ of being applicable to time-variable exposure profiles (EFSA PPR Scientific Opinion, 2018).

4.6. Conclusion

As recently written by EFSA experts~~:~~ ~~,~~ "*uncertainty analysis is the process of identifying limitations in scientific knowledge and evaluating their implications for scientific conclusions*" (EFSA, 2018). ~~Description of uncertainties increases transparency and trust in scientific outputs and is therefore a key for an applied science such as ecotoxicology (Beck et al., 2016). Here~~Inspired by the recent EFSA PPR Scientific Opinion (2018), we evaluated ~~the~~ a combination of mechanism-based models with ~~the~~ a Bayesian inference framework to track uncertainties ~~on~~ of toxicity endpoints used in regulatory risk assessment ~~from~~ with one compound-one species survival ~~bioassay~~bioassays. ~~A lot of other kind~~ We showed that the degree of uncertainty can change dramatically with time and depending on the exposure profile, revealing that single values such as the mean or median may be totally irrelevant for decision making. Description of uncertainties also increases transparency and trust in scientific

outputs and is therefore key in applied sciences such as ecotoxicology. Many other kinds of uncertainties emerge ~~all~~ along the decision chain, from the hazard identification to the characterization of risk. Focusing on uncertainty~~should be of~~ , such as through a Bayesian approach, should be a concern at every ~~steps, and~~step and, above all, for any information returned by mathematical-computational models.

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