Modeling explicitly and mechanistically LC50 as a function of time for risk assessment

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Abstract

A mechanistic model, which explains how toxic effects depend on the duration of exposure, has been developed. Derived from Debtox, it expresses the hazard rate as a function of the toxic concentration in the organism. Using linear approximations in accordance with the general simplifications made in Debtox, the LCx (concentration that induces x% of lethality), and in particular the LC50, are expressed explicitly as functions of time. Only three parameters are required: an asymptotic effect concentration, a time constant and an effect velocity.

More sophisticated (but still analytic) models are possible, describing more complex toxicity patterns, such as an increase of sensitivity with time, or, conversely, an adaptation.

These models can be fitted on the common and widespread LC50 endpoints available from the literature for various aquatic species and chemicals. The interpretation of the values assigned to the parameters will help explain the toxicity processes and to standardize toxicity values from different sources for comparisons.

Keywords

LC50 Mechanistic modeling PNEC

Introduction

Most ecotoxicological studies are based on laboratory assays, performed on a very small sample of the total environmental species, with short exposures, and in artificial conditions. Still, a predicted no-effect concentration (PNEC), which should protect all species, is needed for legal recommendations. Passing from laboratory data to real life situations requires several extrapolations which, at present, are scientifically uncertain.

Our final aim is to better understand the variability of response of different aquatic species to a toxicant, in order to reduce the uncertainty when extrapolating a PNEC from laboratory experiments. The response of test organisms to toxicants depends not only on the concentration to which they are exposed, but also on the duration of that exposure. Moreover, risk assessment should be done for long term exposures. In fact, acute-to-chronic and interspecies extrapolations cannot be considered as independent. Various works have been proposed to model effects as a function of time and concentration, using regression, survival or kinetic approaches. Most of them were designed to use the raw survival data collected in bioassays: the comprehensive data set including the number of survivors associated with each concentration and each observation time ; some of them even require to modify the usual experimental designs. Such data is generally not published in the literature or stored in publicly available toxicity data banks. Consequently, these models cannot be used with most of the published data, and therefore for lots of species. Conversely if the LC50 data found in the literature are used directly, the variability due to the effect of exposure's duration will interfere with the one due to interspecies sensitivities.

Hence our first step is to find a way to take time into account, still using LC50 data. To this aim, a mechanistic model based on the Debtox theory, modified to express LC50 as a function of time, is presented. The temporal evolution of the LC50 endpoints can be predicted and the parameters from this model, can be used to establish differences between species sensibilities to toxicants.

Methods and derivation of an alternative approach

Expressing LC50 as a function of time

Lethality as a function of time and concentration can been seen as a time-concentration-effect surface. The 50% effect section of this surface is the LC50 function of time of exposure. It is instructive to review previous works and to derive the expression of LC50 as a function of time.

Several regression models use an empirical hyperbolic relation first published by Ericksen Jones [1], Green [2] and Sprague [3]: LC50 values are expressed as a linear model of the inverse of time. This is also the case for the models of Mayer et al. [4] (two-step linear regression approach) or Van Wijk and Kraaij [5] (extended log-logistic model). Carter and Hubert [6] used a multivariate regression and have shown, that in practice, an inverse time model is appropriate.

Survival time models focus on a time-to-death point of view. They are derived from industrial reliability surveys and epidemiology. Works of Shirazi and Lowrie [7] or Sun et al. [8], in particular, are based on the use of the Weibull distribution, depending on time and on toxicant concentration. The LC50 is then expressed as a power of time: this is a generalization of the empirical relation found in the regression approaches.

In order to take advantage of the theoretical knowledge from biology, several authors have proposed to use compartments models, which are routinely used in

pharmacokinetics (e.g. Jacquez [9]). In a simple, one-compartment model, the living organism is thought as a box (the compartment) through which a flow of the toxicant exist. The environmental concentration is often assumed as being constant. The solution of the differential equation of the first order uptake/clearance kinetic is the following:

$$C_{org}(t,C) = C\frac{k_{in}}{k_{out}}\left(1-\exp(-k_{out}t)\right) \quad (1)$$

C_{org} and C are the concentrations of toxicant inside the organism and in the external environment respectively, t is the time, k_{in} and k_{out} are the first order uptake and elimination kinetic constants. These coefficients are assumed to depend neither on concentrations nor on time. The works of Kooijman [10] and Chew and Hamilton [11] lead to a similar expression of the LC50 as a function of time. They give an hyperbolic-like shape. More recently, Kooijman and Bedaux [12] have proposed the so called Debtox model, which is also a kinetic based model (see below) in replacement of the "standard" one from Kooijman [10].

Considering the new variable : LC50×time

As just described, the evolution of toxicity with time is often graphically represented by the LC50 versus time plot which has an hyperbolic like shape. Plotting a new variable LC50×t (t being the duration of exposure) versus time, highlights more complex patterns (see Figure 1). In particular, for short times, we can observe a parabolic decrease. For larger times, such a plot begins to increase and seems then to be asymptotically linear.

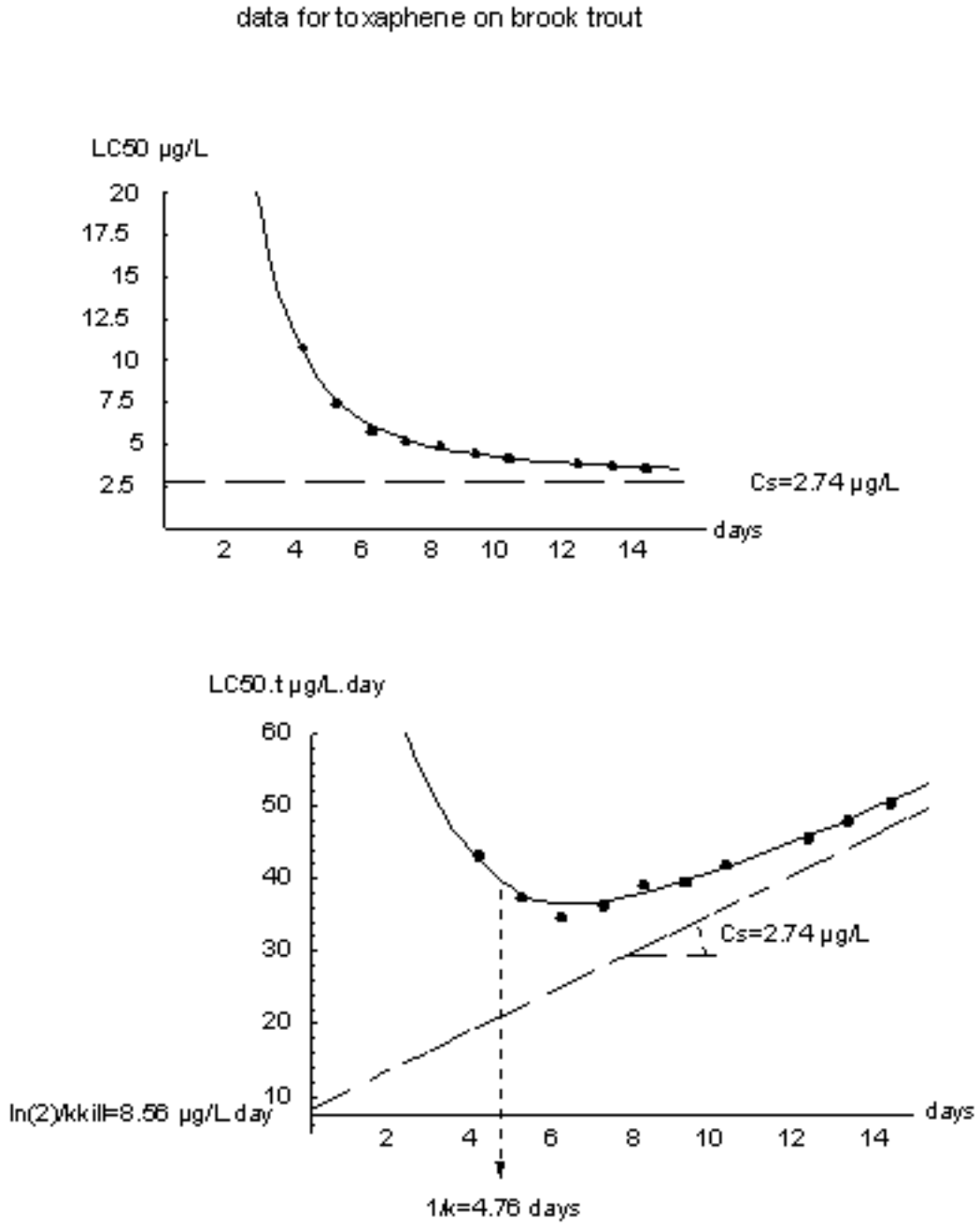

Figure 1: Example of experimental data and fit with our basic model for Toxaphene (CAS 8001352) on *Salvenilus fontinalis* (*Salmonidae*). Data are from Mayer et al. [13] ; C_s= 2.74 (s.d.= 0.14) µg/L, k= 0.210 (s.d.=0.014) day^{-1}, k_{kill}= 0.0813 (s.d.=0.0138) (µg/L)$^{-1}$.day^{-1}.

95 Regression analysis of such transformed data obviously leads to a linear relation with time. These models are correct approximations for long exposures (under the critical assumption that the mode of action will not vary), but cannot explain the shape observed for short exposures. Kooijman [10] and Chew and Hamilton [11] also predicted a linear

asymptotic relation between effective LC50×time and time, but failed to obtain satisfactory predictions for short times since they predict patterns that are always increasing. Debtox is the only model which is precisely able to predict temporal evolution of LC50 versus time.

Expressing LC50 from Debtox

Debtox is based on the DEB (Dynamic Energy Budget) theory developed by Kooijman [14] where the main biological functions: survival, growth or reproduction depend on their energy allocation. Debtox is adapted to the analysis of aquatic toxicity data, assuming that toxicants reduce the efficiency of energy utilization during the biological processes. It is an highly mechanistic way to describe the sensitivity of organisms, relying on simplified but realistic biological assumptions. As a mechanistic model, its parameters can be interpreted according to its biological assumptions. The user can draw several different hypotheses to customize the biological model to his needs.

As in Chen and Selleck [15] kinetic model, Debtox assumes the existence of a threshold concentration below which no effect occurs. For the basic case of survival, when the internal concentration of the toxicant is above this threshold level, the hazard rate h is proportional to this concentration. h is defined as:

$$h(t) = \frac{-1}{S(t)} . \frac{dS(t)}{dt} \tag{2}$$

where S is the survival and t the time. One obtains:

$$\begin{cases} h(C_{org}) = 0 \quad \text{if } C_{org} \le C_{sorg} \\ h(C_{org}) = k_{tox}(C_{org} - C_{sorg}) \quad \text{if } C_{org} \ge C_{sorg} \end{cases} \tag{3}$$

In these equations, the coefficient k_{tox} is the proportionality coefficient linking hazard rate to the internal concentration. It reflects the toxicity of the compound with respect to survival. C_{sorg} is the threshold internal concentration. Internal concentrations are derived from environmental concentrations using a one-compartment first order kinetic model.

The hazard rate reflects the assumption that lethality at the individual level is stochastic.

The survival function is expressed as a function of time and environmental concentration by:

$$\begin{cases} \text{If } C \le C_s: \quad S(t,C) = 1, \\ \text{If } C \ge C_s: \\ S(t,C) = 1 \quad \text{if } t \le t_s \\ S(t,C) = \exp\left[\frac{k_{kill}}{k_{out}}\left(C(1-e^{-k_{out}t} - k_{out}t) - (C-C_s)\ln(1-\frac{C_s}{C}) + C_s(k_{out}t-1)\right)\right] \quad \text{if } t \ge t_s \end{cases} \tag{4}$$

It involves only three parameters: the elimination rate k_{out}, the killing rate k_{kill} and the assymptotic external threshold concentration C_s. C_s corresponds to the internal threshold concentration C_{sorg} divided by the bioconcentration factor (BCF). The killing rate k_{kill} depends on the proportionality factor k_{tox} and on the bioconcentration factor. t_s can be expressed from the other parameters and corresponds to the time –due to the kinetic process- necessary to reach the threshold concentration.

Nevertheless, LC50 cannot be expressed analytically as a function of time from Equations 4. One can perform numerical computations, but they will not allow a full analysis of the function. In particular, the significance of the parameters cannot be demonstrated.

Structure of our basic model

The main idea of our approach is to simplify the kinetic equation, using a two step afine approximation of Equation 1. It is then possible to derive an analytical expression of LC50 versus time.

- for short term exposures (below a time $t=1/k$) the internal concentration C_{org} is considered to be proportional to time and to the environmental concentration C,
- after $t=1/k$, the equilibrium between C_{org} and the environmental concentration C is assumed to be reached.

This is formalized as (see Figure 2):

$$\begin{cases} C_{org}(t,C) = k.BCF.C.t & \text{if } t \le \dfrac{1}{k} \\ C_{org}(t,C) = BCF.C & \text{if } t \ge \dfrac{1}{k} \end{cases} \quad (5)$$

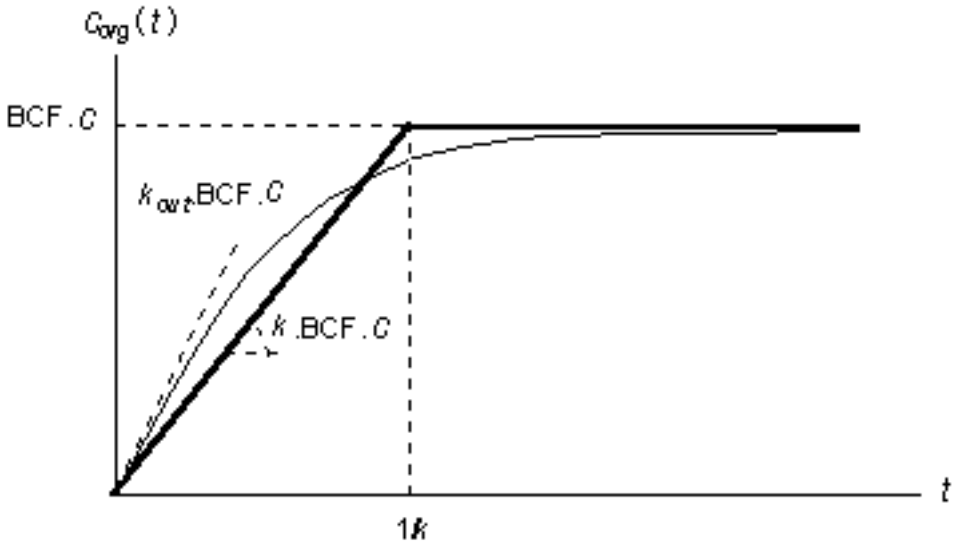

Figure 2: Linear approximation (thick curve) for the one compartment first order kinetic model (thin curve).

This linear approximation is by all means compatible with the general simplifications of Debtox, since the toxicant effect on hazard rate has already a two steps linear structure.

The slope of $C_{org}(t)$ is equal to $k.BCF.C$ (from Equations 5) as long as the hypothesized equilibrium is not reached. The bigger k is, the earlier the effects will be observed.

As for Debtox, the hazard rate is taken proportional to C_{org} as soon as a threshold concentration C_s is reached. From Equations 3, the hazard rate according to the environmental concentration becomes:

$$\begin{cases} \text{if } C \le C_s: \quad h(t,C) = 0 \\ \\ \text{If } C \ge C_s: \\ h(t,C) = 0 \quad \text{if } t \le t_s \quad \text{and } t_s = \dfrac{C_s}{k.C} \\ h(t,C) = k_{kill}\left(k.C.t - C_s\right) \quad \text{if } t_s \le t \le \dfrac{1}{k} \\ h(t,C) = k_{kill}\left(C - C_s\right) \quad \text{if } t \ge \dfrac{1}{k} \end{cases} \tag{6}$$

The parameters C_s and k_{kill} are characteristic of the toxicity of the compound. The smaller C_s is, the more toxic the compound will be. The higher k_{kill} is, the more the toxicity of the compound increases with its internal concentration.

After integrating successively Equations 6, the resulting survival equations are:

$$\begin{cases} \text{If } C \le C_s: \quad S(t,C) = 1 \\ \\ \text{If } C \ge C_s: \\ S(t,C) = 1 \quad \text{if } t \le t_s \\ S(t,C) = \exp\left[-\dfrac{k_{kill}}{2.k.C}\left(k.C.t - C_s\right)^2\right] \quad \text{if } t_s \le t \le \dfrac{1}{k} \\ S(t,C) = \exp\left[-k_{kill}\left((C - C_s)(t - \dfrac{1}{k}) + \dfrac{1}{2.k.C}(C - C_s)^2\right)\right] \quad \text{if } t \ge \dfrac{1}{k} \end{cases} \tag{7}$$

With these new formulations, a LCx can be expressed explicitly as a function of time, since S(t, LC_x)=1-x :

$$\begin{cases} LC_x(t) = \dfrac{\dfrac{-\ln(1-x)}{k_{kill}} + C_s.t + \sqrt{\dfrac{-\ln(1-x)}{k_{kill}}\left(\dfrac{-\ln(1-x)}{k_{kill}} + 2.C_s.t\right)}}{k.t^2} & \text{if} \quad t \le \dfrac{1}{k} \\ LC_x(t) = \dfrac{\dfrac{-\ln(1-x)}{k_{kill}} + C_s.t + \sqrt{\left(\dfrac{-\ln(1-x)}{k_{kill}} + C_s.t\right)^2 - (2.t - \dfrac{1}{k})\dfrac{C_s^2}{k}}}{(2.t - \dfrac{1}{k})} & \text{if} \quad t \ge \dfrac{1}{k} \end{cases} \tag{8}$$

At t=1/k Equations 8 are equal. Near the point t=1/k, the first order Taylor approximations of these two equations are equivalent. This means that the solution, despite its conditional nature, is a continuous function. The LC50×t transformation can be easily expressed for Equations 8. When t is lower than 1/k, LC50×t can basically be expressed as a linear function of the inverse of time. It therefore corresponds to a first decreasing pattern. For the second equation, the LC50×t expression becomes asymptotically a linear dependence upon time (see Figure 1).

The transition time 1/k can then quickly be determined by an examination of the LC50×time plot (see Figure 1). The higher k will be, the earlier the transition will occur. For many real data, the transition time 1/k is shorter than one day, meaning fast convergence to a constant internal concentration of the compound. This certainly explains why the methods we reviewed lead to good predictions, though they cannot explain the decrease on the LC50×time plot.

When time increases, the LC50×t asymptotically tends to a linear function of time, which slope is C_s. The sharper this slope is, the less toxic the compound will be. Its intersection

with the y-axis is then $-\ln(1-x)/k_{kill}$, and it decreases if k_{kill} increases. If the intersection is low the slope of toxicity versus time is high.

According to these simple considerations, the LC50×time pattern can be mechanistically interpreted in a quick and easy way without complex calculations.

Estimation of the parameters

The parameters have been estimated using the LC50×time transformation on Equations 8. Since many local minima are possible, the parameters space has been roughly explored with a Monte Carlo method. By observation of the residuals from random simulations for each parameters, one can constrain the initial range of parameters' values to approach a minimum, and continue the minimization with a traditional algorithm. Likelihood maximization is used, assuming a normal distribution of the errors. Our model is continuous but has two distinct derivatives due to its conditional nature. It is more convenient to use an algorithm which does not require the determination of a gradient of Hessian matrix. The downhill simplex method from Nelder and Mead (Nelder et Mead [16], Press et al. [17]), is robust and well adapted. Confidence on the parameters are derived from the deviance function.

Results: illustrative application

As an application, an example for *Daphnia magna* exposed to cadmium chloride will be presented. From complete survival data, we will first compare the application of our approach with the use of Debtox. We will in particular compare the parameters values obtained with the two methods in order to study how the two approaches differ. We will

also apply our method on LC50 for the same species and chemical, but from an another data source, to check whether the parameters values are still close.

Some raw survival data are presented in Kooijman [10]. Young daphnids were used (<24h) and the bioassay was conducted in hard water, at 20°C, with a semistatic exposure to the toxicant. As illustrated in Figure 3, we fitted this data using Debtox. The no-effect concentration is determined at 14.1 (s.d.=0.69) µg/L, the elimination rate at 0.425 (s.d.=0.050) day^{-1}, the killing rate at 0.0282 (s.d.=0.004) $(\mu g/L)^{-1}.day^{-1}$. By default, a blank mortality rate is also determined at 0.00515 (s.d.=0.001) day^{-1}.

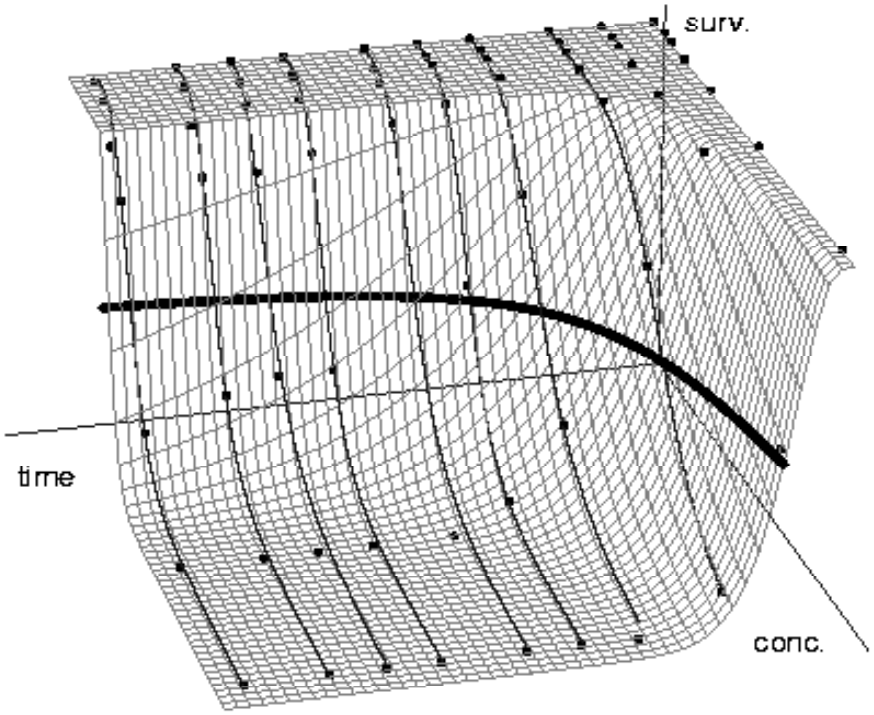

Figure 3: Comparison of our approach with the Kooijman and Bedaux [12] model: explicative exemple on real data. Data from bioassay for cadmium chloride on *Daphnia magna* Kooijman [10] are used. Experimental survivals are plotted in function of concentration and time (dots). Using Debtox software, the surface illustrating the model of Kooijman and Bedaux [12] was fitted to these points. LC50 values were calculated, when possible, at different times using the log-logit transformation : the fits are showed on the figure (thin sigmoid curves for 8 different times). We fitted our basic model to these thus determined LC50 values (dark thick curve).

Using our model, we calculate LC50 at several times using the log-logit transformation. When 50% lethality is not bracketed by experimental points, extrapolation with the logit model is unsafe. This implies that LC50 values was not determined for short times. Note that our model is fitted using 8 values, when Debtox uses 70 values. We find C_s=8.83 (s.d.=0.96) µg/L, k=0.556 day^{-1} and k_{kill}=8.29.10^{-3} (s.d.=1.88.10^{-3}) (µg/L)$^{-1}$.day^{-1} (see Figure 4a). These values are quite similar with the ones given by the Debtox estimate. Note that standard deviation on k can not be calculated since this parameter is poorly estimated when transition 1/k is not present on data. We also compared the previous results with those obtained from an another data source for cadmium chloride on *Daphnia magna*. We use LC50 data determined by Suedel et al. [18]. Adults (2-3 weeks) were used and the assay was conducted in pond water (hardness: 69-87 mg/L $CaCO_3$, temperature: 19.6-24 C°) with a static exposure to the toxicant. The experimental conditions are not strictly equivalent with those of Kooijman [10]. We found C_s=7.63 (s.d.=0.06) µg/L, k=0.459 (s.d.=0.012) day^{-1} and k_{kill}= 6.09.10^{-2} (s.d.=0.30.10^{-2}) (µg/L)$^{-1}$.day^{-1} (see Figure 4b). These values are quite similar to the previously determined ones. The same authors determined a NOEC (with mortality as observed effect) at 5 µg/L after two weeks of exposure, which is quite close to the determined threshold concentrations.

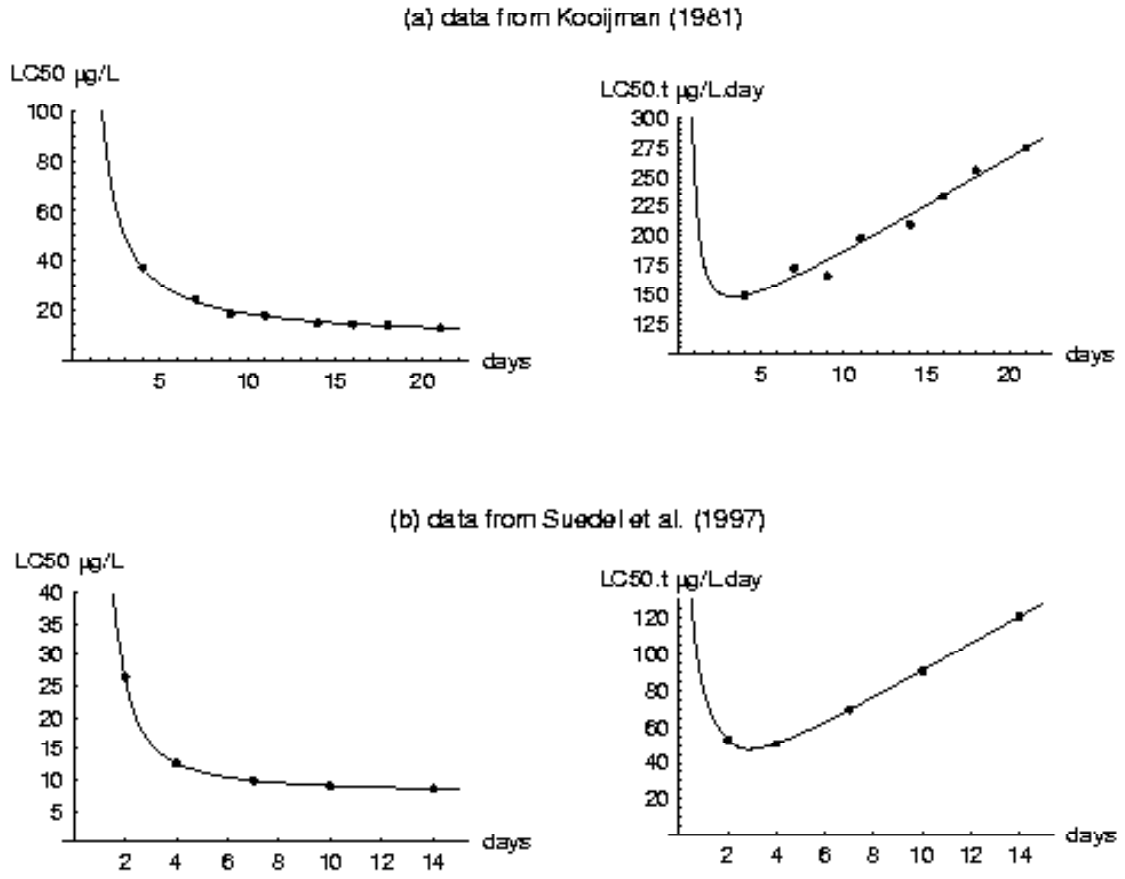

Figure 4: (a) Detail of the fit obtained with our basic model on LC50 values determined from Kooijman [10] for *Daphnia magna* exposed to cadmium chloride (see previous figure). (b) Fit obtained with our basic model on data from Suedel et al. [18].

Discussion and extensions

Giesy and Graney [19] noticed that different shapes of toxicity curves could be observed which differ from the rectangular hyperbola. Moreover, observing the effective LC50×time plot for some data reveals patterns that cannot be explained with our basic model.

For instance, for large times, the LC50×time plot can lose its linear shape and progressively decrease (see Figure 5). This corresponds to a toxicity curve that seems to

tend asymptotically to zero. This decrease can be interpreted as an increasing sensibility with time.

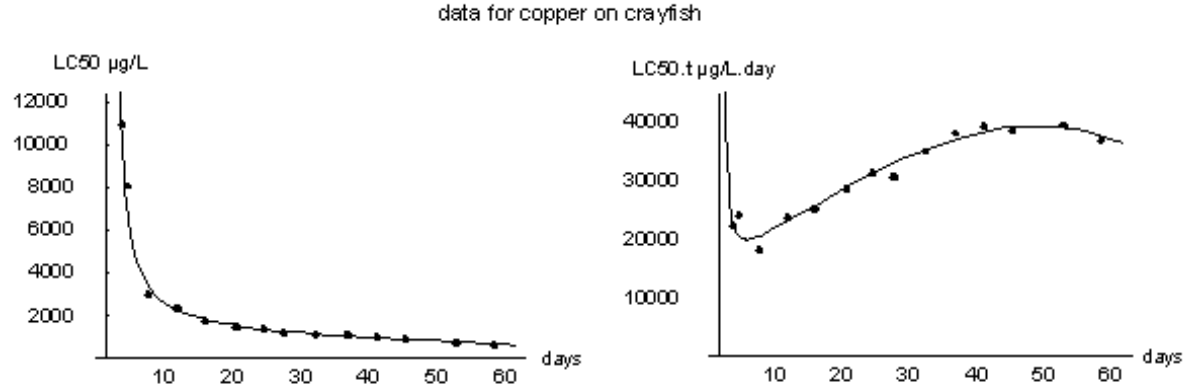

Figure 5: Example of experimental data and fit with the decreasing C_s modelfor copper (CAS 7440508) on *Procambarus clarkii* (*Decapoda*) ; data are from Rice and Harrison [20] ; C_s= 998 (s.d.=118) µg/L, k=0.608 (s.d.=0.186) day^{-1}, k_{kill}= 5.62.10^{-5} (s.d.=0.78.10^{-5}). (µg/L)$^{-1}$.day^{-1}, t_c=57.7 (s.d.=3.1) (µg/L).day^{-1}.

Modifying the mechanistic structure of the model

In a first approach, one may think that this is due to natural mortality. Our basic model can be modified to take this into account. But computing the parameters leads to values that are not relevant from a biological point of view. Indeed, it predicts a natural mortality that is clearly excessive, in particular in the case of acute tests. So, natural mortality is not sufficient to explain the second decrease on the LC50×time plot.

We suggest to interpret this phenomenon as a increasing sensibility, due to a decrease of the threshold concentration C_s with time. It can be seen as a toxic induced aging. Practically, we considered C_s as a decreasing linear function of time. Since C_s cannot become negative we have to define a critical time t_c beyond which C_s is null. This is formalized as:

$$\begin{cases} C_s(t) = C_{s0} - l.t \quad \text{if } t \le t_c \\ C_s(t) = 0 \quad \text{if } t \ge t_c \end{cases} \tag{9}$$

C_{s0} is the initial value of the threshold concentration. l is the slope for the decrease. These two parameters replace the C_s of our basic model. The time t_c is expressed as C_{s0}/l.

Using this new expression of the threshold concentration, the hazard rate and survival function can be expressed using the same hypothesis as previously (equations are given in annex). Equations are still linear, but several cases have to be examined, due to the respective values of t_s, t_c and $1/k$: whether $t_s \le t_c \le 1/k$, $t_s \le 1/k \le t_c$ or $1/k \le t_s \le t_c$. Obviously, as in the basic model, the hazard rates have several steps afine expressions. Following the same mathematical developments, we finally obtain eight conditional equations for the expression of LCx as a function of time: they are given in the annex. They require conditional switches, which can be easily implemented on a computer.

The LC50×time plot decreases for large times, but this can be preceded by an increase (parabolic like shape) (Figure 5), or be always decreasing. These shapes obviously depend on the decrease of C_s (slope of the decrease), but also on the others parameters (see Annex).

An important corollary is that the threshold concentration does not exist anymore for long exposures. This means that chronic exposure to toxicants lead to an irremediable lethality. It would certainly be instructive to identify such compounds. The concerned toxicants are generally considered to be cumulative poisons (Giesy and Graney [19]) or compounds that can be accumulated in buffering tissues with delayed release towards more sensitive biological targets.

Anyway, without any mechanistic interpretation or hypothesis, the observation of a decreasing LC50×time plot should be a sufficient indication to induce cautious analysis.

In such a case, extrapolations based on empirical regression methods, for instance, would then certainly not be relevant since they imply a linear evolution of the LC50×time plot. They will then underestimate the toxicity of the compound.

One can study the adverse hypothesis of an increasing threshold concentration upon time, in a quite similar fashion. This suggests a rising tolerance to the compound which is realistic if an adaptation to the toxicant exists, but also if the compound is bio-metabolised to a less toxic chemical.

An adaptation needs to be interpreted at a macroscopic scale: we cannot distinguish between resistances coming from biological processes, resistances due to a selection that would maintain the less sensitive individuals or degradation of the compound. From a modeling point of view, assuming C_s is increasing, leads to a first increasing then decreasing hazard rate as a function of time. A biological adaptation can easily be interpreted by an increasing threshold (biochemical detoxifications, behavior modification,…) A selection can be understood as a decreasing probability of death with time, i.e. a decreasing hazard rate. A hazard rate, first increasing then decreasing, can also be obtained when the external concentration is diminishing, for instance with non-persistent chemicals (Widianarko and Van Straalen [21]).

The predicted toxicity curves and LC50×time patterns have similar shapes to the ones given by our basic model, but the predicted asymptotic values are higher: this model fits data for which our basic model predicts a C_s smaller than the asymptote of data (Figure 6).

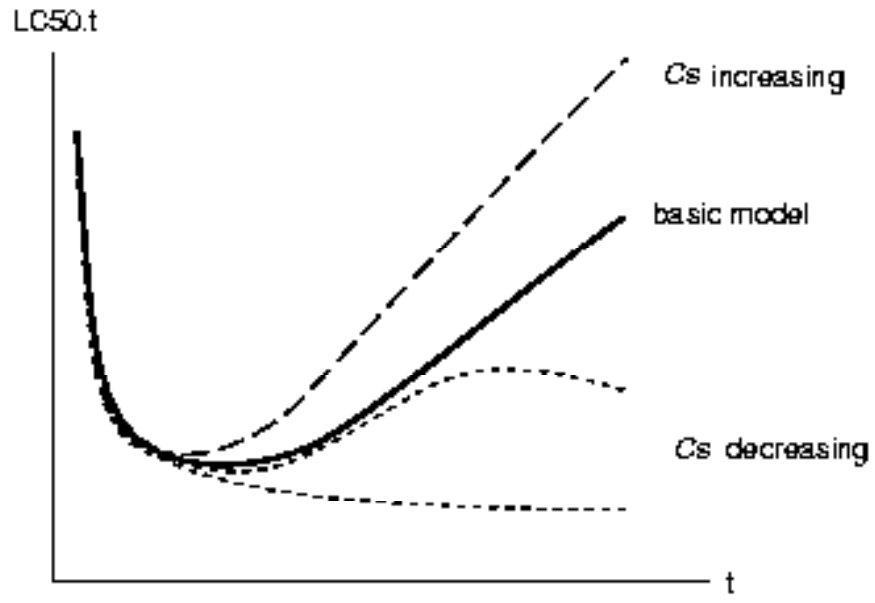

Figure 6: Summary plot for the different models shown on the LC50×time plot.

Interpreting the parameters

This approach is based on mechanistic models. They are obviously simplifications of complex biological mechanisms. We did not try to describe precise toxicological processes, but to make a macroscopic characterization of species sensitivity. Parameters have to be analyzed in view of the biological assumptions of the models. The kinetic constant k should be interpreted on the basis of the first order kinetic one compartment model. A threshold concentration C_s is assumed. C_s is supposed to be homogeneous for all the individuals of the tested population. This assumption imply that C_s correspond to a toxicant threshold concentration below which no effect is supposed to occur. Since bioassays are performed on quite standardized organisms, variations on the biological parameters could be considered as minimal. Unexplained variability, is modeled as stochasticity on individuals lethality, by the use of the hazard rate. Toxicant

concentration is related to the hazard rate by the parameter k_{kill}. k_{kill} is highly correlated with C_s as they both depend on the bioconcentration factor.

A pragmatic approach, to use LC50 data

The current procedures in bioassays consist in observing lethality at fixed times (which can lead to the determination of LC50 endpoints), rather than survival curves (Sprague [3]). There is then a statistical dependence of LC50 data at consecutive times, since they concern the same organisms. Continuing the work of Mayer et al. [4], Lee and al. [22] proposed multifactor probits models. Such models, which require a specific design, are quite heavy: at least five different concentrations with intermediate lethality –i.e. between 10% and 90%- at least observed at four duration times for each concentration.

Survivals models consider raw experimental data as time to death versus concentration. They can remove this dependency difficulty and have an intrinsically greater statistical power. But any biological interpretation is limited. Chew and Hamilton [11] used the first order kinetic model with times to death to predict the time required to cause 50% lethality. This approach is statistically more adapted to these kind of data but requires heavy experiments as continuous observations of deaths are needed.

Our method will be immediately useful when the only available data are the LC50: this is commonly the case in ecotoxicity databases.

Conclusions

Due to its mechanistic nature and its analytical formalization, our model provides an easy way to study the species sensitivities to toxicants using published LC50 data taking time into account.

Parameters values can be interpreted in accordance with the biological assumptions inherited from Debtox. A threshold toxicant concentration below which no effect is supposed to occur is assumed. The hazard rate is proportional to the internal toxicant concentration above this threshold. The internal toxicant concentration is derived using a linear approximation of a one compartment first order kinetic model. An analytical expression of the LC50 as a function of time is thus possible.

It was shown that parameters can be roughly but quickly determined through the LC50×time transformation.

The LC50×time versus time plot also reveals different evolutions in the sensitivity of species with time. These can be interpreted at a macroscopic scale using various hypotheses as decreasing or increasing threshold concentrations.

Each species sensitivity is associated with the 3 or 4 parameters values of the models. The next step is to analyze these parameters values in order to find out what characterizes the species sensitivity to toxicants and whether they can be related to the toxicants' properties, or species' biological attributes (for instance taxonomy).

Annex

Equations for the decreasing C_s model

The analytical solution is a conditional function. It is expressed by the eight following equations.

We define $m = -\frac{\ln(1-x)}{k_{kill}}$, $\alpha = \frac{C_{s0}^2}{2l}$, $\beta = 2t - \frac{1}{k}$, $k' = \frac{1}{k}$

If $t \le t_c \le 1/k$:

$$C = k'.\frac{m + C_{s0}t + \sqrt{m\left(m + 2C_{s0}t\right)}}{t^2} - l.k'$$

If $t_c \le t \le 1/k$:

$$C = k'.\frac{m + \alpha - \frac{lt^2}{2} + \sqrt{\left(m + \alpha - \frac{lt^2}{2}\right)^2 + 2l.m.t^2}}{t^2}$$

If $t_c \le 1/k \le t$:

$$C = \frac{m + \alpha - \frac{l.k'}{2}\beta + \sqrt{\left(m + \alpha - \frac{l.k'}{2}\beta\right)^2 + 2l.m.k'.\beta}}{\beta}$$

If $t_s \le t \le 1/k \le t_c$:

$$C = k'.\frac{m + C_{s0}t + \sqrt{m\left(m + 2C_{s0}t\right)}}{t^2} - l.k'$$

If $t_s \le 1/k \le t \le t_c$:

$$C = \frac{m + C_{s0}t - \frac{lt^2}{2} - \frac{l.k'}{2}\beta}{\beta} + \frac{\sqrt{\left(m + C_{s0}t - \frac{lt^2}{2} - \frac{l.k'}{2}\beta\right)^2 - \beta\left(-2l.m.k' + k'.(lt - C_{s0})^2\right)}}{\beta}$$

If $1/k \le t_s \le t \le t_c$:

$$C = C_{s0} - lt + \sqrt{2l.m}$$

If $t_s \le 1/k \le t_c \le t$:

$$C = \frac{m + \alpha - \frac{l.k'}{2}\beta + \sqrt{\left(m + \alpha - \frac{l.k'}{2}\beta\right)^2 + 2l.m.k'.\beta}}{\beta}$$

If $1/k \le t_s \le t_c \le t$:

$$C = C_{s0} - lt + \sqrt{(C_{s0} - lt)^2 + 2l.m}$$

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