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A biology-based dynamic approach for the reconciliation of acute and chronic toxicity tests: Application to *Daphnia magna*

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EXECUTIVE SUMMARY

There is a need to integrate existing dose-response data in a coherent framework for extending their domain of applicability as well as their extrapolation potential. This integration would also reduce time and cost-consuming aspects of these tests and reduce animal usage. In this work, based on data extracted from literature, we have assessed the advantages that a dynamic biology-toxicant fate coupled model for *Daphnia magna* could provide when assessing toxicity data, in particular, the possibility to obtain from short-term (acute) toxicity test to long-term (chronic) toxicity values and *vice versa*; and the possibility of toxicity data reconciliation from several sources taking into account the inherent variability of *Daphnia magna* populations. Implicitly in this approach is the assumption that the mode of action of the toxicant does not change after prolonged exposure. The results show that the prediction errors are considerably reduced when compared with the factor from 2 to 5 obtained using acute-to-chronic ratios (ACR). However, due to the scarcity of complete sets of experimental data a more general validation has not been possible.

CONTENTS

1. INTRODUCTION

Risk assessment is based on hazard and exposure assessment. If the exposure levels do not exceed the predicted no-effect concentrations for a selected group of species, it is assumed that the risks posed by a chemical are acceptable. The approach developed for hazard assessment uses normally dose-response analysis of standardized toxicity test to extract relevant values, such as NOEC (Not Observed Effect Concentration) and EC_{50}, and relies on the use of assessment factors (AF) to derive a predicted no-effect concentration value (PNEC). This approach is largely descriptive and a lot of process information from the toxicity tests is lost. In addition, the standard values derived from these tests (NOEC, EC_x, LC_{50}) change with exposure time as a function of the tested species and the toxicant (Roex et al., 2000). Nevertheless, toxicity tests performed according to the standard test protocols contain valuable process information that could be used in a more appropriate way (Jager e al. 2004).

There is the need to integrate these dose-response data in a coherent framework for extending the domain of applicability as well as their extrapolation potential. This integration should also reduce the time and cost-consuming aspects of these tests and reduce animal usage.

Several approaches have been developed to predict acute-to-chronic ratio (ACR). McKim (1995) analyzed early life stage (ELS) toxicity tests to estimate the maximum acceptable toxicant concentration (MATC) in fish and concluded that this was possible within a factor of two. Analysis of the ECETOC aquatic toxicity (EAT) database by Länge et al. (1998) showed that ACRs of around 15 to 25 may be appropriate for use in risk assessment in contrast with the value of 100 proposed by Heger et al. (1995) and the AF of 1000 used in European legislation when deriving a PNEC from acute toxicity data (EC, 2003). Roex et al. (2000), using population growth parameter as a standardized chronic end-point and chemicals classified as nonpolar narcotics, polar narcotics, specially acting compounds and heavy metals, concluded that nonpolar narcotic chemicals demonstrated the smallest variation in ACRs and acute test could be used to derive chronic endpoints, whereas for the other classes species sensitivity was more important than mode of action to determine the ACRs.

A complementary approach to data analysis and integration of acute and chronic toxicity datasets, independently of the mode of action and organism, consists on the coupling with biology-based models (BBMs) that include toxic effect models. These models could explain the differences of sensitivity found for different organisms when calculating ACRs (Heger et al., 1995) and provide an efficient means of using the available data in an integrated manner from routine toxicity tests, by including in the model explicit assumptions regarding the processes underlying the toxic effect. In addition these models, once calibrated with a data set could provide toxicity measures independent of exposure time and could extrapolate toxicity values and therefore being able to predict long term exposure from acute toxicity test or *vice versa*, thus reducing animal testing and the time and costs of

chronic toxicity tests. Furthermore, by coupling the biology with a toxicity model, insights into the mechanisms underlying the toxic response are gained. Finally, these models could, in principle, be calibrated with toxicity data at lower concentrations and therefore reducing the number of high dose experiments.

Biology-based models have become effective tools in estimating and managing ecological risks (Suter, 1993; Bartell, 1996; Pastorok et al., 2003), but the application to assess toxicological tests was started by Kooijman and co-workers (Kooijman and Bedaux, 1996abc; Jager et al., 2004, Jager et al. 2006) with the development of DEBtox (Kooijman and Bedaux, 1996a) that was included into a OECD document (OECD, 2006) for the statistical analysis of ecotoxicity data.

In addition to include biology-based models, it is also important to add the dynamics of the toxicant or at least an estimation of the rate at which the compound changes during the test. For this reason a fate model should be coupled with the biology-model.

In this work, based on data extracted from literature, we have assessed the advantages that a dynamic biology-fate coupled model - in this case a DEB model of *Daphnia magna* combined with a simple uptake-depuration plus degradation kinetics- could provide when assessing toxicity data, in particular, the possibility to obtain from short-term (acute) toxicity test (24 and 48 h) long-term (chronic, 21 days) toxicity values and *vice versa*; and the possibility of toxicity data reconciliation from several sources. Implicitly in this approach is the assumption that the mode of action of the toxicant does not change after prolonged exposure. As a first approach, we have studied mortality as end point, but we describe also how other end points as reproduction could be studied as already proposed by Kooijman and Bedaux (1996c). The results show that the prediction errors are considerably reduced, when compared with the factor from 2 to 5 obtained using acute-to-chronic ratios (ACR). However, due to the scarcity of complete sets of experimental data a more general validation has not been possible. This lack of experimental data is not due to a scarce number of experiments, but at how experiments are reported since to validate this approach complete dose-response curves are needed and not few points like NOEC and EC_{50}, which are the most frequent values reported.

This work could contribute to the development of an integrated testing approach for risk assessment and to the three Rs principle (Replacement, Reduction and Refinement) when extended to vertebrate species by replacing the biology-based model of the corresponding species.

2. METHODS AND APPROACH

2.1. DATA SETS

Acute and chronic toxicity data on *Daphnia magna* from several organic chemicals of different use groups including some nanoparticles were examined, between them plant protection products

(pyridine, chlordecone), veterinary antibiotics (oxolinic acid, streptomycin, tiamulin and tylosin) and industrial chemicals in the New Chemicals Database (NCD). The approach was always the same; one set of data –acute or chronic- was selected to fit the model's parameters, whereas the second set was used to compare with the predictions using the parameters obtained from the former data set. In the case of predicting chronic from toxic, the approach may be considered as an extrapolation, whereas when moving from chronic to acute it should be an interpolation.

Data on chronic toxicity for pyridine were obtained from Santojanni et al. (1995), whereas acute toxicity data were extracted from the USA Environmental Protection Agency ECETOX database (http://cfpub.epa.gov/ecotox). Data concerning chronic and acute toxicity for T-Lite^{TM} SF-S – a nanoparticle ~50 nm length and ~100 nm width titanium dioxide, aluminium hydroxide and simethicone/methicone polymer- were obtained from a recent publication from Wiench et al. (2009).

Table 1. Ecotoxicity studies on Chlordecone (Kepone®). All values in mg L^{-1}; plus-minus the standard deviation and the number of reported data points (in parenthesis).

Time (h)	NOEC	MATC	LOEC	EC_{50}	LC_{50}	NR-LETH
24				0.6		
48	0.05			0.30±0.20 (9)	0.31±0.26 (4)	
120 (5 d)						0.15
168 (7 d)	0.05	0.07	0.1	0.1		
336 (14 d)	0.025	0.035	0.05	0.06		
504 (21 d)	0.0112	0.020	0.026±0.0023 (2)	0.03		

Data for chlordecone (Kepone®, CAS 143-50-0), which is a banned plant protection product used originally as an insecticide for leaf-eating insects, ants and cockroaches, and as a larvicide for flies, were obtained using the OECD QSAR Application Toolbox (http://www.oecd.org/env/existingchemicals/qsar) and references therein. The data are summarized in Table 1. The data set for acute and long-term toxicity of veterinary antibiotics to *Daphnia magna* was obtained from Wollenberger et al. (2000) and are summarized in Table 2. The data set for acute and long-term toxicity to *Daphnia magna*, Table 3, were extracted from the New Chemicals Data base (NCD) which was formerly maintained by the European Chemicals Bureau within the European Commission's Joint Research Centre (http://ecb.jrc.ec.europa.eu/new-chemicals/). The data were submitted by industry in a harmonized format as a part of the notification process for each new chemical substance that was manufactured or imported into the European Union (EC, 1979). Amongst others, the data provided information on physical and chemical properties, toxicological and ecotoxicological effects. The data is confidential, for this reason the name of the substance is not disclosed in the report. On 1st of June 2008 this notification scheme has been revoked and replaced by a new regulation (REACH, EC 2006). According with the notification scheme, acute toxicity test were carried out following OECD (1981) Guideline 202 (Daphnia sp. Acute immobilisation test), whereas long term toxicity were carried out following the OECD guideline 2002 Part B, updated now as OECD

(1996) guideline 211 (*Daphnia magna* reproduction test). In this study, data for acute and long term toxicity for *Daphnia magna* were extracted from the NCD. Initially data that contained acute and long term toxicity for *Daphnia magna* for 152 substances were found. After a preliminary quality control check on acute toxicity data, i.e. NOEC, EC_{50} and EC_{100} should be different, NOEC<EC_{50}<EC_{100}, only 28 substances were found for which 48 h acute toxicity NOEC, EC_{50} and EC_{100} were available.

Table 2. Ecotoxicity studies on oxolinic acid, streptomycin, tiamulin and tylosin. All values in mg L^{-1}; with 95% confidence limits (in parenthesis).NR-LETH stands for time interval between initial exposure to the dose and death.

Compound	**Oxolinic acid**					
Time (h)	**NOEC**	**EC_{10}**	**EC_{50}**	**LOEC[a]**	**LC_{40}[b]**	**NR-LETH[c]**
24		3.0 (1.9-3.9)	5.9 (4.8-7.3)			
48		2.5 (1.7-3.2)	4.6 (3.8-5.7)			
240 (10 d)						3.0
336 (14 d)					0.75	
504 (21 d)	0.38					
Compound	**Streptomycin**					
Time (h)	**NOEC**	**EC_{10}**	**EC_{50}**	**LOEC**	**LC_{50}**	**NR-LETH**
24		408 (192-574)	947 (778-1181)			
48		120 (59-185)	487 (346-721)			
456 (19 d)						64
504(21 d)	32					
Compound	**Tiamulin**					
Time (h)	**NOEC**	**EC_{10}**	**EC_{50}**	**LOEC**	**LC_{50}**	**NR-LETH**
24		53 (37-61)	81 (70-115)			
48		32 (25-35)	40 (36-43)			
120 (5 d)						32.4
168 (7 d)						16.2
Compound	**Tylosin**					
Time (h)	**NOEC**	**EC_{10}**	**EC_{50}**	**LOEC**	**LC_{50}**	**LC_{90}**
24				700		
48		483 (308-576)	680 (568-759)			
120 (5 d)						180
288 (12 d)					90	
504(21 d)	45					

[a]LOEC is the Lowest Observed Effect Concentration: the concentration that has a statistically significant adverse effect
[b]LC_x concentration in water that is estimated to be lethal to x% of the test organisms
[c]NR-LETH stands for time interval between initial exposure to the dose and death.

Table 3. Dataset extracted from NCD (all concentrations in mg L^{-1}). Due to the confidentiality of the dataset, the identity of the compounds is not disclosed.

Compound	**24 h**	**48 h**			**21 days**		**Compound**	**24 h**	**48 h**			**21 days**	
	EC_{50}	**EC_{50}**	**EC_{100}**	**NOEC**	**EC_{50}**	**NOEC**		**EC_{50}**	**EC_{50}**	**EC_{100}**	**NOEC**	**EC_{50}**	**NOEC**
1	>2.2	0.58	2.2	0.4	0.8	0.44	**15**	-	67.6	105	>26	32.2	12
2	-	8.2	43.1	4.2	>0.79	>0.79	**16**	80	60	100	32	19	1.9
3	0.018	0.008	0.016	0.002	>0.016	0.0043	**17**	74	29	56	18	6.9	1.9
4	0.33	0.13	0.56	0.056	0.074	0.042	**18**	>0.28	0.19	>0.28	0.052	>0.02	>=0.0098
5	-	1.6	3.1	1.1	0.434	0.131	**19**	21	19	46	4.6	>16	1.6
6	13	1.8	4.3	<1.2	>0.03	0.03	**20**	3.5	2.3	>3	1.4	0.4	-
7	-	16	44.2	4.9	6.2	1.9	**21**	40.8	25.8	60	11.8	34.9	-
8	-	1.5	>5	0.36	<0.02	0.02	**22**	76	66	>100	10	16.9	4.6
9	0.49	0.006	0.1	0.003	0.171	0.0056	**23**	7	6.3	16	1.9	2.94	0.625
10	222	190	340	100	46	22.5	**24**	198	115	220	50	>0.0017	>=0.0017
11	-	25	50	12.5	15.1	6.63	**25**	>16.3	8.62	16.3	0.594	>4.5	-
12	0.0175	0.0175	>0.052	0.0008	>0.00128	>0.00128	**26**	24	22	32	10	0.71	0.32
13	3.7	0.58	3.2	<3	>0.16	0.0256	**27**	>101.5	70.5	>=100	54.4	>9.02	1.82
14	7.8	4.2	14.5	<3	2	0.97	**28**	9.9	1.8	17	0.18	0.6	0.147

2.2. DEB MODEL OF DAPHNIA MAGNA

The DEB theory (Kooijman, 2000) provides the basis for the description of the relations between feeding, maintenance, growth, development and reproduction in organisms. In DEB this description is carried out using mass and energy budgets normally expressed as ordinary differential equations. Following Kooijman (2000) the basic allocation pathways are shown in Figure 1. As it can be observed, structural body mass, reserves and maturity are the state variables. Food is assimilated as energy reserves or excreted as faeces. Once assimilated one fraction κ (κ -rule, Kooijman 2000) of these reserves is used for growth and somatic maintenance and the rest for maturation or reproduction, with somatic maintenance having the precedence over other energetic needs. This theory has been extensively tested for different kind of organisms, e.g. mollusks, fish, birds, etc. (Kooijman, 2000).

During this report the notation and symbols follow those in Kooijman (2000), therefore:

- Lower and upper case symbols are related via scaling;
- Quantities that refer to unit of volume are expressed within brackets []; those that refer to unit of biosurface area within braces {};
- Rates have dots.

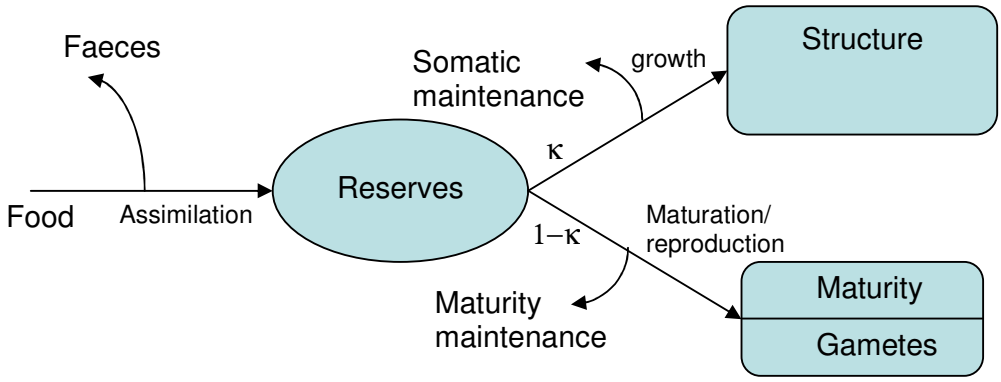


Figure 1. Representation of the energy fluxes following the DEB approach (Kooijman, 2000).

The state variables of the DEB model are: Structural Volume, V (cm^3), Energy reserves, E (J), and Energy allocated to development and reproduction, R (J).

The Energy reserves can be expressed as the difference between the assimilation energy rate ($\dot{p}_A$,J d^{-1}) and the energy utilization rate ($\dot{p}_C$, J d^{-1}):

$$\frac{dE}{dt} = \dot{p}_A - \dot{p}_C \qquad (1)$$

where the assimilation energy rate may be expressed as:

$$\dot{p}_A = \{\dot{p}_{Am}\} f \cdot k(T) \cdot V^{2/3} \qquad (2)$$

where $\{\dot{p}_{Am}\}$ is the maximum surface area-specific assimilation rate (J cm^{-2} d^{-1}) –see Table 2- and f is the functional response of assimilation to food concentration:

$$f = \frac{[Chla]}{[Chla] + [Chla]_K} \tag{3}$$

where $[Chla]_K$ is the half saturation coefficient in μg l^{-1} (see Table 3) and k(T) is a temperature dependence defined as (Kooijman, 2000):

$$k(T) = \frac{\exp\left(\frac{T_A}{T_I} - \frac{T_A}{T}\right)}{\left[1 + \exp\left(\frac{T_{AL}}{T} - \frac{T_{AL}}{T_L}\right) + \exp\left(\frac{T_{AH}}{T_H} - \frac{T_{AH}}{T}\right)\right]} \tag{4}$$

The energy utilization rate, $\dot{p}_C$ (J d^{-1}), may be expressed as (Kooijman, 2000):

$$\dot{p}_C = \frac{[E]}{[E_G] + \kappa[E]}\left(\frac{[E_G]\{\dot{p}_{Am}\} \cdot V^{2/3}}{[E_m]} + [\dot{p}_M] \cdot V\right) \tag{5}$$

where $[E]$ is the energy density, $[E]=E/V$, $[E_G]$ is the volume-specific cost for structure (J cm^{-3}), $[E_m]$ is the maximum energy density in the reserve compartment (J cm^{-3}), κ is the fraction of energy utilization rate spent on maintenance plus growth, and $[\dot{p}_M]$ is the maintenance costs (J cm^{-3} d^{-1}) which is also function of the temperature, i.e. $[\dot{p}_M] = k(T) \cdot [\dot{p}_M]_m$.

According to Kooijman (2000) a fixed fraction of energy is allocated to somatic maintenance and growth while the rest is used for maturation reproduction (see Fig. 1). However, maintenance has priority over growth and when there is not enough food growth stops. Therefore, the change in structural volume, V, is given by:

$$\frac{dV}{dt} = \frac{\kappa \cdot \dot{p}_C - [\dot{p}_M] \cdot V}{[E_G]} \tag{6}$$

Concerning the energy allocated to development and reproduction, Kooijman (2000) showed that it can be expressed as:

$$\frac{dR}{dt} = (1-\kappa)\dot{p}_C - \left(\frac{1-\kappa}{\kappa}\right)\min(V, V_P)[\dot{p}_M] \tag{7}$$

where V_P, is a threshold value of the structural volume for the transition juvenile/adult (the subscript P refers to puberty).

However under a controlled situation with constant or high food density the equation expressing reproduction rate as a function of body length may be expressed as (Kooijman and Bedaux, 1996c):

$$R = \frac{R_m}{1 - l_p^3}\left(\frac{1+l}{2}l^2 - l_p^3\right) \tag{8}$$

where R_m is the maximum reproduction rate, l is the normalized body length ($l=l/L_m$), l_p is the normalized length at puberty ($l_p=L_p/L_m$) and L_m is the maximum length. Since food density is constant, it is possible to write the expression of the body length as a function of time as (Kooijman, 2000):

$$L(t) = L_m - (L_m - L_b)e^{-\gamma \cdot t} \quad (9)$$

where L_b is the body length at birth and γ is the von Bertalanffy growth rate.

2.3. TOXICITY AND EFFECTS MODELS

2.4.1. Effects of chemicals on survival

The direct effects of a chemical concentration, c, on survival may be expressed as (Billoir et al., 2007):

$$q(t,c) = \exp\left(-\int_0^t h(\tau,c)d\tau\right) \quad (10)$$

where q is the probability of surviving until time t and h is the hazard rate at time τ which can be written as:

$$h(\tau,c) = \begin{cases} k_t(c_q - NEC) + m & \text{if } c > NEC \text{ and } \tau > \tau_0 \\ m \end{cases} \quad (11)$$

where c_q is the internal concentration of the toxicant in the organisms and NEC is the no effect concentration.

2.4.2. Effects of chemicals on reproduction

Even though in this work we have not considered effects on reproduction, this could also be included in a similar way. Following Kooijman and Bedaux (1996c), it is possible to distinguish between two types of effects: direct and indirect. In direct effects (Hazard and Costs models), only reproduction is affected, i.e. the R function (Eq. 8) changes, whereas indirect effects maintenance, growth or assimilation are affected which in turn decrease reproduction, i.e. Eqs.8-9 are modified.

- *The Hazard and costs models*

The effects on reproduction occur during an increase in mortality during oogenesis or an increase in the energy costs per egg, then following Kooijman and Bedaux (1996c) it is possible to write for these two cases the following equations:

$$R_H = R \cdot \exp\left[-\left(\frac{c_q - NEC_r}{c_H}\right)\right] \quad (12)$$

$$R_C = R \cdot \frac{1}{\left(1 - \frac{c_q - NEC_r}{c_C}\right)} \quad (13)$$

where R is the reproduction rate without the toxicant, Eq. (8), NEC_r is the no observed effect of reproduction and c_H and c_C are tolerance concentrations for the hazard and the costs models, respectively.

- *The Maintenance, Growth and Assimilation models*

In this case it is possible to write a stress function as (Kooijman and Bedaux, 1996c):

$$s(c_q) = \frac{c_X}{c_q - NEC_r} \tag{14}$$

where c_X is a tolerance concentration.

Deriving Eq. (9) as a function of time and rearranging terms, it is possible to obtain the variation of the normalized body length as:

$$\frac{dl}{dt} = \gamma(1-l) \tag{15}$$

The introduction of the different indirect effects on the variation of the body length and reproduction, Eq. (8) gives for the maintenance, growth and assimilation models the following equations:

$$\begin{aligned} &\frac{dl}{dt} = \gamma[1-l(1+s)] \\ &R_M = \frac{R}{(1+s)^2} \end{aligned} \tag{16}$$

$$\begin{aligned} &\frac{dl}{dt} = \gamma(1-l)\frac{2}{2+s} \\ &R = \frac{R_m}{1-l_p^3}\left(\frac{1+l+s}{2+s}l^2 - l_p^3\right) \end{aligned} \tag{17}$$

$$\begin{aligned} &\frac{dl}{dt} = \gamma(1-l-s) \\ &R_A = R(1+s)^3 \end{aligned} \tag{18}$$

These models have been implemented in the DEBtox software (Kooijman and Bedaux, 1996c) (http://www.bio.vu.nl/thb/deb/deblab/debtox) as well as an estimation of the parameters using the maximum likelihood method to assess which is the most probable effect.

2.4. FATE MODEL

To apply the DEB model for toxicity assessment, the tissue-concentration, c_q, is necessary. This value may be obtained solving the following ode at abundant food (Kooijman, 2000), which is typical in long-term toxicological tests:

$$\frac{dc_q}{dt} = \frac{c \cdot k_a}{l} - c_q\left(\frac{k_a}{l} + \frac{3}{l}\frac{dl}{dt}\right) \tag{19}$$

where c is the dose concentration and k_a is the scaled elimination rate. It is implicitly assumed that uptake and elimination follow one compartment kinetics rate and that contaminant from food intake can be also included in this general equation.

In addition, we also consider an exponential decrease in the concentration of the contaminant during the experiment:

$$c = c_0 \cdot e^{-k_d \cdot t} \tag{20}$$

2.5. MATRIX POPULATION MODELS

The use of continuous ordinary differential equations ignores population structure by treating all individuals as identical. The existence of demographically important differences among individuals is obvious. Matrix population models (Caswell, 1989) integrate population dynamics and population structure and they are very useful when the life cycle is described in terms of size classes or age classes. There are fundamentally two types of approaches, the age classified model and the stage classified model. The first one assumes age-specific survival and fertility are sufficient to determine population dynamics. On the other hand, if the vital rates depend on body size, and growth is sufficiently plastic that individuals of the same age may differ appreciably in size, then age will provide little information about the fate of an individual (e.g. fish models, see Zaldívar and Campolongo, 2000). In the age-based type of modelling the matrix **A**, called Leslie matrix, which describes the transformation of a population from time t to time t+1,

$$\boldsymbol{n}_{t+1} = \boldsymbol{A}\,\boldsymbol{n}_t \tag{21}$$

has the following structure:

$$\mathbf{A} = \begin{bmatrix} 0 & F_2 & \ldots & \ldots & F_q \\ P_1 & 0 & 0 & \ldots & 0 \\ 0 & P_2 & 0 & 0\ldots & 0 \\ \ldots & \ldots & \ldots & \ldots & \ldots \\ 0 & 0 & 0\ldots & P_{q-1} & G_q \end{bmatrix} \tag{22}$$

where $\boldsymbol{n}_t$ is a vector describing the population at each stage at time t, P_i is the probability of surviving from the i-th age class to the next i, G_i is the probability of surviving and growing in the same age class, and F_i is the fecundity rate per unit time (d), $i = 1,2,...,q$. For the case of the *Dapnia magna* model, we have considered 20 age classes (one day duration, except the last one) with different mortalities for each class (see next Section). In this case it is possible to write:

$$P_i = \frac{q_{i+1}}{q_i} \tag{23}$$

where q_i , Eq. (10), is the probability of surviving for day i to day $i+1$ and

$$G_{20} = P_{20} \tag{24}$$

In a similar way, the fecundity of each class, using the reproduction rate Eq. (8), may be written as:

$$F_i = \int_i^{i+1} R(t)dt \tag{25}$$

Incorporation of interaction between species at different stages can be easily done (Cushing, 1998; Zaldívar and Campolongo, 2000). With this approach toxic effects introduced at population level can be extended to ecosystem level. The introduction of DEB models into matrix population models has been developed recently (Lopes et al., 2005; Klanjscek et al., 2006; Billoir et al., 2007).

2.6. OPTIMIZATION PROCEDURE

There are three to four parameters that have to be calculated from toxicity data sets and then used to perform predictions. These are: k_a, the exchange rate constant between the environment and the internal concentrations in *Daphnia magna* which controls the dynamics of the toxicant internal concentrations; k_d, the constant for the exponential decrease of the contaminant during the experiment (sometimes reported in the data sets); *NEC*, No-effect concentration for survival, and the k_t, the killing rate of the toxicant. To obtain these parameters the integrated model, i.e. contaminant fate, biology and contaminant effects (survival), was run and the results compared with toxicity data (dose-response curves). Constrained optimization using the Optimization Toolbox™ from MATLAB® was performed to minimize the error between modelled and fitted dose-response curves.

As explained before, one data set (chronic or acute) was used to fit these parameters, whereas the other data set was used to test the validity of the approach.

3. RESULTS

3.1. AGE-CLASSIFIED DAPHNIA MODEL

Since we are interested in using acute(24 and 48 h) and chronic toxicity data sets (21 days), we have divided the age-based model in a Leslie matrix of 20 age classes and a time step of one day in a similar way as in Billoir et al. (2007). The parameters of the age-classified model for *Daphnia magna* were compared with experimental data on growth, reproduction and mortality. Figure 2 shows the comparison between experimental data and model results. The growth parameters were taken from Kooijman and Bedaux (1996) and Kooijman (2009), whereas reproduction was modified to optimize the fit, but the value is quite close to that of Koijman and Bedaux (1996): 28.9 d^{-1}. Natural mortality was calculated for each class to fit the curve obtained by Santojanni et al. (1995). This also allowed calculating the decrease rate in the last age, G_{20}, since Daphnia magna can live more than 21 days.

Since we are interested in analyzing ecotoxicological experiments, we have assumed that the experiment was carried out under a controlled situation with constant temperature and high food density. For these reasons, the model does not consider the effects of both parameters of the dynamics of the population.

As it can be observed, there is a good agreement between simulated and experimental results and therefore, the age-classified model seems to capture the dynamics of *Daphnia magna* concerning growth, reproduction and mortality. These parameters were kept during all the subsequent simulations. Once we have a model of the species of interest, in this case *Daphnia magna*, we can now try to simulate the experimental conditions in the toxicological experiments, trying to reproduce them "*in silico*".

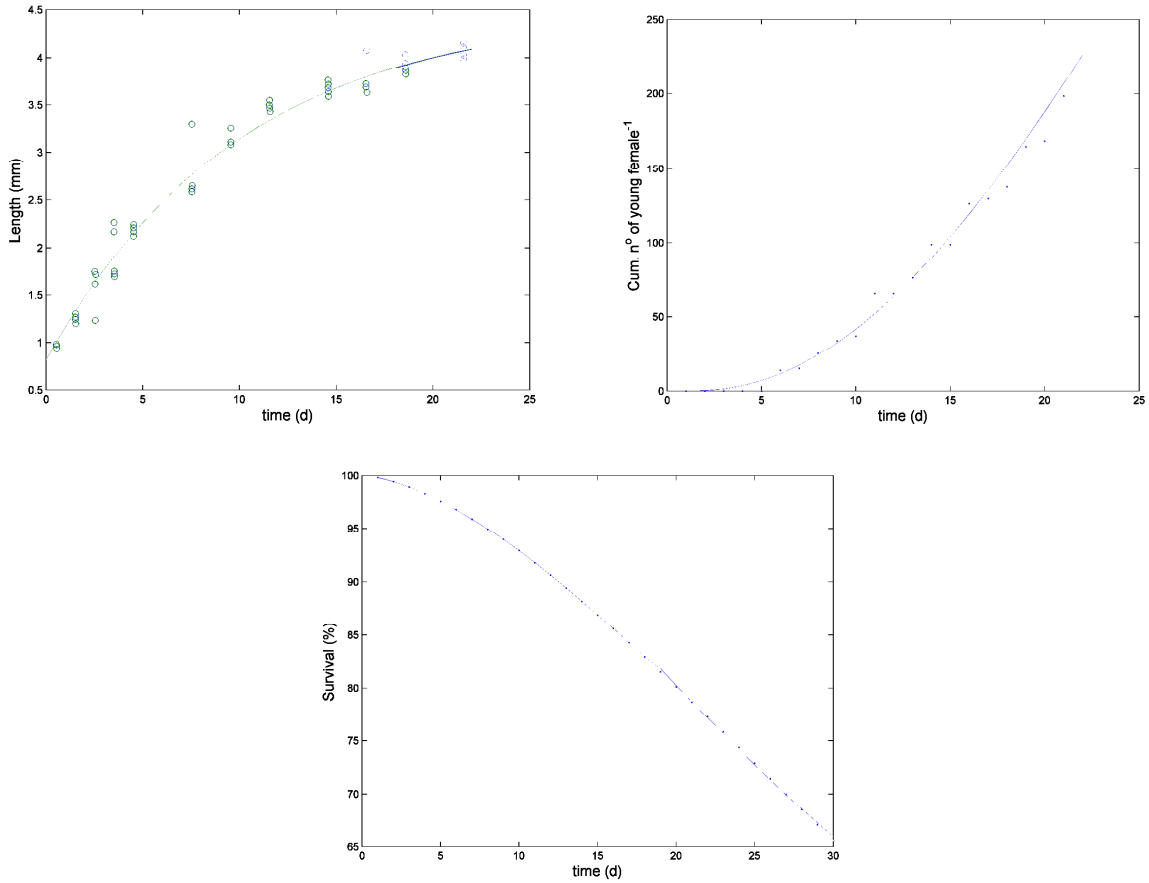


Figure 2. a/ Simulated (continuous line) and experimental (dots) data (Kooijman, 2009) on *Daphnia magna* length; b/ Simulated and experimental data (Kooijman and Bedaux, 1996) from cumulative number of young per female for *Daphnia magna;* c/ Simulated and experimental data (Santojanni et al., 1995) on survival as a function of time. Parameters: L_m=4.50 mm; L_b=0.18 mm; L_p=0.4 mm; R_m=24.9 d^{-1}; γ=0.1; m_i = (0.0019, 0.0038, 0.0052, 0.0063, 0.0073, 0.0082, 0.0091, 0.0099, 0.0106, 0.0114, 0.0120, 0.0127, 0.0133, 0.0140, 0.0146, 0.0151, 0.0158, 0.0163, 0.0128, 0.0197);$G_{20} = P_{20}$.

3.2. CASE STUDIES

Even though the main interest of this approach would consists on infer chronic data from acute toxicity test, we have also performed the opposite calculation. This was due to the quality and the number of data in each set. Since we need to fit 3-4 model parameters we need a minimum amount of data to perform the optimization of these parameters.

3.2.1. From chronic to acute toxicity: Pyridine and nanomaterial case studies

To assess the validity of this integrated modelling approach, we have used the survival data provided by Santojanni et al. (1995) when Daphnia magna was exposed to several concentrations of pyridine: 25, 50 and 100 mg L^{-1}. The objective was to evaluate if a single set of parameters was able to model all survival experiments. In this case we used nonlinear optimization with constraints (parameters should not have negative values) to obtain a set of parameters that was able to fit all experimental results, see fig. 3. There are two parameters related to the toxic effects: the no effect concentration (*NEC*) and the

killing rate of the compound (k_t) and two others related with the kinetics of the contaminant: the exchange rate of the chemical between the water and the organism (k_a) and the degradation rate (k_d), which was assumed to follow a decreasing exponential function with time and which was initialized 3 times a week according with the experimental procedure to renew the test solutions.

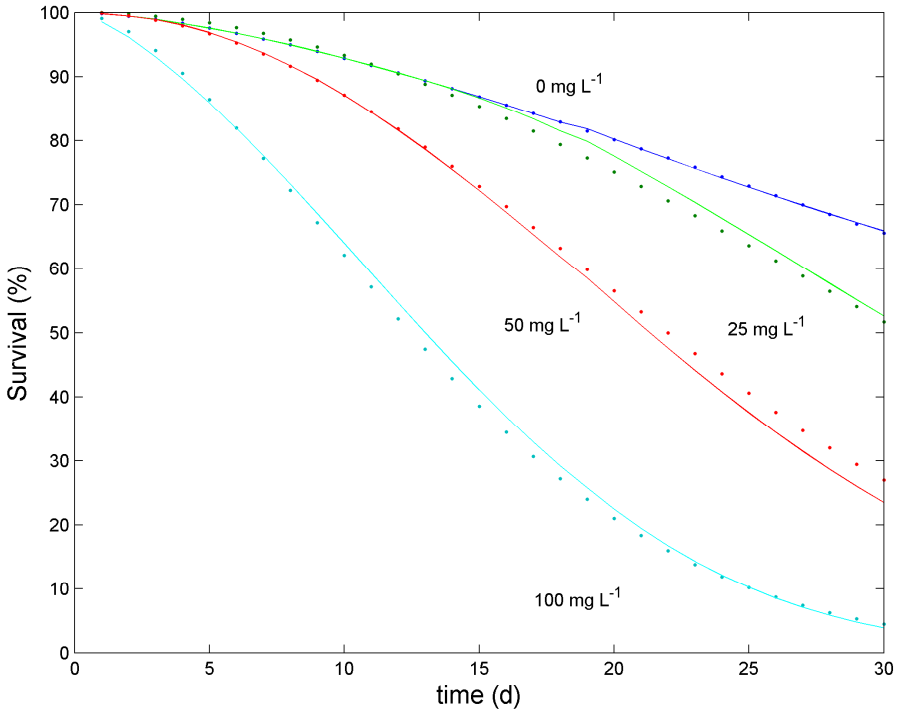


Figure 3. Percentage of individuals surviving in different pyridine concentrations as a function of time. Fitted data by the combined age-based and fate and effects models (continuous line). Results from fitting several Weibull equations (see Santojanni et al., 1995 –Table 1) to experimental data (dots). Parameters: NEC = 1.2604 mg L^{-1}, k_t = 0.021 L mg^{-1} d^{-1}; k_a = 0.0111 d^{-1}; k_d = 2.63 d^{-1}.

Acute toxicity data from ECOTOX (http://cfpub.epa.gov/ecotox) on LC_{50} for 24 and 48 h experiments on Daphnia magna were obtained. The following values are reported for pyridine: LC_{50}^{24h}=2114 mg L^{-1} (Dowden and Bennet 1965); LC_{50}^{48h}= 944 (Dowden and Bennet 1965), 1120 1140, 1210, 1570 and 1940 mg L^{-1} (Canton and Adema, 1978). The model then was run for one and two days at different concentrations of pyridine. Figure 4 show the results. As it can be seen the predicted values for LC_{50} at 24 and 48 h (1833 and 859 mg L^{-1}, respectively) are slightly lower than the experimental values, but still acceptable with 13.3% and 35% error, respectively.

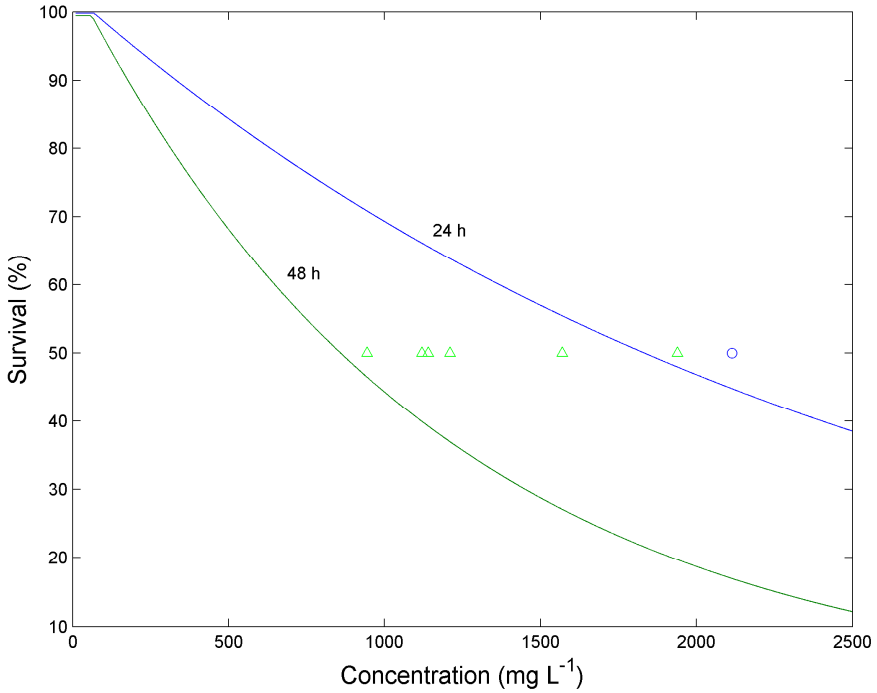
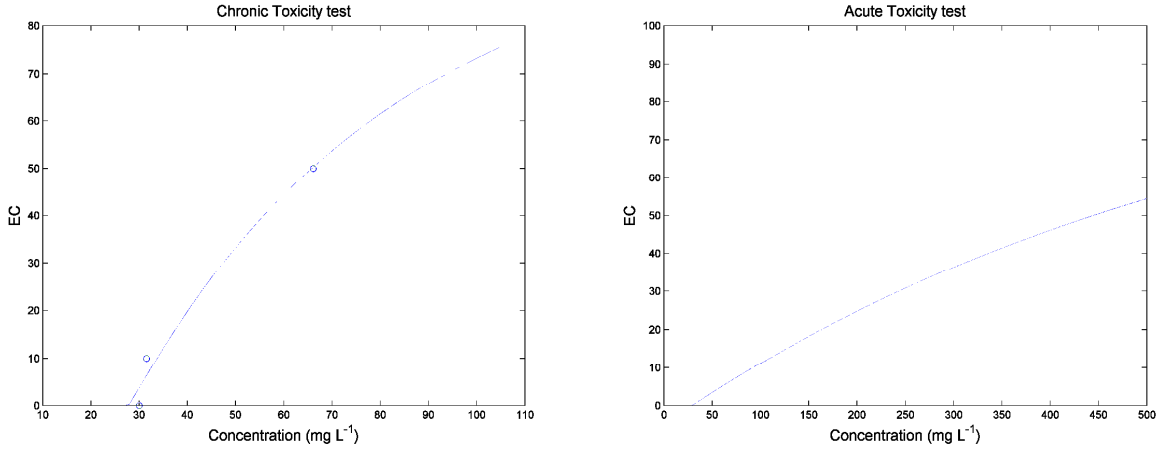


Figure 4. Simulated survival for 24 (blue line) and 48 (green line) h test with pyridine, and experimental values for 24 h (circles) and 48 h (triangles) from Dowden and Bennet (1965) and Canton and Adema (1978).

A similar situation as with pyridine was also found for the nanomaterial data set. Data concerning toxicity, mortality endpoint, after exposure to T-Lite^{TM} SF-S – a nanoparticle ~50 nm length and ~100 nm width titanium dioxide, aluminium hydroxide and simethicone/methicone polymer- have been recently presented by Wiench et al. (2009). The following values were reported: Acute toxicity (OECD guideline 202): EC_{10}>100 mg L^{-1}, EC_{50}> 100 mg L^{-1}; Chronic toxicity (OECD guideline 211): NOEC= 30 ; EC_{10} =31.5 (18.8-52.9, 95% confidence interval); EC_{50}=66.1 (42.3-103.3) mg L^{-1}.



a/ b/

Figure 5. a/ Experimental(circles and 95% confidence interval) and fitted EC values (mortality end point) for chronic (21 days) toxicity test. b/ Predicted EC values for 48 h acute toxicity test (horizontal line 100 mg L^{-1}). Parameters: NEC = 27.78 mg L^{-1}, k_t = 8.78·10^{-4} L mg^{-1} d^{-1}; k_a = 41.12 d^{-1}; k_d = 0.0 d^{-1}.

In this case, we were interested in assessing the results of the model with a scarce number of data points as well as to test if nanomaterials behave in some way different from organics for this type of test. We assumed that the degradation rate was zero and we fitted the other three parameters of the model with the chronic toxicity data set, then we used these parameters to develop a dose-response curve for the acute toxicicty test. Fitted results are shown in Fig. 5a, whereas the predicted values are shown in fig 5b. As the value reported were EC_{10} and EC_{50}> 100 mg L^{-1}, we can only confirm that the approach gives a valuable result within the range of experimental results even for this case where few experimental data are available. We predicted an EC_{10}= 92.4 mg L^{-1} and an EC_{50}= 450 mg L^{-1}.

3.2.2. Data Reconciliation: Chlordecone and case study

Before moving for acute to chronic toxicity prediction we were also interested in using the fate-biology based modelling approach for analyzing existing data sets and to investigate if this approach provided coherent results over all published data. Data reported for chlordecone in the OECD QSAR tool come from six different sources and span over a period of 20 years. The acute and chronic data sets described in Table 1, with the exception of the lethal tests, were used to fit the toxicity and fate parameters. Figure 6 shows the results, whereas Figure 7 shows the reproduced lethal tests. Even though chlordecone is one of the banned plant protection products for which more data are available, experimental values are still scarce and show a great variability. However, the simulated results are in general agreement, considering the reported variability and the, sometimes, contradictory values.

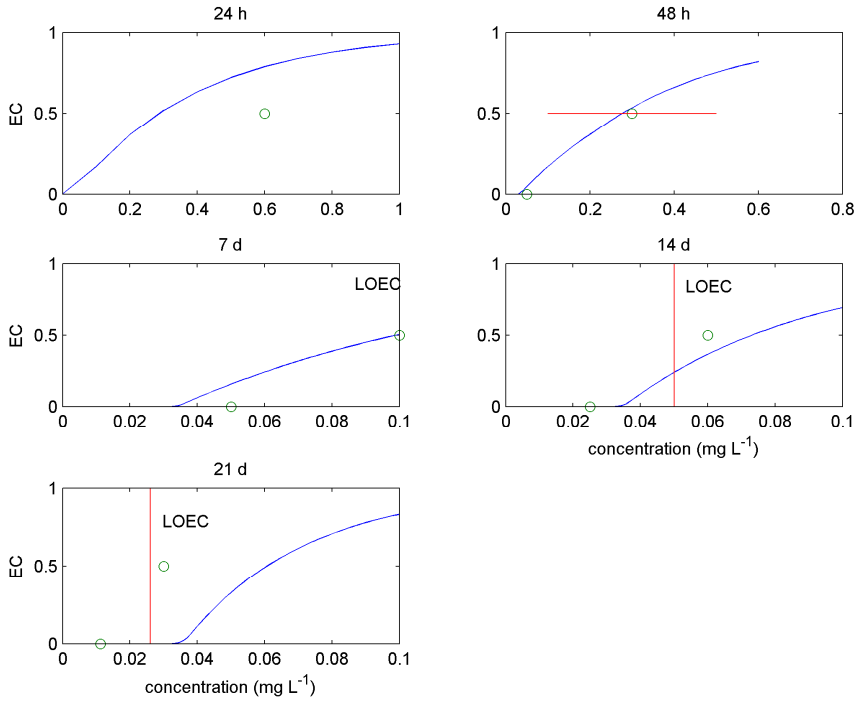


Figure 6. a/ Experimental(circles and standard deviation) and fitted EC values (mortality end point) for acute and chronic toxicity tests. Parameters: *NEC* = 0.028 mg L^{-1}, k_t = 3.267 L mg^{-1} d^{-1}; k_a = 3.58 d^{-1}; k_d = 4.05 d^{-1}.

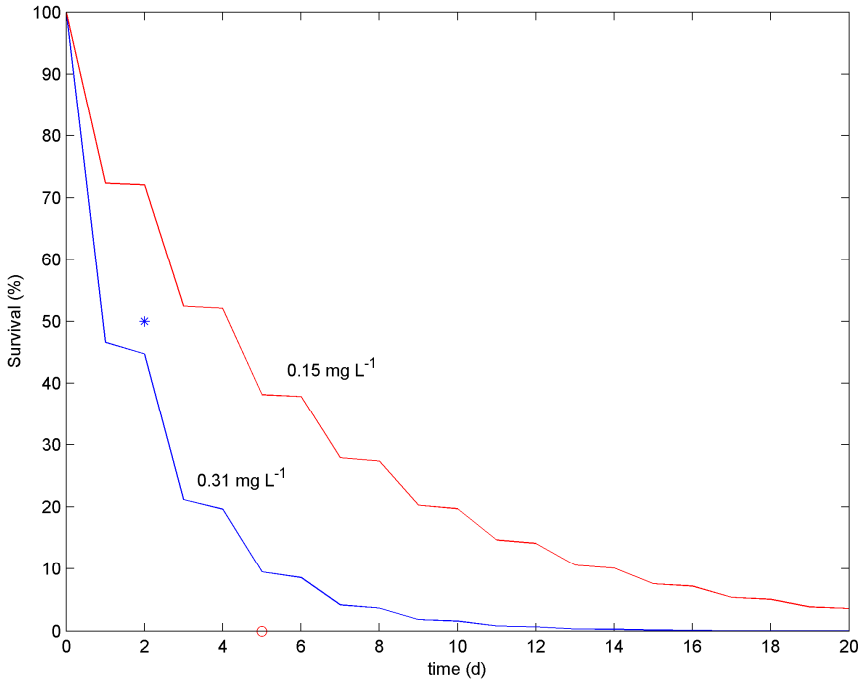


Figure 7. Simulated survival under 0.15 and 0.31 mg L^{-1}. Experimental values LC_{50} 48 h (asterisk) with 0.31 mg L^{-1} and LC_{100} 5 days (circle) with 0.15 mg L^{-1} using the parameters from fig. 6.

3.2.3. From acute 24h and 48 h to chronic toxicity: veterinary antibiotics case study

Data concerning acute toxicity of oxolinic acid, streptomycin, tiamulin and tylosin, Table 3, were used to fit the coupled biology-fate model and then to predict chronic toxicity data. Figures 8-11 show the fitted values whereas figs. 12-15 the predicted chronic values and dynamics. Despite the scarce number of data points from acute toxicity test used to fit the model parameters, the prediction of the chronic toxicity tests are generally in reasonable agreement. For Oxolinic acid, Fig. 12, the severity is slightly over estimated; for Streptomycin, Fig.13, the predictions and experimental values are in good agreement, whereas for Tiamulin and Tylosin (Figs. 14 and 15) the severity of the effect is underestimated.

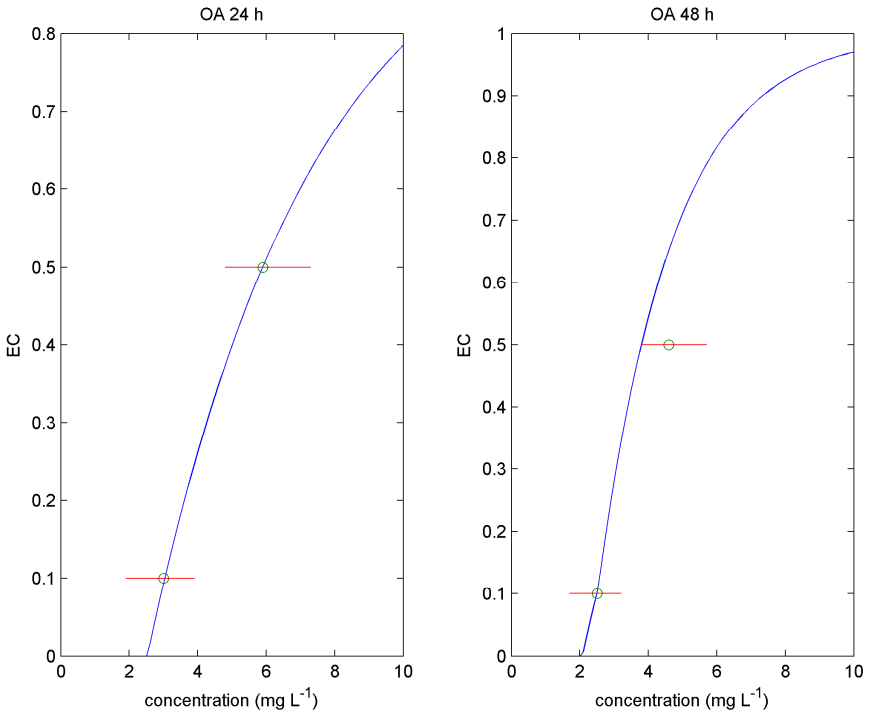


Figure 8. Acute toxicity data for oxolinic acid (experimental value and 95% confidence intervals) and simulated optimized values. Parameters: *NEC* = 0.36 mg L^{-1}, k_t = 1.458 L mg^{-1} d^{-1}; k_a = 0.094 d^{-1}; k_d = 1.22 d^{-1}.

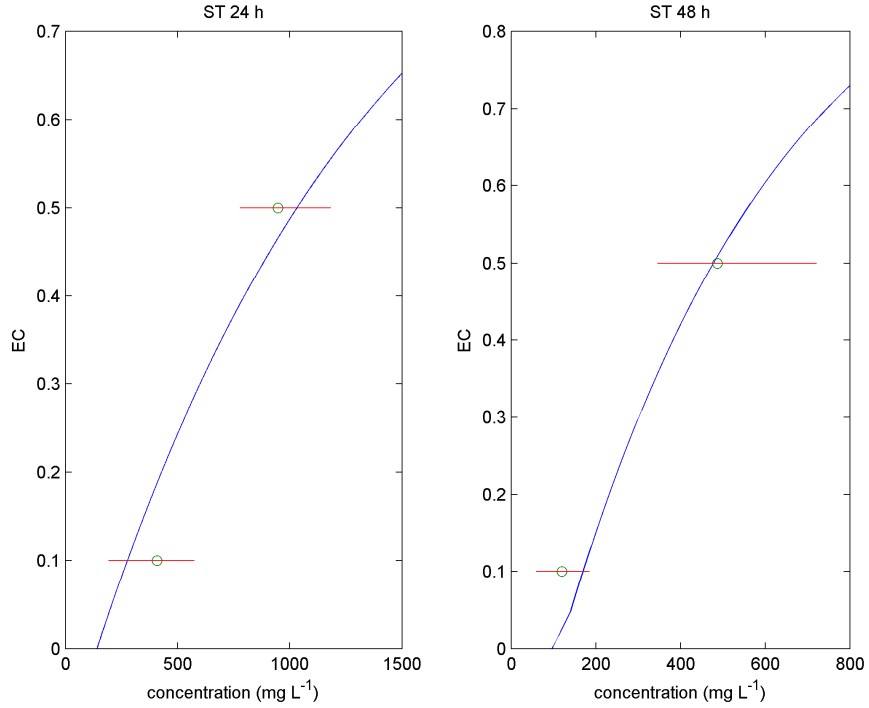


Figure 9. Acute toxicity data for Streptomycin (experimental value and 95% confidence intervals) and simulated optimized values. Parameters: *NEC* = 1.90 mg L^{-1}, k_t = 0.05 L mg^{-1} d^{-1}; k_a = 0.009 d^{-1}; k_d = 0.96 d^{-1}.

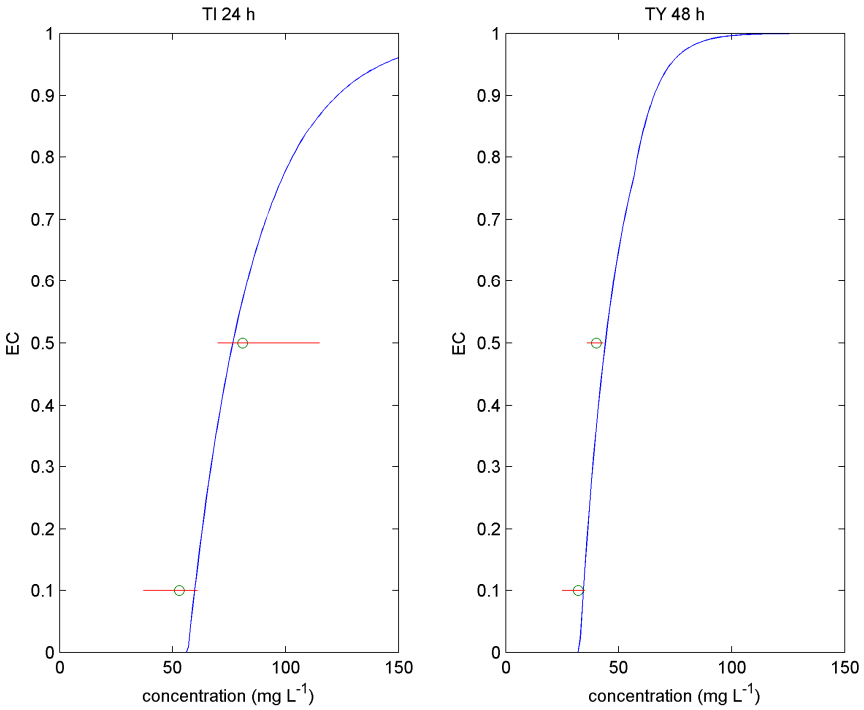


Figure 10. Acute toxicity data for Tiamulin (experimental data and 95% confidence intervals) and simulated optimized values. Parameters: *NEC* = 11.14 mg L^{-1}, k_t = 0.18 L mg^{-1} d^{-1}; k_a = 0.13 d^{-1}; k_d = 0.0 d^{-1}.

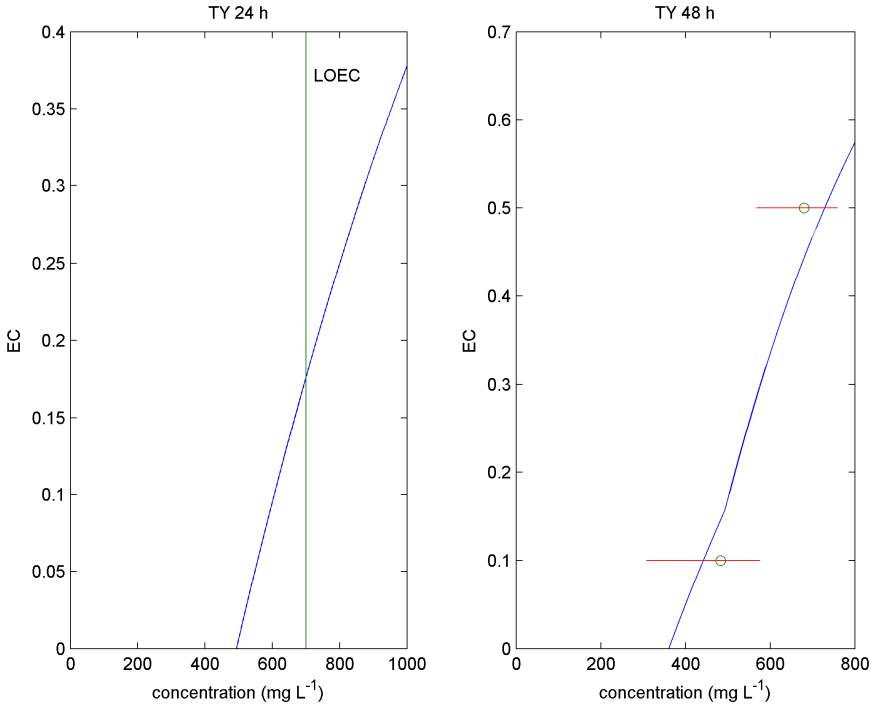


Figure 11. Acute toxicity data (experimental data and 95% confidence intervals) and simulated optimized values. Parameters: *NEC* = 12.24 mg L^{-1}, k_t = 0.04 L mg^{-1} d^{-1}; k_a = 0.015 d^{-1}; k_d = 1.24 d^{-1}.

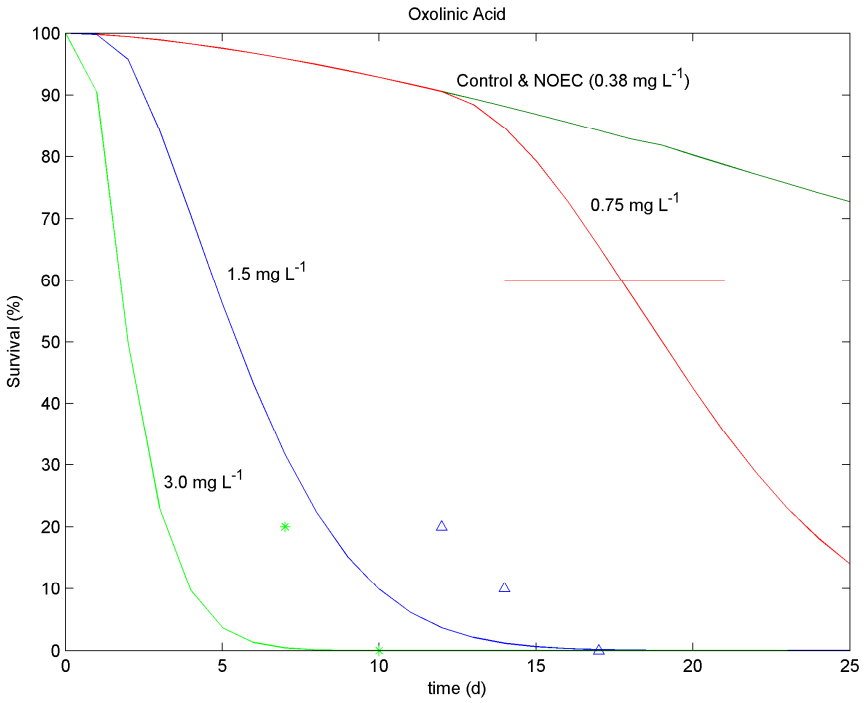


Figure 12. Experimental values (points) and simulated survival for several concentrations. Parameters from fig. 8.

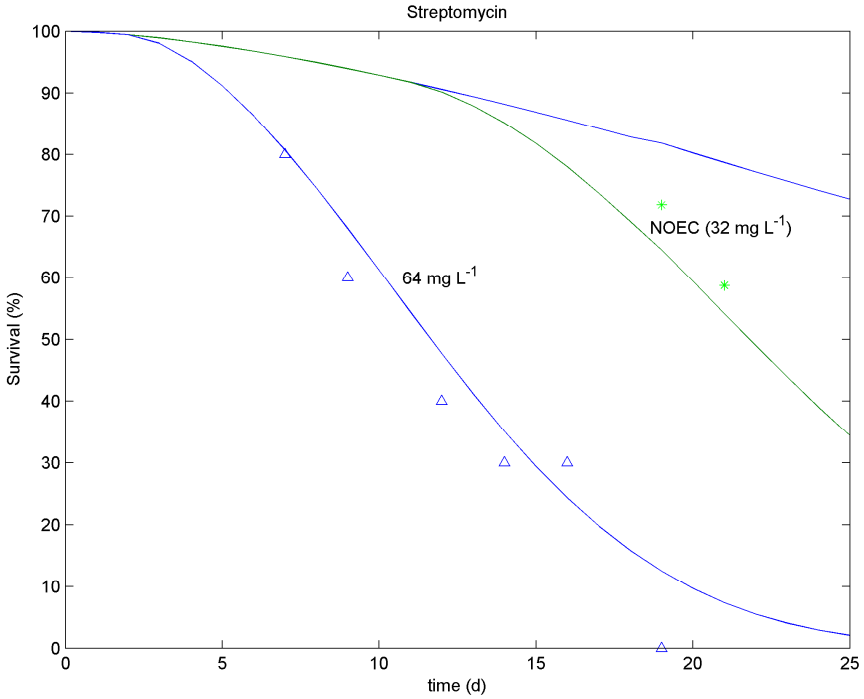


Figure 13. Experimental values (points) and simulated survival for several concentrations. Parameters from fig. 9.

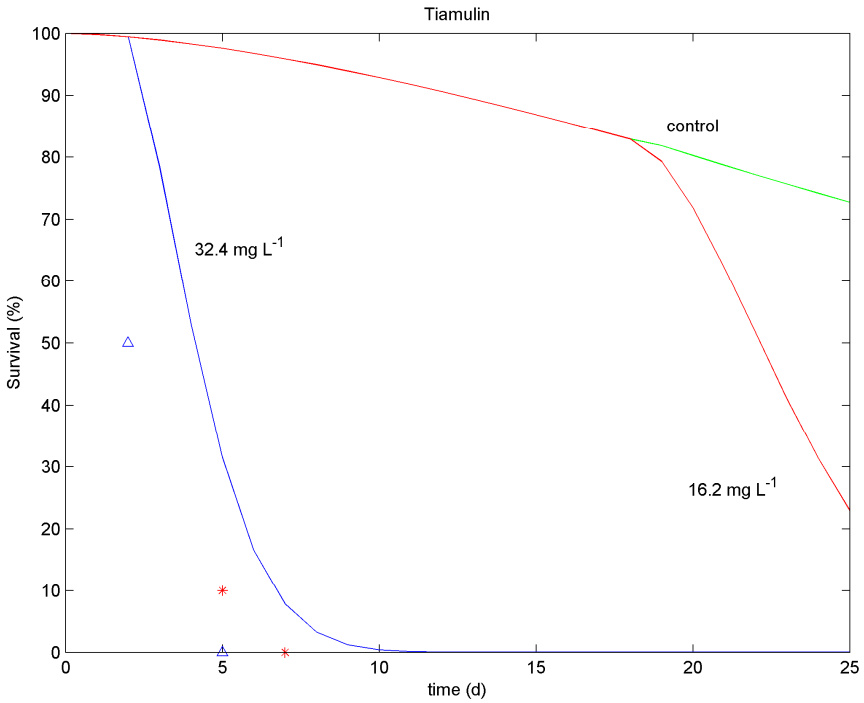


Figure 14. Experimental values (points) and simulated survival for several concentrations. Parameters from fig. 10.

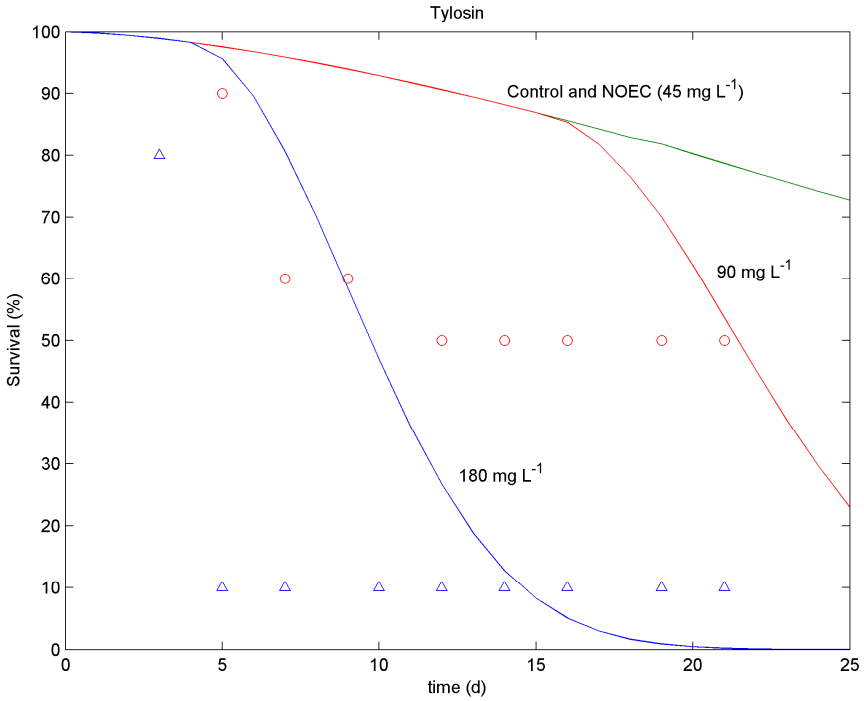


Figure 15. Experimental values (points) and simulated survival for several concentrations. Parameters from fig. 11.

4. DISCUSSION

Despite the scarce number of data points to fit model parameters, we have showed that this integrated approach is able to produce useful results and to provide good estimations when predicting chronic from acute toxicity experiments or *vice versa*. In addition, the dynamics of the different experiments can be studied in detail using the fitted data and then the model can be used to guide the design of the experimental conditions. Furthermore, the model can be used to identify experimental outliers that are not easily observed when comparing different test performed by different laboratories and/or to distinguish between typical biological variability.

Better results could possibly be obtained with more experimental data points. In this case, we could be able to improve the estimation of the model parameters and provide as assessment of the uncertainties in their determination. This is important because of the strong non-linear coupling between the estimated model parameters, e.g. a higher mortality rate with a lower exchange rate could, in principle, provide similar results.

An effect that we have not considered in the model, but that could be easily implemented is the difference in the fate model between acute and chronic experiments. Whereas 24 and 48 h experiments are normally carried out without food supply, this is not the case for long-term experiments. In this case, the contaminant will also enter into the organisms by feeding. This type of effects have been reported by Klüttgen et al. (1996) and by Herbrandson et al. (2003a,b). Under these conditions, the contaminant will distribute between dissolved and particulate phase and it will enter into the organism by predation (Dueri et al., 2009; Marinov et al., 2009). However, this could be easily incorporated in the fate model adding a supplementary term that consider feeding, the drawback being that the fate equation should have more parameters to fit and then due to the scarcity of data points, the fitting would be more problematic.

4.1. THE PROBLEMS OF FITTING TOXICITY DATA SETS WITH FEW DATA POINTS: THE NCD DATA SET

Even though in principle, our idea was to use the New Chemical Data (NCD) base to statistically test the approach, we have had several problems due to the amount of data points and the existence of multiple minima during the optimization, when using such a reduced number of points. To illustrate the problems with the toxicity data sets, we have fitted the data using traditional dose-response curves. Examples of these are (Backhaus et al., 2004):

- Weibull:

$$f(x) = 1 - \exp[-\exp(\theta_1 + \theta_2 \log_{10} x)] \quad (26)$$

- Box-Cox transformed Weibull:

$$f(x) = 1 - \exp\left[-\exp\left(\theta_1 + \theta_2 \frac{x^{\theta_3} - 1}{\theta_3} \right) \right] \quad (27)$$

- Morgan-Mercier Flodin:

$$f(x) = 1 - \frac{1}{1 + \theta_1 \cdot x^{\theta_2}} \quad (28)$$

- Logit

$$f(x) = \frac{1}{[1 + \exp(-\theta_1 - \theta_2 \log_{10} x)]} \quad (29)$$

- Generalized Logit:

$$f(x) = \frac{1}{[1 + \exp(-\theta_1 - \theta_2 \log_{10} x)]^{\theta_3}} \quad (30)$$

where θ_1, θ_2,and θ_3 are parameters of the dose-response curves. These curves have a sigmoidal shape and a lower (L) and upper (U) asymptotes with values of 0 and 1 (0-100%), respectively. For example Table 4 summarizes the fitted parameters for the Weibull function, Eq. (26), whereas fig. 16 show experimental versus fitted results for the 28 NCD retained compounds.

Table 4. Fitted Weibull parameters, Eq. (26).

Compound	**θ_1**	**θ_2**	**Compound**	**θ_1**	**θ_2**
1	2.395	11.67	15	-14.9	7.97
2	-6.291	6.483	16	-13.98	7.667
3	10.17	4.998	17	-13.67	9.095
4	4.197	5.151	18	7.817	11.34
5	-2.73	11.58	19	-5.454	4.017
6	-3.097	10.7	20	-5.025	13.03
7	-5.127	3.969	21	-8.258	5.592
8	-0.9306	3.318	22	-23.2	12.55
9	2.999	1.51	23	-3.632	4.122
10	-16.63	7.141	24	-12.55	5.924
11	-9.13	6.404	25	-7.709	7.849
12	6.077	3.658	26	-12.8	9.305
13	1.19	6.58	27	-31.29	16.73
14	-8.401	12.89	28	-0.855	1.941

The main problem is that with three data points it is difficult to assess the goodness of the fit since there are multiple values that will give the same error. To illustrate this problem, Fig. 17 show the results provided by Eqs. (26)-(30) to Compound n° 1 of the NCD. All the curves provide a fit with r^2=1 and sum of squares due to errors (or residuals) quite similar, with values of $9.38 \cdot 10^{-7}$, $1.824 \cdot 10^{-8}$, $2.695 \cdot 10^{-6}$, $7.235 \cdot 10^{-7}$ and $1.141 \cdot 10^{-8}$, respectively. Other goodness of fit statistics, e.g. adjusted r-square, root mean squared error, produce also similar results. However, the shape and form of the curves may be quite different and results will differ when used to estimate chronic toxicity.

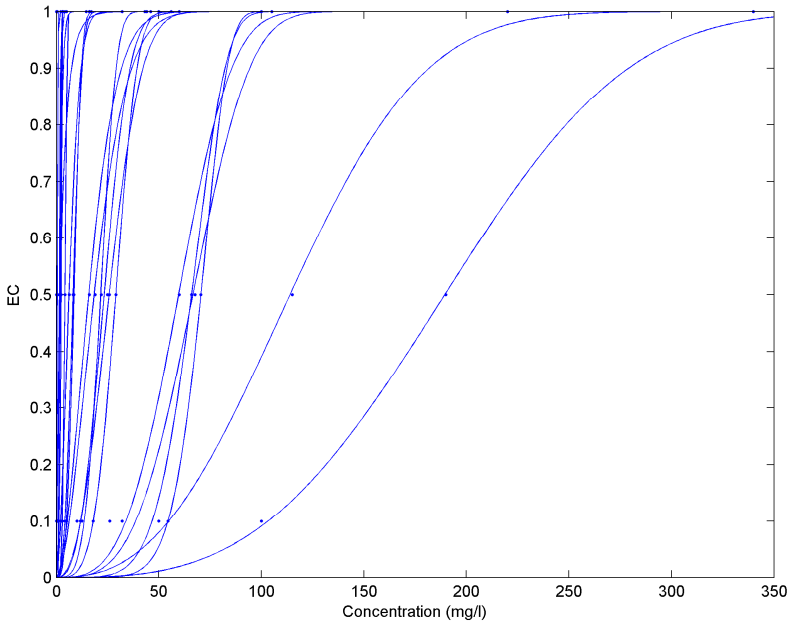


Figure 16. Individual concentration response curves and data from NCD for the *Daphnia magna* toxicity of the 28 compounds. Fitting functions from Table 4.

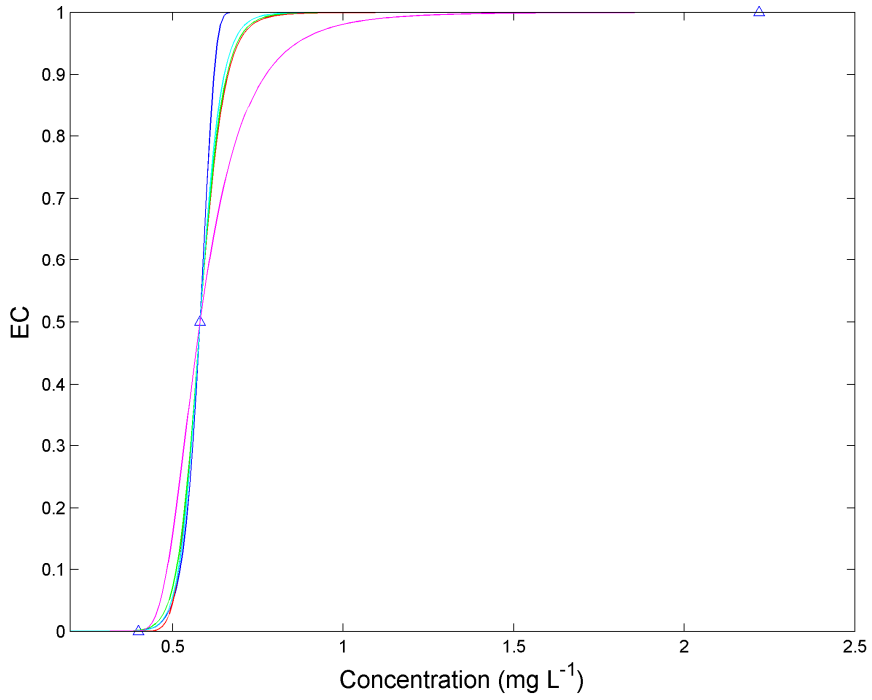


Figure 17. Fitted – Eqs. (26)-(30)- and experimental values (triangles) dose-response curves for compound n° 1 of the NCD.

The reduced number of data points is also the same problem we have observed using the *Daphnia magna* model instead of the mathematical correlations provided by Eqs. (26)-(30). In this case, since the data provided an estimation of k_d as the reported % loss in concentration during the test period, we

have used the chronic data to estimate only *NEC*, k_t and k_a parameters. With three data points we are not in the position to assess the validity of the optimum parameters. For example in Fig. 18 it is possible to observe a nearly perfect fit –evaluated as the square difference between observed and calculated EC-values – between model and experimental values. These parameters will predict a EC_{50}^{24h} of 5.6 and $NOEC^{21d}$ of 0.95 mg L^{-1} whereas the experimental values reported are 9.9 and 0.15 mg L^{-1}, respectively. However, a number of combinations of these parameters (*NEC*, k_t and k_a) that will produce a similar fit are possibler, as it was observed during the optimization procedure, and therefore different predictions will be obtained. This problem is not due to the approach, but to the way data are reported in these experiments.

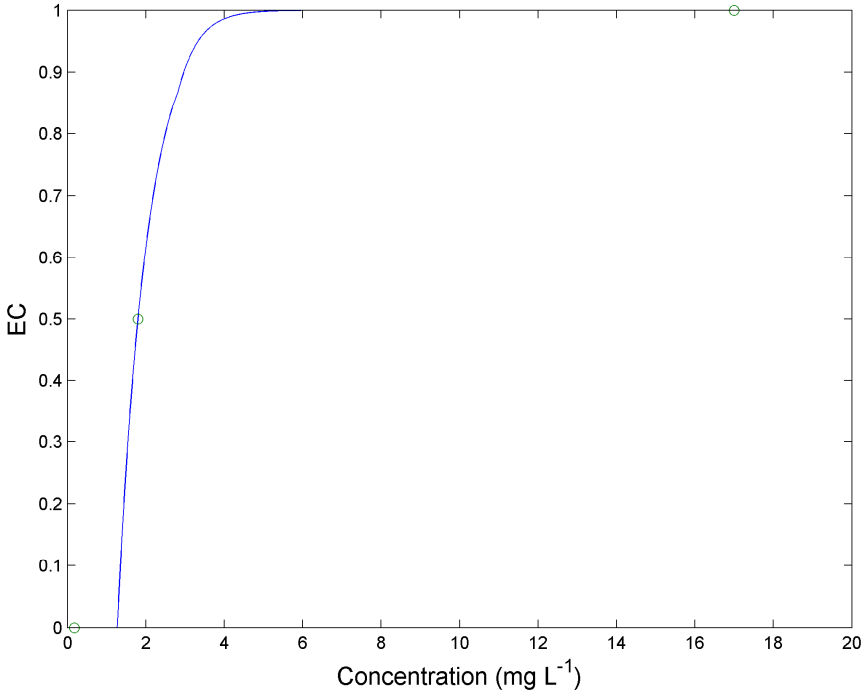


Figure 18. Reported and calculated EC for the compound n° 28 of the NCD. Parameters: *NEC* = 0.37 mg L^{-1}, k_t = 4.54 L mg^{-1} d^{-1}; k_a = 0.06 d^{-1}; k_d = 0.26 d^{-1}.

4.2. RATE LIMITING PROCESSES

One fundamental aspect that is often neglected in toxicological test is the dynamics of the different process occurring, i.e. decomposition, uptake, depuration, degradation, feeding, growth, reproduction, etc. The rate at which these processes occur depends on the physico-chemical properties of the toxicant as well as on the species characteristics and these rates will have an effect on the internal concentration of toxicant in the organisms and therefore on the results of the test. In the limiting cases of the parameters, the whole process will be controlled by the slower process. For example, if uptake is the slower process, whereas product decomposition is faster we can conclude that the product is not toxic

whereas the only conclusion would that the product is unstable. To illustrate some of these effects, we have carried out several simulations changing the different parameters of the model.

Figure 19 shows the effects on the internal concentration, c_q, when changing k_a for a 48h acute toxicity test with *Daphnia magna* assuming no growth (red lines) and growth (blue lines), and no change in the water concentration, c=100 mg L^{-1}. As it can be seen k_a has a strong influence on the toxicant concentration values reached internally. Also the dilution effects due to growth (blue versus red lines) can be observed. For higher k_a values these aspects become irrelevant since the transfer of contaminant into the organism is no longer the rate limiting process and internal and external concentrations are equivalent.

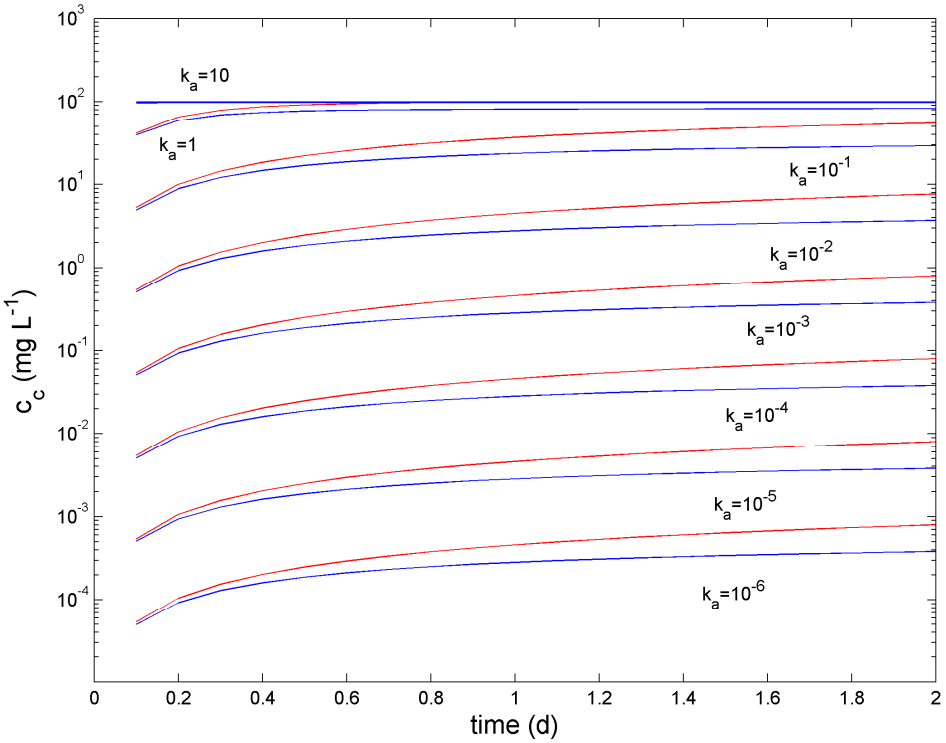


Figure 19. Internal concentration, c_c (mg L^{-1}), in Daphnia magna as a function of exchange rate constant k_a (d^{-1}) assuming no-growth (red lines) and growth (blue lines) and a constant external concentration of 100 mg L^{-1}.

Another important process is the rate of decomposition/disappearance of the chemical during the acute or chronic tests and how to correct the results if the decomposition is higher than 80% are provided in the OECD Guidance.

In our model, we have considered that the decomposition follows an exponential decreasing function, Eq. (20). Furthermore for the chronic test we have considered the renewal of the medium three times per week. Figure 20 shows the importance of this effect for the internal concentrations experienced by *Daphnia magna*. As it can be observed, when there is no decomposition, k_d = 0, with an exchange rate constant of 10 d^{-1}, the internal and external concentrations are practically the same after a short period

of time. However, already with k_d=1 d^{-1}, 63.2 % decrease after one day, the internal concentration in *Daphnia magna* never reaches the external value and decreases exponentially during all the experiment; with k_d =3 d^{-1}, 95% decrease after one day, the toxicant concentration in *Daphnia magna* is practically zero at the end of the two days experiment. This effect can be observed in the shape of the simulated survival curve in fig. 7 during a chronic test, where the mortality occurs only when there is a medium replacement since according with our optimization parameters, it disappears quite rapidly, k_d = 4.05 d^{-1}.

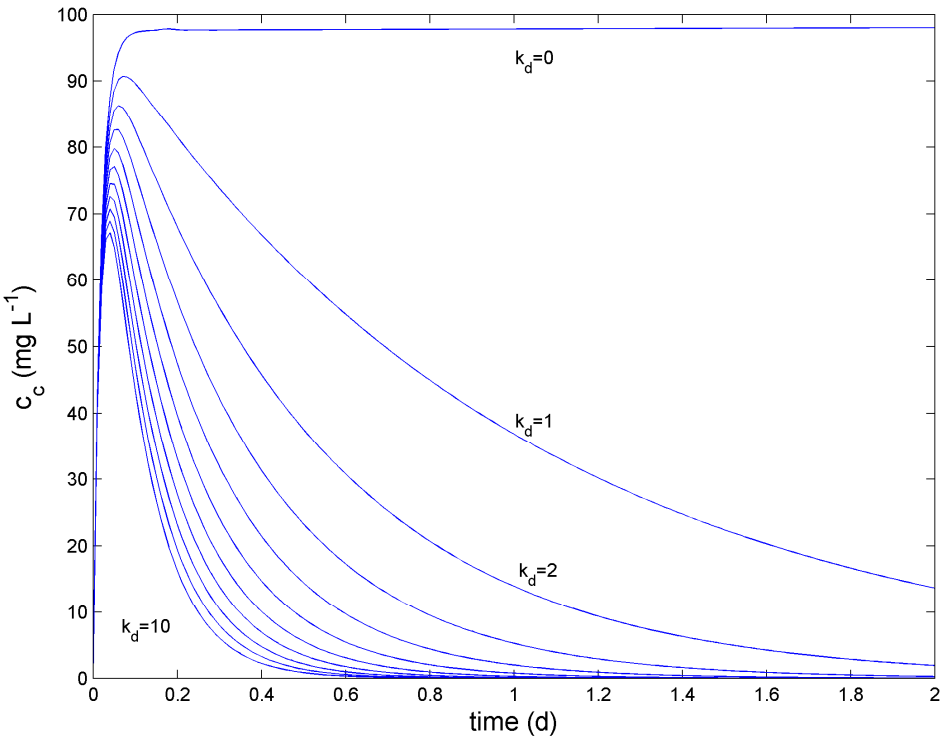


Figure 20. Internal concentration, c_c (mg L^{-1}), in *Daphnia magna* as a function of the decomposition rate constant k_d (d^{-1}) (0-10) assuming an exchange rate constant k_a=10 (d^{-1}) and an initial external concentration of 100 mg L^{-1}.

The interplay of these effects make the optimization process problematic since the model is quite sensitive to the values of these parameter and may jump from a 100% survival to 0% survival when there is a change in the rate limiting process. For example, Figure 21 shows the internal concentration of *Daphnia magna* when changing the initial concentration between 10 and 100 mg L^{-1} for a NEC of 50 mg L^{-1}. As it can be observed, only for initial concentrations higher than 60 mg L^{-1}an effect will occur and, due to the product degradation, this effect will only occur for a limited amount of time during the two days experiment, for example ~5.5 hours with c_0 =100 mg L^{-1}.

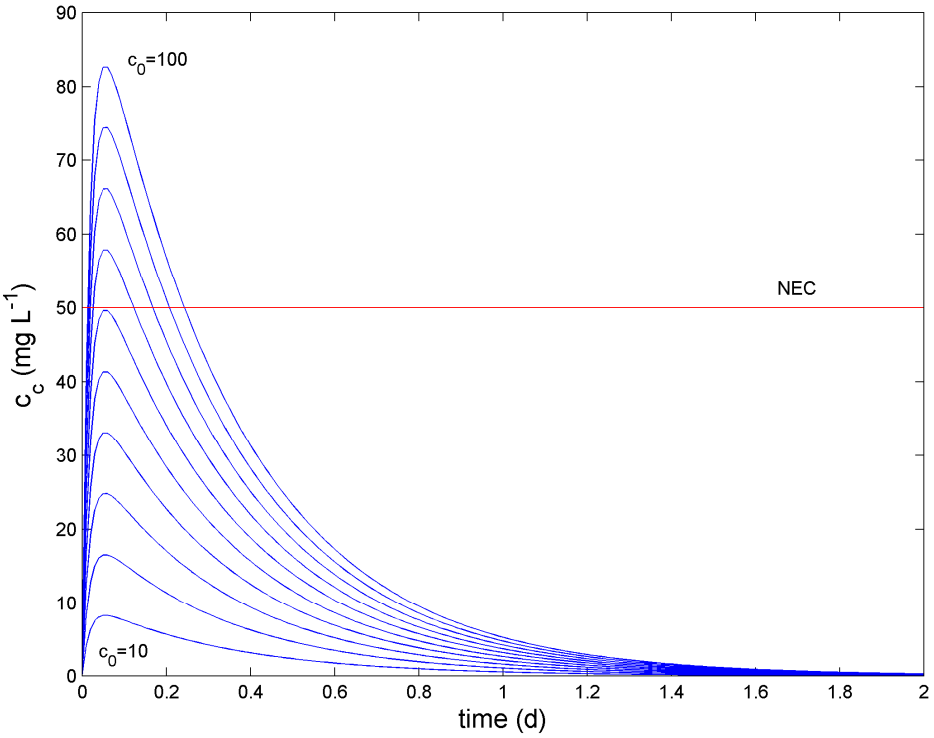


Figure 21. Variation in the internal concentration, c_c (mg L^{-1}), in *Daphnia magna* as a function of the initial concentration in water for a decomposition rate constant k_d (d^{-1}) of 3, an exchange rate constant k_a=10 (d^{-1}) and a NEC of 50 mg L^{-1}.

5. CONCLUSIONS

The introduction of biology and fate dynamics in the analysis of acute and chronic toxicity provides a wide amount of information and new data that, in principle, could help in reducing the number of experiments with animals as well as the required dose levels.

With data rich results, e.g. Santojanni et al. (1995), we have shown that the same parameters were able to fit all their experiments. In addition, the errors for predicting the acute data reported by other authors were 13.3% and 35% for 24 and 48h, respectively. It should be pointed out that for the case of 48 h, the maximum error between the mean value and the reported values is already 32%. Similar results were obtained for the nanomaterial case study. However, in this case, the information provided by the experiment was lower. Then the same approach was used to perform data reconciliation from several authors and experimental procedures, and finally to predict chronic data from acute values. As it can be observed from the simulated results relatively good agreement is obtained. The values predicted by the model are an improvement when compared with the factor of 5 obtained using acute to chronic ratios (Länge et al., 1998).

An important remark is the necessity to produce and report more information from each experiment or set of experiments performed. This would improve considerably the fitting to the model's parameters.

Though, even with few data points, the model can be useful already in predicting starting conditions for the chronic toxicity testing and *vice versa*, therefore reducing the number of test as it has been shown with the example of the nanomaterial.

In addition and to complement and allow the interpretation of experimental data, this approach provides also the internal toxicant concentration in the organisms and therefore could help in the interpretation of critical body residues experiments. Furthermore, the introduction of a process based modelling approach is able to show the interplay between the different effects and how the dynamics will affect the outcome of the toxicological test.

Finally, this approach could easily be extended to other standardized test by replacing the *Daphnia magna* DEB model with the corresponding species model (fish, birds, etc.). The parameters for a considerable number of species are already available in literature (Kooijman, 2000).

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Abstract. There is a need to integrate existing dose-response data in a coherent framework for extending their domain of applicability as well as their extrapolation potential. This integration would also reduce time and cost-consuming aspects of these tests and reduce animal usage. In this work, based on data extracted from literature, we have assessed the advantages that a dynamic biology-toxicant fate coupled model for *Daphnia magna* could provide when assessing toxicity data, in particular, the possibility to obtain from short-term (acute) toxicity test to long-term (chronic) toxicity values and *vice versa*; the possibility of toxicity data reconciliation from several sources and the inherent variability of *Daphnia magna* populations. Implicitly in this approach is the assumption that the mode of action of the toxicant does not change after prolonged exposure.

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