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From simple toxicological models to prediction of toxic effects in time

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Abstract The ability to predict the effects of toxicants in organisms with reasonable accuracy depends to a great extent on the toxico-kinetic models used to describe such effects. Toxic effects of organic chemicals and heavy metals have been described adequately using a hyperbolic model that considers the concentration of the toxicant and the time of exposure only. Such a model relies on the median time to effect (ET_{50}) of a chemical to estimate effects at any exposure time, but cannot make predictions for concentrations other than those tested experimentally. A complementary log-to-log model can calculate all ET_{50} values for a toxicant, thus enabling the hyperbolic model to predict any level of effect for any combination of concentrations and times of exposure. The parameter values used in both models are obtained from experimental bioassays where the time-to-effect of a toxicant is recorded regularly in addition to standard acute or chronic toxicity data. These models will facilitate the risk assessment of chemicals by (1) predicting effects under any combination of time and concentrations, and (2) reducing to a minimum the experimental efforts required to obtain comprehensive ecotoxicity data.

Keywords Modelling · Risk assessment · Exposure · Time–dose relationship · Insecticides

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Introduction

Prediction of toxic effects of chemicals on organisms is the primary aim of ecotoxicology (Truhaut 1977). Traditional approaches based on the dose–response relationship typically consider toxic effects at fixed exposure times, so the estimation of the doses or concentrations required to produce any level of effect (percentile) can only be done for such times. Because toxicity is a process that takes place in time (Mackay et al. 1992), this traditional approach is, however, unable to allow extrapolation from the measured endpoints (e.g. 96-h LC_{50}) to effects that may occur at other times of exposure (e.g. 60-h LC_{50}), thus limiting our predicting ability enormously. Not surprisingly, current toxicological databases are mere collections of endpoint values obtained at fixed, unrelated times of exposure, and as such these values cannot be linked to make predictions for the wide range of exposures in the environment, and so they are of little relevance in risk assessment.

In order to overcome this handicap, an increasing number of researchers are using a variant of the traditional toxicity testing protocol which includes the measurement of effects at several times of exposure, not just at 24, 48 or 96 h. This time-to-event (TTE) approach is very powerful, as it provides information on the acquired doses as well as the exposure times needed for a toxic compound to produce any level of effect on the organisms tested (Newman and McCloskey 1996). Consequently, extrapolations and predictions of toxic effects for any combination of concentration and time are now made possible.

The inclusion of exposure times in toxicology is not new (see for instance Bliss 1937; Sprague 1969), but its use in standard toxicity testing has been neglected until recently. One of the drivers of this new approach has been the realisation that exposure to most pollutants often occurs in

episodic events or pulses rather than continuously (Pascoe and Shazili 1986; McCahon and Pascoe 1990). Indeed, to analyse and compare the impact of toxicants applied as pulses or in a continuous manner the length of time required for recording effects needs to be considered. Much modelling has been derived from such investigations on exposure to contaminants (Hickie et al. 1995; Chaisuksant et al. 1997; Hoang et al. 2007), and a review on their application to aquatic risk assessment can be found in Reinert et al. (2002). Whilst toxicokinetic models such as PULSETOX (Hickie et al. 1995) and DEBtox (Kooijman 2000) are based on mechanistic assumptions that consider rates of uptake and transfer of chemicals between compartments (McCarty and Mackay 1993; Bedaux and Kooijman 1994), other methods are empirical and typically consist of regression lines fitted to transformed experimental data. In both cases, the rates, constants and coefficients used in the models are specific for the toxic compounds and organisms concerned, and must be obtained from experimental data sets.

Following the same line of research, we demonstrated in our previous work (Sánchez-Bayo and Goka 2007) that toxicity effects in time can be described by a hyperbolic model that uses the Michaelis-Menten mathematical expression. The model could be applied to acute as well as long-term (chronic) exposures to organic or heavy metal contaminants, and performed equally well with lethal or non-lethal endpoints in a range of aquatic organisms, from algae to zooplankton crustaceans and fish. In essence, the hyperbolic model is a simplified version of the 4-parameter sigmoid model commonly used in toxicology (Brisbin 1990; Forbes et al. 2001). The great advantage of the hyperbolic model is its reliance on two variables only: concentration of the toxicant and time of exposure, both of which are known to the researcher. The model equation requires, however, to input the time at which 50% of the effect occurs (ET_{50}), and this parameter must be calculated for each chemical concentration in the TTE bioassays using any of the standard procedures available (e.g. probit, logit or Weibull models). In the end, the hyperbolic model relies as much on specific sets of experimental data as do other models. Although this is by no means a weak feature of any model, it does not allow the hyperbolic model to estimate effects in time for concentrations other than those tested. How can we then extrapolate for the effects at unknown concentrations?

This paper aims at providing a method for calculating ET_{50}s of unknown concentrations of toxic compounds, which can subsequently be used in the hyperbolic model to estimate toxic effects in organisms at any time of exposure for any level of concern. Alternatively, the calculated ET_{50}s can be used with other models as well, since they represent median effect values for given concentrations. The outcomes find practical application in risk assessments, as they enable the prediction of direct toxic effects for any combination of dose or concentration and time (Suter et al. 1987), whether they result from pulses, post-acute or continuous exposure to toxic chemicals.

Material and methods

Experimental data

The data of interest are the median effective times (ET_{50}s) for various toxic chemicals and organisms. Such data were either reported as such or could be calculated from the raw experimental data published in research papers, as indicated below. Also included are unpublished data from our own experiments.

The toxic chemicals considered here are the metals copper, zinc, cadmium ($CdCl_2$) and the non-metallic, essential element selenium. Among the organic toxicants, comprehensive sets of toxicity data with time in the literature consulted were only found for the neonicotinoid insecticides imidacloprid and thiacloprid. All concentrations refer to external concentrations in the aqueous media. Mortality was the endpoint reported in all cases, so the ET_{50}s reported here are in fact LT_{50}s, although the former acronym will be used throughout as the model is meant to apply in general.

Data on single pulse exposures of *Daphnia magna* Straus to copper, zinc and selenium were taken from Hoang et al. (2007), who indicate the regression equations (in Fig. 1 of that paper) that allow calculation of ET_{50}s for a range of concentrations of the respective elements. Their equations were derived from series of exposures that represented 202 different combinations of metals, metal concentrations, pulse duration and number of pulses. Exposure durations for single pulses—the only ones considered here—varied from 24 h for selenium to 150 h (over 6 days) for copper and 500 h (21 days) for zinc. Mortality was measured throughout those periods, but the exposures ended between days 1 and 6, so acute as well as latent effects (Brent and Herricks 1998) during the post-exposure periods are both included here. The reader is referred to that paper for a complete explanation of the experimental setup and conditions used by the authors.

Chronic toxicity data for *D. magna* exposed to $CdCl_2$ for 21 days was taken from Kooijman (1981). The aqueous media in this case was refreshed at regular intervals to ensure a constant concentration of the toxic substance in the media, as recommended for chronic toxicity tests. The results show the cumulative effect of the toxicant over time, which follow first order kinetics. Mortality of the cladocerans at each of the five concentrations tested was

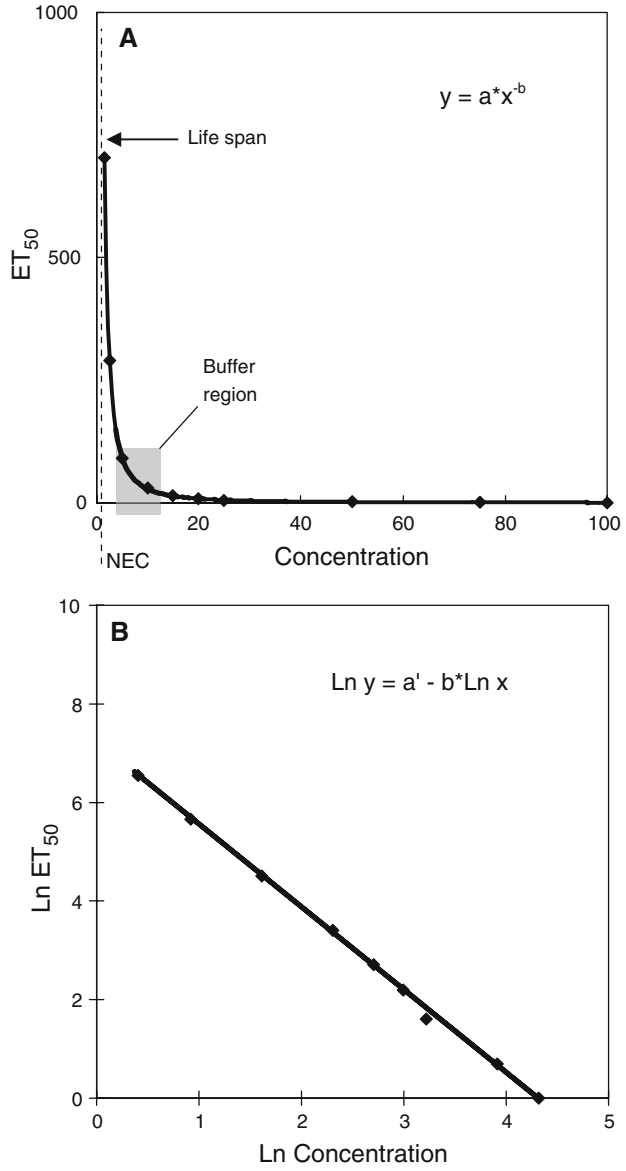


Fig. 1 **a** The relationship between toxicant concentration in the media and time to 50% effect (ET_{50}) in the organisms exposed follows a hyperbolic curve asymptotic on the *y* axis; in reality, this asymptote is determined by the no-effect concentration (NEC), while the upper limit of the curve is determined by the life span of the organism. **b** The same relationship becomes a straight line when using the logarithms of both variables. See text for an explanation of the 'buffer' region

recorded at several intervals during the entire experimental period, and the corresponding ET_{50}s were calculated based on the published raw data, and were also reported in our previous paper (Sánchez-Bayo and Goka 2007).

For fish, data on exposure of guppies (*Poecilia reticulata* Peters) to a diluted pulse of zinc was obtained from Widianarko et al. (2001). Survival data were recorded at 12 h intervals for the duration of the experiment (144 h). In this case, replacement of 10% of the zinc solution with clean water was done every 12 h, thus introducing a progressive dilution factor that affected survival: the authors report a levelling off in mortality after 60 h for the lowest concentration tested (4.94 mg/l).

Short-term exposure (4 days) of the freshwater ostracod *Cypridopsis vidua* O. F. Muller to imidacloprid was carried out in our laboratories. The data was pooled from three different bioassays, which involved four replicates for each of the concentrations tested in accordance with standard guidelines for cladocerans (OECD 1993). The conditions of the assays are described in Sánchez-Bayo and Goka (2006), and the original data set and calculated ET_{50}s were reported in Sánchez-Bayo and Goka (2007). The same experimental conditions were used with toxicity tests of *D. magna* cultures exposed to gradient concentrations of imidacloprid, but in this case post-exposures from 4 to 10 days were used.

Finally, data for seven species of aquatic arthropods exposed to thiacloprid were taken from Beketov and Liess (2008), where the reader can find a detailed description of their experimental conditions. The authors reported the survival of three species of crustaceans (the cladoceran *D. magna*, the isopod *Asellus aquaticus* L. and the amphipod *Gammarus pulex* L.) and four species of aquatic insect larvae (the dragonfly *Sympetrum striolatum* Charpentier, caddisfly *Notidobia ciliaris* L., blackfly *Simulium latigonium* Rubtsov and mosquito *Culex pipiens molestus* Froskal). Exposure durations varied between 17 and 30 days for crustaceans, and 11–15 days for all larval insects so they represent acute toxicity as well as post-exposure effects of a single pulse. As the original data values could not be obtained in this case, survival of each species was estimated from the graphic plots; this procedure introduced an error in the calculation of ET_{50}s, which must therefore be considered as approximate values, but still provided a good data set for the purpose of this paper.

Data analysis

A median effective time to cause 50% mortality of the organisms was calculated for each of the concentrations tried in the bioassays, using a lognormal regression on the above survival data against time of exposure. For survival of *Daphnia* exposed to copper, zinc and selenium, Hoang et al. (2007) provide in Fig. 1 of their paper the equations needed to calculate the ET_{50} values.

To describe the relationship between toxicant concentrations and their corresponding ET_{50}s, a plot of such values on the normal scale revealed a hyperbolic curve that is asymptotic on the *y* axis (Fig. 1a). Indeed, at a concentration of zero the time required to cause 50% effect on a group of organisms is theoretically infinite. In reality, the

asymptote is the no-effect concentration (NEC) as defined by Kooijman et al. (1996). It is also evident that the life span of the organism determines the upper limit of the curve. This type of curve is best described by a power function of the type

$$y = a \times x^{-b} \quad (1)$$

For ease of calculation the coefficients a and b can be obtained by linear regression on the natural logarithm transformed data of both y and x variables (Fig. 1b); in our case the dependent variable (y) was the ET_{50} and the independent variable (x) the external chemical concentration C

$$\ln ET_{50} = a' - b \cdot \ln C \quad \text{where } a' = \ln(a) \quad (2)$$

A characteristic of this type of curve is the existence of a 'buffer' region, close to the origin in Fig. 1a, where small changes in concentration do not translate in ample variations of the corresponding time to effect. However, departures from this narrow region will result in either extended periods to cause an effect when the concentrations are below the critical range, or very little time difference when the concentrations are far beyond this range. This feature cannot be appreciated in the linear plot of Fig. 1b. Equation (2) is similar to the model described by Zhao and Newman (2004), and this point will be discussed below.

Model validation

The above empirical model allows calculation of ET_{50} values for a given toxicant concentration and viceversa. To validate the model, a good fit between the estimated and reported ET_{50}s should be obtained. But because most toxicological data are reported as LC_{50}s rather than ET_{50}s, and this is the case with the data presented here, the estimated ET_{50} values are of no apparent use for this validation. However, this obstacle is overcome when considering that any LC_{50} represents the lethal concentration for 50% of the organisms at a particular time, whereas an ET_{50} is the actual time for the indicated LC_{50}. All that is needed is to match them in time. To this purpose, the hyperbolic model (Sánchez-Bayo and Goka 2007) was used to extrapolate LC_{50} values for the reported times used by the authors for each organism and toxicant considered.

Having done so, the two sets of estimated and reported LC_{50}s could now be compared by a linear regression. As reported LC_{50}s vary on the logarithmic scale, the regression was done on the logarithmic transformed values. Validation could only be performed with the neonicotinoid insecticides, since LC_{50} values for the metals were not reported by the respective authors.

Results

Estimated ET_{50} values for several concentrations of chemical elements tested on *D. magna* and *Poecilia reticulata* are indicated in Table 1. The parameters of the fitted model are indicated in Table 2. In general, the goodness of fit of the model is above 80%, and for selenium $r^2 = 0.998$. For *Daphnia*, a graphic representation of the model (Fig. 2) shows that the regression lines for Zn and $CdCl_2$ are almost parallel, indicating that the rate of uptake of these two toxicants by this organism is very similar: the only difference is the inherent toxicity of each, which is higher for $CdCl_2$ and lower for Zn. The low fit in the case of guppies ($r^2 = 0.796$) may be due to the dilution already mentioned above.

Values of estimated ET_{50}s for the neonicotinoid insecticides thiacloprid and imidacloprid for several species of

Table 1 Estimated times to 50% mortality (ET_{50}) for the waterflea *D. magna* and guppy (*P. reticulata*) after exposure to a gradient of metal concentrations

Species	Chemical	Concentration (μg/l)	ET_{50} (h)
Daphnia magna	Copper[a]	28	782
		32	156
		48	77
		56	48
		64	30
	Zinc[a]	59	3,912[d]
		125	768
		250	47
		500	32
		750	23
	Selenium[a]	158	78
		800	17
		1,200	12
		1,600	9
		2,000	6
	$CdCl_2$[b]	3.2	7,010[d]
		5.6	1,392
		10	901
		18	270
		32	136
		56	45
Poecilia reticulata	Zinc[c]	5,600	23,322
		10,000	12,893
		18,000	6,775
		32,000	141

[a] Hoang et al. (2007)

[b] Kooijman (1981)

[c] Widianarko et al. (2001)

[d] Estimated effect times longer than life span of *D. magna*

Table 2 Parameters of the regression equation $\ln(ET_{50}) = a + b \cdot \ln(C)$ fitted to the data shown in Fig. 2 and Table 1

Species	Chemical	Intercept (a)	Slope (b)	r^2	n
Daphnia magna	$CdCl_2$	3.6×10^4	−1.657	0.982	6
	Copper	3.3×10^7	−3.355	0.893	5
	Selenium	1.1×10^4	−0.969	0.998	5
	Zinc	1.6×10^7	−2.114	0.923	5
Poecilia reticulata	Zinc	8.6×10^{14}	−2.742	0.797	4

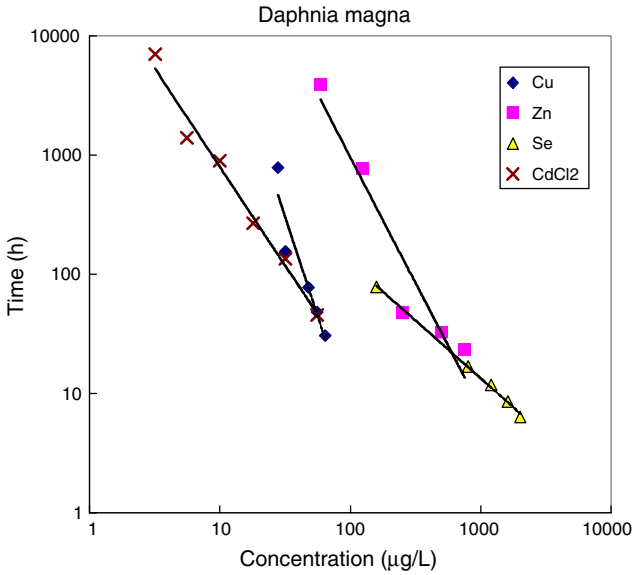


Fig. 2 Time to 50% mortality for *Daphnia magna* exposed to different concentrations of metals. Data sources: Cu, Zn and Se from Hoang et al. (2007); $CdCl_2$ from Kooijman (1981)

aquatic organisms are indicated in Table 3, and the relationship between these values and the corresponding concentrations is shown graphically in Fig. 3. A good fit to the model was obtained, with r^2 values above 0.76 in all cases (Table 4).

Unlike some chemical elements, the regression lines obtained for these organic toxicants are not parallel. This can be interpreted as an indication of different sensitivities among organisms and compounds, because the mode of action of the two insecticides is the same. For instance, *Daphnia* is much more tolerant than *Cypridopsis* when exposed to imidacloprid; the ostracods appear to be not only very sensitive but also have a lower gradient of response than *Daphnia* to this insecticide. Among the arthropods shown in Fig. 3, the amphipod *G. pulex* is the most sensitive species and dragonfly nymphs the least to the insecticide thiacloprid.

Table 5 shows the LC_{50}s reported for the two neonicotinoids tested on these species of aquatic organisms. The corresponding predicted values were estimated using the

Table 3 Estimated times to 50% mortality (ET_{50}) for several aquatic arthropod species after exposure to gradient concentrations of two neonicotinoid insecticides

Species	Insecticide	Concentration (µg/l)	ET_{50} (days)
Cypridopsis vidua	Imidacloprid[a]	4	5.2
		16	3.0
		64	3.3
		250	2.3
		1,000	2.0
		4,000	0.9
Daphnia magna	Imidacloprid[b]	250	384.7[d]
		750	69.7
		2,220	18.6
		6,700	15.0
		20,000	18.4
		60,000	3.0
Gammarus pulex	Thiacloprid[c]	99	63.6
		364	16.7
		988	6.5
		3,100	3.2
		9,520	0.9
Simulium latigonium	Thiacloprid[c]	2.1	23.4
		4.2	11.5
		6.7	12.1
		10.9	1.5
Sympetrum striolatum	Thiacloprid[c]	7.2	20.6
		8.0	17.2
		12.7	13.0
		113.3	3.2

[a] Sánchez-Bayo and Goka (2007)

[b] New data, this paper

[c] Beketov and Liess (2008)

[d] Estimated effect times longer than life span of *D. magna*

hyperbolic model of Sánchez-Bayo and Goka (2007). A regression line on their transformed logarithms indicates a very good fit between the two sets of data ($r^2 = 0.97$), which can also be seen graphically in Fig. 4. This provides a great deal of confidence in the models used: the empirical log-to-log equation describing the relationship between ET_{50} and chemical concentrations as well as the hyperbolic model used to predict the LC_{50}s.

Discussion

The time required for a toxicant to cause a 50% effect in organisms can be easily determined for any concentration of toxicant using data from experimental bioassays. It has been demonstrated in this paper that a simple log-to-log

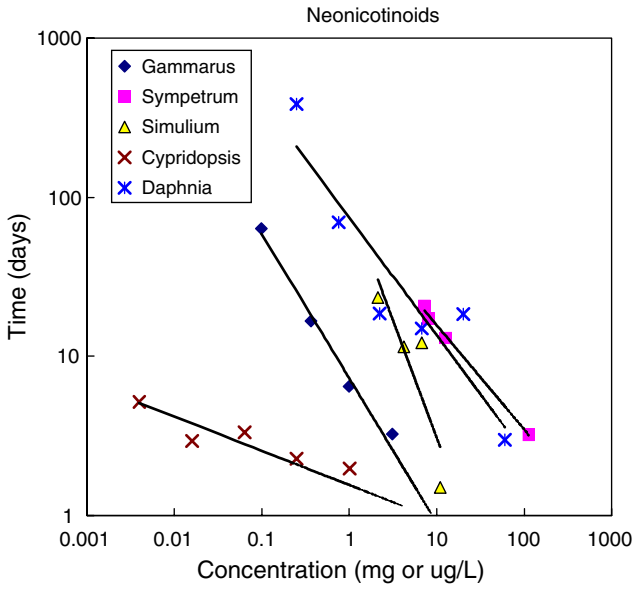


Fig. 3 Time to 50% mortality for several arthropod species exposed to different concentrations of the insecticides imidacloprid (*Cypridopsis vidua* and *Daphnia magna*) and thiacloprid (other species). Concentrations for *Sympetrum* and *Simulium* species are in µg/l; for all other species in mg/l. Data sources: imidacloprid from Sánchez-Bayo and Goka (2006); thiacloprid from Beketov and Liess (2008)

Table 4 Parameters of the regression equation $\ln(ET_{50}) = a + b \cdot \ln(C)$ fitted to the data shown in Fig. 3

Species	Chemical	Intercept (a)	Slope (b)	r^2	n
Cypridopsis vidua	Imidacloprid	1.55	−0.215	0.872	6
Daphnia magna	Imidacloprid	74.44	−0.742	0.863	6
Gammarus pulex	Thiacloprid	7.25	−0.909	0.992	5
Simulium latigonium	Thiacloprid	91.29	−1.476	0.755	4
Sympetrum striolatum	Thiacloprid	70.50	−0.655	0.997	4

relationship (Eq. 2) applies to both variables, the ET_{50} and the external chemical concentration. The specific coefficients of this relationship can be easily derived from time-to-event experimental bioassays, a variant of standard toxicity tests in which effect levels are recorded throughout a period of time (Newman and McCloskey 1996). This straightforward relationship has advantages over other models proposed to date (Table 6), which typically require the calculation of rates and other parameters derived from experimental data. In order to understand the usefulness of this simple approach, its applications and consequences, let us compare it with other models that are being used to describe the effects of toxicants with time.

The first models that examined the influence of time of exposure on the toxic effects on organisms can be attributed to Brown et al. (1969) and Mancini (1983). Basically, these authors aimed at determining the time required to observe a specific level of effect (i.e. percent mortality) after exposure of aquatic organisms to known concentrations of toxicants. For a given concentration of either a single compound or a mixture of compounds, a linear relationship between the percent mortality and the natural logarithm of the time needed to achieve such effect was found. The underlying kinetics of the model are explained by Mancini in terms of uptake and depuration (detoxification) rates in a single compartment, which effectively determine the internal dose responsible for the observed mortality (Connolly 1985). These rates are calculated from the experimental bioassay data, thus introducing additional parameters which are specific for the species and chemicals tested, a feature common to the majority of toxicological models. The bioaccumulation model of Mancini could be employed to calculate the probable mortality of fish from exposure to any time history of toxic concentrations, provided that such concentrations remained constant throughout the period of exposure, which is hardly the case in natural environments.

In order to estimate the mortality due to fluctuating exposures, Breck (1988) introduced the damage–repair model based on the kinetic assumptions of the above bioaccumulation model. In Breck's model, damage to an individual accumulates with exposure time, and death of an individual occurs when the damage level reaches that individual's threshold level. The mathematical expression of his model uses only the concentration levels in the external media and the time of exposure to calculate the percent mortality (Table 6), and also indicates a way to estimate the median time to death (t_{50} or ET_{50}) which is very similar to the Eq. (2) proposed here. The coefficients for such equations could be obtained from experimental data, although their calculation is not as straightforward as it seems. Hoang et al. (2007) have recently produced a model that predicts mortality as a function of the time duration in single or multiple pulse exposures. Their model is based on first order kinetics and introduces a mortality rate constant that is specific for each compound, and a recovery time to predict effect levels (Table 6).

Dixon and Newman (1991) analysed the time-to-death after exposure to heavy metals, and Newman and Aplin (1992) explained the advantages of this approach in ecotoxicology, which subsequently was called time-to-event analysis or TTE (Newman and McCloskey 1996). Further work by Newman and colleagues on this subject has led to a better understanding of the toxic processes in time. The relationships among the three variables of interest (i.e. concentration of toxicant, time of exposure and percent level of effect) are usually analysed by means of regression equations fitted to the experimental data. Typically, the natural logarithms of either variable are used, and besides

Table 5 Comparison of reported median lethal concentrations (LC_{50}) and their 95% confidence intervals (CI) for several aquatic arthropods with their predicted values using the hyperbolic model

Species	Compound	Time (days)	Reported LC_{50} (μg/l)	CI (μg/l)	Predicted LC_{50} (μg/l)
Asellus aquaticus	Thiacloprid[a]	1	>698.5	–	948
		4	299	142.5–627.2	332
		19	153.4	58.8–399.6	102
Culex pipiens	Thiacloprid[a]	1	7.4	NR	9.6
		4	7.1	NR	5.5
		7	5.8	4.5–7.3	4.4
Cypridopsis vidua	Imidacloprid[b]	1	3,951	NR	7,790
		2	391	193–792	310
		4	7.1	2.1–24	12
Daphnia magna	Thiacloprid[a]	1	7,200	NR	5,018
		4	4,400	3,580–5,400	4,553
		14	4,400	3,580–5,400	3,818
		30	4,100	3,430–4,900	3,606
	Imidacloprid	2	64,873	NR	129,315
		10	9,500	NR	14,861
Gammarus pulex	Thiacloprid[a]	1	>9,520.0	–	8,845
		4	580	450–740	1,925
		17	190	170–210	392
Notidobia ciliaris	Thiacloprid[a]	1	7.7	NR	24
		4	7	6.5–7.7	10
		15	6.8	6.1–7.5	4.2
Simulium latigonium	Thiacloprid[a]	1	10.1	NR	21.3
		4	7.8	6.6–9.2	8.3
		11	5.5	3.7–8.2	4.2
Sympetrum striolatum	Thiacloprid[a]	1	>113.3	–	661
		4	47.6	NR	79.8
		11	31.2	23.8–40.8	17

NR = not reported, as CI were not reliable

[a] Beketov and Liess (2008)

[b] Sánchez-Bayo and Goka (2006)

the necessary equation coefficients, other parameters (e.g. body size) that result in better predictions are often included. In essence, their models aim at predicting the proportion of organisms dying at any combination of concentrations and exposure times (Zhao and Newman 2004, 2006), although other effects such as recovery in time can be analysed as well. Response surfaces can be generated to indicate predicted level of effects with time; such levels can be seen graphically whenever the chemical concentration is plotted against the time of exposure (Fig. 5a). The survival model equation of Zhao and Newman (Table 6) is similar to Eq. (2) and to the second equation in Breck's model, the main difference being that the latter two models specifically estimate the median time to death instead of lethality at any other time. Thus, our approach is also very similar to the one undertaken by these authors, as we all intend to predict the effects of varying concentrations of toxicants with time, but the main advantage of our Eq. (2) is that it does not require corrections by error coefficients as indicated by other authors (Newman 1995).

In spite of the obvious applications of the former models for estimation of risks under varying concentrations of either single toxic chemicals or their mixtures in the environment, many authors have adopted a different approach and sought to design mechanistic models based on similar toxicokinetic principles. One such model is PULSETOX (Hickie et al. 1995), specifically designed to deal with pulses. Its authors point out that acute lethality tests are essentially toxicokinetic bioassays where the response of the organism incorporates the accumulation of the critical body residues (CBR) in addition to the time to respond. As in Mancini's bioaccumulation model, the rates of uptake and depuration are estimated first from the experimental data, and then are used as inputs in the model equation in order to estimate the inverse of the median lethal concentration ($1/LC_{50}$) with increasing times of exposure (Table 6). The PULSETOX model assumes that a CBR threshold must be reached for the toxic effects to take place, and that the bioaccumulation rate for a given chemical is constant over time. A more general model, the Dynamic Energy Budget or DEBtox is based on similar

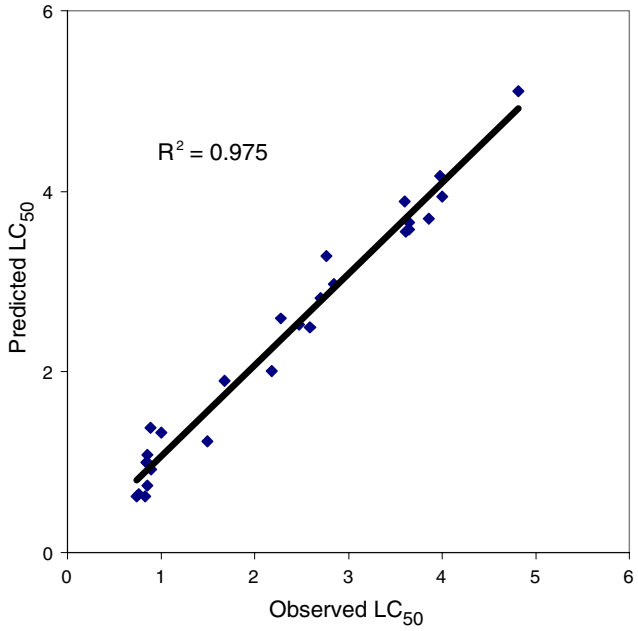


Fig. 4 Validation of the relationship between concentration and time to 50% mortality described in this paper. Predicted LC_{50} values were obtained from their corresponding times to mortality using the hyperbolic model (Sánchez-Bayo and Goka 2007)

kinetic assumptions (Bedaux and Kooijman 1994) and was developed by Kooijman and colleagues (Kooijman and Bedaux 1996; Péry et al. 2002) to estimate endpoints such as LC_{50}, reproduction effects or growth EC_{50} in time. The relationship between effective internal doses and external concentrations is dependent on the chemical characteristics of the compounds, which in their model are represented by the specific bioconcentration factor of each compound (Table 6). However, the application of DEBtox to environmental risk assessment required further development, and Bonnomet et al. (2002) developed a function to estimate LC_{50}s with time of exposure using the fundamental basis of DEBtox. Their model generates an asymptotic curve similar to the one obtained with PULSETOX and our hyperbolic model. In a way, the curve produced by the power Eq. (1) is similar to that of PULSETOX and Bonnomet's asymptotic curves, but with the axes swapped around. Our model is designed to estimate ET_{50} from known concentrations, whereas all other models in Table 6 estimate effective concentrations for known times of exposure—obviously, the opposite term of the equation can be calculated as well.

Another toxicokinetic model was proposed by Yu et al. (1999) to analyse the toxicity of halobenzenes to aquatic organisms with increasing time of exposure. Using the same assumptions on accumulation and uptake of organic compounds explained earlier by Mortimer and Connell (1994), the life expectancy reduction model estimates the internal lethal concentration (IL_{50}) as a function of the time of exposure, and describes it by a linear relationship with the natural logarithm of the median time to death (Table 6). The simplicity of this linear model is evident, but the normal life expectancy of the organisms, which is used as a reference point, may be variable under different environmental conditions, so the applicability of the model in environmental risk assessment may be fraught with difficulties.

One way or another, all these kinetic models enable the prediction of standard median toxicological endpoints (i.e. LC_{50}, LD_{50}, EC_{50}) with time of exposure, a useful but insufficient outcome because in risk assessment of chemicals the prediction of toxicity endpoints other than the median values is also important. After all, the decreasing value of median toxicity endpoints with time can be worked out using existing, conventional toxicity data, as Sánchez-Bayo (2006) has shown for a large number of organic pollutants tested on zooplankton crustacean taxa.

For prediction of different effect levels with time, the approaches of Breck, Newman and colleagues indicated above are preferred because they use directly (i.e. without the need to calculate rates of any kind) the experimental data obtained from toxicity bioassays (Fig. 5a). Using the same approach, I found our hyperbolic model (Sánchez-Bayo and Goka 2007) performed well for predicting the toxicity effects of a variety of compounds and organisms (Fig. 5b). The advantage of the hyperbolic model over any other model indicated above is its mathematical simplicity and the fact that it includes a median toxicity value (either LC_{50} or ET_{50}) as an essential input to generate the response curves. The hyperbolic model is more useful when applied to analysis of toxicity in time, as it can predict any level of effect for a given chemical concentration at different times, in which case only the ET_{50} value is required. However, because experimental data can only generate a handful of datasets, and each concentration is used to estimate a single ET_{50} value, the hyperbolic model cannot predict any other effect values (e.g. LC_x) for concentrations other than those tested. To be able to estimate the latter values for unknown concentrations, the relationship between median effective time and concentration must be known, and Eq. (2) describes this relationship. Thus, the model presented in this paper is complementary to the hyperbolic model. The following example may help understand this point.

Let's imagine that three concentrations of a toxicant, namely C_1, C_2 and C_3, were used in a TTE bioassay with a particular aquatic organism, and that survival or another toxic effect was recorded during 21 days of exposure. A plot of the percent effect of each concentration against time is well described by the hyperbolic model (Fig. 5b), so the response curves based on the calculated ET_{50}s for each concentration (a, b and c respectively) represent the

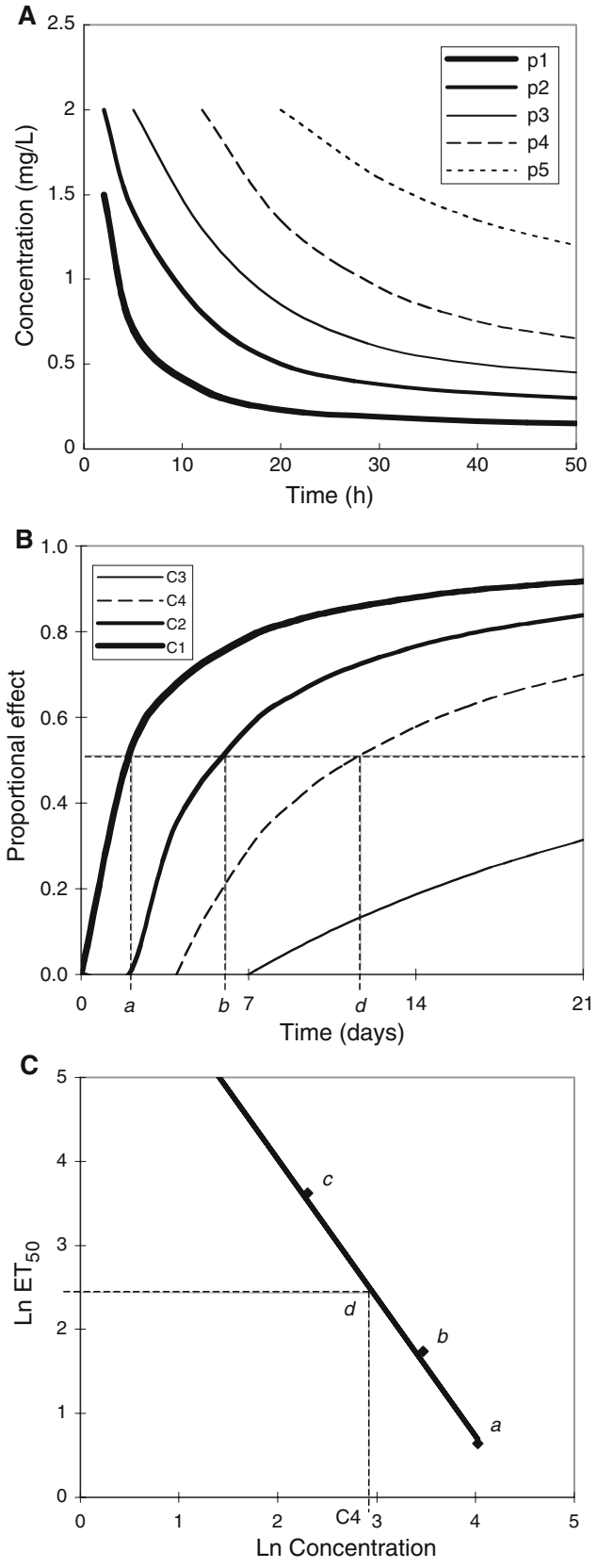
Table 6 Summary of models that allow estimation of toxic effects in organisms with time of exposure (t) to chemical concentrations (C)

Model	Expression(s)	Parameters	Authors
Bioaccumulation	$C_{N(t)}/k_u = C_w/k_r[1-\exp(-k_r t)]$	$C_{N(t)}$ = internal concentration after t exposure C_w = external concentration (media) k_u = toxic uptake rate k_r = detoxification rate	Mancini (1983)
Damage–repair	$P = a + b\ln(C) + d\ln(t)$ $\ln(t_{50}) = (P_{50}-a)/d - y\ln(C)$	P = probit (% mortality) P_{50} = probit (50% mortality) t_{50} = median time to death a, b, d and y = coefficients	Breck (1988)
PULSETOX	$1/LC_{50} = 1/CBR \cdot k_1/k_2(1-e^{-k2t})$	CBR = critical body residue k_1 = uptake rate k_2 = clearance rate	Hickie et al. (1995)
DEBtox	$dc_i/dt(t) = k_e[C_e(t)-c_i(t)]$ $c_i(t) = C_i(t)/BCF$	C_i = internal concentration (tissue) C_e = external concentration (media) c_i = scaled concentration k_e = elimination rate BCF = bioconcentration factor	Kooijman and Bedaux (1996)
Life expectancy reduction	$ILC_{50} = a\ln(LT_{50}/NLT_{50})$	ILC_{50} = internal LC_{50} LT_{50} = exposure time for 50% lethality NLT_{50} = average normal life expectancy of organism a = coefficient (slope)	Yu et al. (1999)
Lethality in time	$C_{org}(t, C) = k \cdot BCF \cdot C \cdot t$ if $t \leq 1/k$ $BCF \cdot C$ if $t \geq 1/k$	C_{org} = internal concentration (organism) k = rate constant BCF = bioconcentration factor	Bonnomet et al. (2002)
Survival model	$\ln T = a - b\ln C + \sigma L$	T = time to death σL = error term a, b = regression coefficients	Zhao and Newman (2004)
Single or multiple pulses	$\ln(M_m) = -K_m t_r + \ln(M_{m0})$	M_m = mortality at exposure time t K_m = mortality rate constant t_r = recovery time M_{m0} = mortality when $t_r = 0$	Hoang et al. (2007)
Hyperbolic	$P = P_{max} \cdot C/(ET_{50} + C)$ *$\ln(ET_{50}) = a + b\ln(C)$	P = % effect P_{max} = maximum effect (i.e. 100%) ET_{50} = median time to effect a, b = regression coefficients	Sánchez-Bayo and Goka (2007) and this paper

* Indicates the reference for the model

predictions for the time series. Now, let's also imagine that the same toxicant was found in certain environmental waters at different concentrations (C_4) from the ones tested, so the hyperbolic model would be unable to predict the effects of C_4. Using Eq. (2), the regression on the known ET_{50}s against their respective concentrations (Fig. 5c) allows us to estimate the ET_{50} for C_4 (d in Fig. 5c), and once d is known the hyperbolic model can now predict any level of effect for the C_4 concentration at any time (dashed line in Fig. 5b).

Application of the hyperbolic model and its complementary Eq. (2) in risk assessment of chemicals is useful in situations where single pulse exposures are the norm. Indeed, the short-term acute toxicity and the latent effects that may take place in prolonged post-exposures can be predicted using the hyperbolic model, while the estimation of the required ET_{50} parameter can be done using the Eq. (2) for any chemical concentration of interest. For scenarios in which repeated pulses occur within a short period, so that there is not sufficient time for recovery, other models such as those proposed by Breck (1988) and Hoang et al. (2007) may be more appropriate. In any case, the relationship between the toxicant concentration in the media and the time for 50% effect on the exposed



◀**Fig. 5** **a** Time-to-event toxicity experiments allow the generation of response surfaces indicating the survival levels (p_x in *legend*) for different concentrations with time of exposure (after Zhao and Newman 2004). **b** The hyperbolic model (Sánchez-Bayo and Goka 2007) produces similar response curves, and for each concentration tested, C_1, C_2 and C_3, a curve (*solid lines*) is generated from its median time-to-death: *a*, *b* and *c*, respectively in the example shown. **c** For any other concentration, the regression equation between the already tested concentrations and their ET_{50}s allows the determination of other median effect values (*d* for C_4), which in turn can be incorporated in the hyperbolic model to produce its own response curve (*dashed line* in **b**)

organisms is a useful one and can be applied interchangeably to extrapolate values between these two variables. Alternatively, the ET_{50}s can be used with any of the other time-models indicated above.

The data used in this paper to validate this model refer to a disparate array of individual chemicals with different modes of action, and yet in all cases the predictions of the model conformed very well to the toxicity endpoints found in the literature consulted (Table 5; Fig. 4). Although this relationship has been shown here for single compounds, there is no objection in principle to its application to mixtures of toxicants as well.

Whether Eq. (2) may be applicable to continuous, chronic exposure at relatively constant concentrations of toxicant is still uncertain, although I have shown above that some data for chronic exposure of *D. magna* (Table 1) fit this model well. The dynamics of uptake, bioaccumulation and depuration of chemicals by the organisms concerned must be taken into account in such cases, as well as the possible adaptation to constant levels of chemical stress (Grant 1998), because all these factors would change substantially the actual time needed for mortality or other toxic effects to be apparent. After all, chronic effects of toxicants are better expressed by sublethal endpoints such as reproduction indicators (Daniels and Allan 1981; Walthall and Stark 1997) and biomarkers of physiological and metabolic changes (Peakall 1992).

Conclusion

The relationship between chemical concentration of a toxicant in the media and the time to produce effects in 50% of the organisms exposed has been described by a log-to-log parametric equation using data for heavy metals and organic toxicants. This simple relationship enables the prediction of the median effective time (ET_{50}) for any chemical concentration in the media, and conversely to estimate the concentration of toxicant needed to cause 50% effect at any time of exposure. Whatever the parameter

sought, this relationship is very useful for risk assessment, as it allows the extrapolation of values that can be used with any of the toxicity models available to predict toxic effects in most environmental situations. Specifically, the estimated ET_{50}s can be used as inputs of the hyperbolic model in order to predict acute and latent effects of toxicants under single pulse exposures.

References

Bedaux JJM, Kooijman SALM (1994) Statistical analysis of bioassays based on hazard modelling. Environ Ecol Stat 1:303–314. doi:10.1007/BF00469427

Beketov MA, Liess M (2008) Acute and delayed effects of the neonicotinoid insecticide thiacloprid on seven freshwater arthropods. Environ Toxicol Chem 27:461–470. doi:10.1897/07-322R.1

Bliss CI (1937) The calculation of the time–mortality curve. Ann Appl Biol 24:815–852

Bonnomet V, Duboudin C, Magaud H, Thybaud E, Vindimian E, Beauzamy B (2002) Modelling explicitly and mechanistically median lethal concentration as a function of time for risk assessment. Environ Toxicol Chem 21:2252–2259. doi:10.1897/1551-5028(2002)021<2252:MEAMML>2.0.CO;2

Breck JE (1988) Relationships among models for acute toxic effects: applications to fluctuating concentrations. Environ Toxicol Chem 7:775–778. doi:10.1897/1552-8618(1988)7[775:RAMFAT]2.0.CO;2

Brent RN, Herricks EE (1998) Postexposure effects of brief cadmium, zinc and phenol exposures on freshwater organisms. Environ Toxicol Chem 17:2091–2099. doi:10.1897/1551-5028(1998)017<2091:PEOBCZ>2.3.CO;2

Brisbin IL Jr (1990) Applications of a modified Richards sigmoid model to assess the uptake and effects of environmental contaminants upon birds. In: Kendall RJ, Larcher TE (eds) Wildlife toxicology and population modeling. Lewis Publishers, Boca Raton, pp 161–170

Brown VM, Jordan DHM, Tiller BA (1969) The acute toxicity to rainbow trout of fluctuating concentrations and mixtures of ammonia, phenol and zinc. J Fish Biol 1:1–9. doi:10.1111/j.1095-8649.1969.tb03837.x

Chaisuksant Y, Yu Q, Connell D (1997) Internal lethal concentrations of halobenzenes with fish (*Gambusia affinis*). Ecotoxicol Environ Saf 37:66–75. doi:10.1006/eesa.1997.1524

Connolly JP (1985) Predicting single-species toxicity in natural water systems. Environ Toxicol Chem 4:573–582. doi:10.1897/1552-8618(1985)4[573:PSTINW]2.0.CO;2

Daniels RE, Allan JD (1981) Life table evaluation of chronic exposure to a pesticide. Can J Fish Aquat Sci 38:485–494. doi:10.1139/f81-070

Dixon PM, Newman MC (1991) Analyzing toxicity data using statistical models for time-to-death: an introduction. In: Newman MC, McIntosh AW (eds) Metal ecotoxicology. Concepts and applications. Lewis Publishers, Chelsea, pp 207–242

Forbes VE, Calow P, Sibly RM (2001) Are current species extrapolation models a good basis for ecological risk assessment? Environ Toxicol Chem 20:442–447. doi:10.1897/1551-5028(2001)020<0442:ACSEMA>2.0.CO;2

Grant A (1998) Population consequences of chronic toxicity: incorporating density dependence into the analysis of life table response experiments. Ecol Model 105:325–335. doi:10.1016/S0304-3800(97)00176-2

Hickie BE, McCarty LS, Dixon DG (1995) A residue-based toxicokinetic model for pulse–exposure toxicity in aquatic systems. Environ Toxicol Chem 14:2187–2197. doi:10.1897/1552-8618(1995)14[2187:ARTMFP]2.0.CO;2

Hoang TC, Tomasso JR, Klaine SJ (2007) An integrated model describing the toxic responses of *Daphnia magna* to pulsed exposures of three metals. Environ Toxicol Chem 26:132–138. doi:10.1897/06-081R.1

Kooijman SALM (1981) Parametric analyses of mortality rates in bioassays. Water Res 15:107–119. doi:10.1016/0043-1354(81)90190-1

Kooijman SALM (2000) Dynamic energy and mass budgets in biological systems. Cambridge University Press, Cambridge

Kooijman SALM, Bedaux JJM (1996) The analysis of aquatic toxicity data. VU University Press, Amsterdam

Kooijman SALM, Bedaux JJM, Slob W (1996) No-effect concentrations as a basis for ecological risk assessment. Risk Anal 16:445–447. doi:10.1111/j.1539-6924.1996.tb01091.x

Mackay D, Puig H, McCarty LS (1992) An equation describing the time course and variability in uptake and toxicity of narcotic chemicals to fish. Environ Toxicol Chem 11:941–951. doi:10.1897/1552-8618(1992)11[941:AEDTTC]2.0.CO;2

Mancini JL (1983) A method for calculating effects on aquatic organisms of time varying concentrations. Water Res 17:1355–1362. doi:10.1016/0043-1354(83)90264-6

McCahon CP, Pascoe D (1990) Episodic pollution: causes, toxicological effects and ecological significance. Funct Ecol 4:375–383. doi:10.2307/2389599

McCarty LS, Mackay D (1993) Enhancing ecotoxicological modeling and assessment. Body residues and modes of toxic action. Environ Sci Technol 27:1719–1728. doi:10.1021/es00046a001

Mortimer MR, Connell DW (1994) Critical internal and aqueous lethal concentrations of chlorobenzenes with the crab *Portunus pelagicus* (L.). Ecotoxicol Environ Saf 28:298–312. doi:10.1006/eesa.1994.1054

Newman MC (1995) Quantitative methods in aquatic ecotoxicology. CRC Publishing, Boca Raton

Newman MC, Aplin MS (1992) Enhancing toxicity data interpretation and prediction of ecological risk with survival time modeling: an illustration using sodium chloride toxicity to mosquitofish (*Gambusia holbrooki*). Aquat Toxicol 23:85–96. doi:10.1016/0166-445X(92)90001-4

Newman MC, McCloskey JT (1996) Time-to-event analyses of ecotoxicity data. Ecotoxicology 5:187–196. doi:10.1007/BF00116339

Organization for Economic Cooperation and Development (1993) OECD guidelines for testing of chemicals. Procedure 202—*Daphnia* sp., Acute immobilisation test and reproduction test. Paris, France

Pascoe D, Shazili NAM (1986) Episodic pollution—a comparison of brief and continuous exposure of rainbow trout to cadmium. Ecotoxicol Environ Saf 12:189–198. doi:10.1016/0147-6513(86)90010-2

Peakall D (1992) Animal biomarkers as pollution indicators. Chapman & Hall, Cornwall

Péry ARR, Flammarion P, Vollat B, Bedaux JJM, Kooijman SALM, Garric J (2002) Using a biology-based model (DEBtox) to analyse bioassays in ecotoxicology: oportunities and recommendations. Environ Toxicol Chem 21:459–465. doi:10.1897/1551-5028(2002)021<0459:UABBMD>2.0.CO;2

Reinert KH, Giddings JM, Judd L (2002) Effects analysis of time-varying or repeated exposures in aquatic ecological risk assessment of agrochemicals. Environ Toxicol Chem 21:1977–1992. doi:10.1897/1551-5028(2002)021<1977:EAOTVO>2.0.CO;2

Sánchez-Bayo F (2006) Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda,

Copepoda and Ostracoda. Environ Pollut 139:385–420. doi:10.1016/j.envpol.2005.06.016

Sánchez-Bayo F, Goka K (2006) Influence of light in acute toxicity bioassays of imidacloprid and zinc pyrithione to zooplankton crustaceans. Aquat Toxicol 78:262–271. doi:10.1016/j.aquatox.2006.03.009

Sánchez-Bayo F, Goka K (2007) Simplified models to analyse time- and dose-dependent responses of populations to toxicants. Ecotoxicology 16:511–523. doi:10.1007/s10646-007-0158-9

Sprague JB (1969) Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity. Water Res 3:793–821. doi:10.1016/0043-1354(69)90050-5

Suter-II GW, Rosen AE, Linder E, Parkhurst DF (1987) Endpoints for responses of fish to chronic exposures. Environ Toxicol Chem 6:793–809. doi:10.1897/1552-8618(1987)6[793:EFROFT]2.0.CO;2

Truhaut R (1977) Ecotoxicology: objectives, principles and perspectives. Ecotoxicol Environ Saf 1:151–173. doi:10.1016/0147-6513(77)90033-1

Walthall WK, Stark JD (1997) A comparison of acute mortality and population growth rate as endpoints of toxicological effect. Ecotoxicol Environ Saf 37:45–52. doi:10.1006/eesa.1997.1521

Widianarko B, Kuntoro FXS, Van Gestel CAM, Van Straalen NV (2001) Toxicokinetics and toxicity of zinc under time-varying exposure in the guppy (*Poecilia reticulata*). Environ Toxicol Chem 20:763–768. doi:10.1897/1551-5028(2001)020<0763:TATOZU>2.0.CO;2

Yu Q, Chaisuksant Y, Connell D (1999) A model for non-specific toxicity with aquatic organisms over relatively long periods of exposure time. Chemosphere 38:909–919. doi:10.1016/S0045-6535(98)00220-3

Zhao Y, Newman MC (2004) Shortcomings of the laboratory-derived median lethal concentration for predicting mortality in field populations: exposure duration and latent mortality. Environ Toxicol Chem 26:2147–2153. doi:10.1897/03-557

Zhao Y, Newman MC (2006) Effects of exposure duration and recovery time during pulsed exposures. Environ Toxicol Chem 25:1298–1304. doi:10.1897/05-341R.1