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Empirical Comparison of Seven two-parameter Sigmoid Equations for the Evaluation of the Concentration-response Curves from Standard Acute Ecotoxicity Assays

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ABSTRACT: The acute ecotoxicity of a set of mixture samples (washing powders, wastes, fuel extracts etc.) was assessed using four acute ecotoxicity assays with aquatic organisms (*Daphnia magna*, *Poecilia reticulata*, *Artemia salina* and *Desmodesmus subspicatus*). The experimental concentration-response curves were fitted by seven two-parameter sigmoid equations using non-linear regression. The regression performance of the equations was compared in three categories (overall fit, mid-range fit, and low-effect fit) using non-parametric statistics. The best overall fit was achieved by Weibüll, Bolztman (i.e. logistic), modified Gompertz and log-Weibüll equations. The best low-effect fit was achieved by a modified Gompertz curve. Those equations transforming concentrations to log c fitted significantly worse than those not transforming them. The obtained EC50 values calculated by all of the equations were comparable to those calculated by the probit model. The results show that regardless of the knowledge of the susceptibility distribution or the mechanisms of toxic action simple two-parameter equations fit the data from acute ecotoxicity assays well and might be used for their evaluation.

Key words: Acute ecotoxicity, Concentration-response curve, Sigmoid curve, Non-linear regression, Susceptibility distribution

INTRODUCTION

Ecotoxicological acute-toxicity assays are widely used for safety purposes, (the risk assessment of chemicals, wastes etc.) among others (Callahan *et al.*, 1994; Gendig *et al.*, 2003; Isnard *et al.*, 2001; Jeram *et al.*, 2005; Oshode *et al.*, 2008; Chibunda, 2009). The primary data from these assays are represented by a concentration-response curve, generally a sigmoid (Bliss, 1935; Finney, 1971; Christensen, 1985; Kalantari and Ghaffari, 2008). The main outputs are the values of NOEC (no observed effect concentration, i.e. the highest tested concentration not affecting the tested population significantly), LOEC (lowest observed effect concentration, i.e. the lowest tested concentration affecting the tested population significantly), EC50 (the concentration affecting half of the tested population), and sometimes analog ECXX values (concentrations affecting XX% of the tested population). While ECXX values are calculated by a regression of the concentration-response curve, the NOEC and LOEC values are determined by statistical hypotheses testing with all of the corresponding negatives (the dependence on the experiment setup, the favoring of poorer experiments, the underestimation of toxicity etc.). In recent years, research effort has been invested in the substitution of the NOEC and LOEC values by adequate regression ECXX values (Maure and Caux, 1997; Isnard *et al.*, 2001).

The first and likely the most commonly used method for fitting the CR curves is the method of probits (Bliss, 1934a, 1934b, 1935; Finney, 1971). However, the integrated normal distribution used for the probit calculation cannot be expressed in analytic form. An alternative to the probit method might be a fitting of CR curve by analytically expressible sigmoid equations. This approach might bring some advantages. A database storing of the equation parameters enables estimations on the toxic effect at

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any concentration. In some cases, the equation parameters might be interpreted in terms of the mechanisms of the interaction between the toxicant and organism (Hill, 1910; Christensen and Chen, 1985; Callahan *et al.* 1994). If the used equation possesses a low number of parameters, then the number of experimental points might be reduced along with the test costs and the lives of the test organisms. Unlike the probit model designed for fitting quantal data (Finney, 1971), simple sigmoid equations might be used for fitting both the quantal and continuous data. Simple sigmoid equations can form also basis for more complicated mathematical models involving e.g. more toxicants (Tichý *et al.* 2002), time factor (Sun *et al.* 1995, Brown and Foureman, 2005), joint induction and toxicity (Trögl *et al.* 2007) etc.

The shape of a CR curve results from the susceptibility distribution among the individuals and the toxic action mechanism. Nevertheless, it is often the case that neither of the two prerequisites is known, especially when assessing the toxicity of complex, poorly-defined mixtures, such as wastes. In these frequent cases, it makes sense to use simple models irrespective of the toxic action mechanisms selected empirically based on the fit quality (Moore and Caux, 1997). Christensen (1984) found the linearized Weibüll model to be better than the probit model. Gendig *et al.* (2003) stated the same for a proposed test of nitrification inhibition. On the contrary, Fowles *et al.* (1999) found the probit model to be better than the Weibüll model for fitting the acute toxicity data of higher organisms. Isnard *et al.* (2001) compared several models in the fitting of low effects and found the Weibüll model to be the best option for such purposes. Moore and Caux (1997) evaluated a set of 198 data sets; the two-parameter logit, probit, and Weibüll models fitted the best in 41.2%, 17.6%, and 41.2% of the cases, respectively. Scholze *et al.* (2001) proposed a general method for selecting the best model for each sample individually. Such an approach is however laborious and not practical in routine analyses.

In this study, we aimed to compare seven simple two-parameter sigmoid equations empirically for fitting the CR curves especially of complex mixture samples from four acute ecotoxicity assays. The fitting ability was assessed in three categories: the overall fit of the whole CR curve, the low-effect fit of up to 20% experimental inhibition (important especially for estimating safety limits), and the mid-range fit in the range of 20%–80% experimental inhibition (important especially for the accurate calculation of EC50 values). The comparison was based on non-parametric statistics using ranks of fit. We have focused on analytically expressible equations and therefore excluded the probit model from the comparison. Nevertheless, the EC50 values and their confidence intervals calculated using the tested equations were compared to those calculated by two variants of the probit model.

MATERIALS & METHODS

All of the experiments were carried out in Empla ecological laboratories (www.empla.cz) accredited in accordance with the EN ISO/EC 17025 standard. The toxicity of potassium dichromate (a standard toxicant) was determined regularly for quality control. The details on the exposure times, number of organisms, replicates etc. are provided in Table 1.The *Daphnia magna* and *Poecilia reticulata* assays were carried out according to European standards (EN ISO 6341 and EN ISO 7346, resp.). The organisms in the defined media were exposed to a concentration series of the toxicant and after the exposure time had elapsed the percentage of affected individuals was calculated. This procedure was followed by a non-standardized *Artemia salina* assay. The *Artemia* cysts (Sanders, Premium quality) were stored in a refrigerator (~10°C) in a tightly sealed plastic bag to avoid water access. Both hatching and toxicity testing proceeded in rested tap water that had been doped with 25 g/L of sodium chloride and whose pH was adjusted to 8 using sodium hydroxide. The cysts were hatched in Erlenmayer flasks under roughly aerated condition at 22–25°C for 24 hours. A Petri dish (with a 60 mm diameter) filled with 10 nauplii in 5 ml of the test solution was used for each experimental point. The tests were carried out in duplicate or quadruplicate. Four to six dishes without toxicant were used as a control. The test was carried out at 22±2 °C under illumination without aeration and feeding. After 48 hours of exposure, the percentage of immobilized individuals was calculated. The *D. subspicatus* assay was carried out according to the EN ISO 8692 standard. The growth curve in mineral medium was monitored for at least 72 hours. The inhibition was calculated as a ratio of the specific growth rates of the affected culture and control (as an average from three replicates) in the exponential growth-phase.

Used nine samples consist of various chemical mixtures (washing powders, wastes, fuel extracts etc.) which Empla customers have assessed for ecotoxicity (EC50 values). Only samples with EC50 < 100 mg/L (the legislative limit for an environmental risk) were used for analyses. This condition was tested in a

Table 1. Ecotoxicity assays: details

Organism	Exposure time [hrs]	Monitored endpoint	Individuals per experimental point	Tested concentrations[a]	Replicates
A. salina	48	Immobilization	10	5-6	2
D. magna	48	Immobilization	10	5	2
D. subspicatus	72 (exponential phase)	Inhibition of growth	N/A	5	1
P. reticulata	96	Lethality	7	5	1

[a]controls not included

preliminary test with a sole concentration of 100 mg/L in triplicate. A further basic test intended for EC50 determination was carried out only when the average effect from the preliminary test exceeded 50%. The water extracts were prepared by mixing the sample with deionized water (1kg dry weight per 10 liters of water) in plastic bottles. The samples were then vertically shaken for 24 hours at 10 rpm and 20°C and filtered through a 5 μm paper filter. The appropriate organism-dependant salts were added to the extract in order to prevent osmotic stress for the organisms and to maintain the mineral composition in accordance with EN ISO 6341, EN ISO 8692 and EN ISO 7346 standards. For *Artemia salina*, 25 g/L of NaCl was added.

The equations used for fitting the CR curves are listed in Table 2. The original or traditional forms of these equations were reformulated so that they would have an EC50 value as one of the two regression parameters. The non-linear regression was calculated using a QC Expert 2.5 (TriloByte statistical software, s.r.o., Pardubice, Czech Republic, www.trilobyte.cz) by the least squares method. Along with other related values, the protocol provided includes 95% confidence (asymptotic) intervals for the regression parameters and value of Akaike information criterion (AIC, Akaike, 1974). The controls without toxicant were excluded from the CR curve calculations.

The probit model was fitted using BioStat 2009 software (AnalystSoft, Inc., Vancouver, Canada, www.analystsoft.com). Two variants, with and without the logarithmic transformation of the concentration, were calculated. The non-parametric statistics were calculated using the Statistica 8.0 (StatSoft, Inc., Tulsa, USA, www.statsoft.com) software package. Overall fit was compared based on the AIC values derived for a mutual comparison of the regression models (Akaike, 1974). The low-effect fit and mid-ranged fit were compared based on the values of the average squares for the experimental points with the measured effect of <20% and 20%–80%, respectively. Comparable values for the tested equations were ranked from 1 (best) to 7 (worst) for further non-parametric comparisons. The average ranks were assigned in the case of equal values. The ranks were compared by Wilcoxon pair comparison (Wilcoxon, 1945) for dependent values (α=0.05). For each tested criterion, the equations were first sorted in ascending order by their average ranks, all of the corresponding Wilcoxon tests were calculated, and the obtained dissimilarity probabilities were arranged in a distance-type matrix. Finally, a cluster analysis was performed in order to delimit the quality groups. The equations were clustered by a single linkage using a matrix of the probabilities. The groups obtained with a calculated distance of >0.75 were considered different.

RESULTS & DISCUSSION

The average ranks of the regression performance of the equations used are summarized in Table 3. For better illustration, the curves for Sample 2 (mixture detergent) from the *D. magna* assay are plotted in Fig. 1.

Overall fit. Based on the AIC values, the best overall-fit of the data from all four assays was achieved by the Weibüll equation (4). Statistically comparable results were however achieved also by the Boltzmann equation (1), modified Gompertz equation (6) and Log-Weibüll equation (5). The Hill equation yielded a significantly worse fit. The worst overall fit was achieved by the Log-Hill (3) and Log-Gompertz (7) equations. When the assays were evaluated, similar results were obtained.

Low-effect fit. The quality of the fit in the range of up to 20% experimental inhibition showed results slightly different from the overall fit. In this case, the tested equations were split into two quality groups. The best fit was achieved by the modified Gompertz equation (6); the other six equations fitted mutually comparably but significantly worse than the modified Gompertz equation. The individual assessment of the *A. salina* and *D. magna* assays followed a similar pattern. Slightly different results were obtained for the *D. subspicatus* assay. The fit of the modified Gompertz

Table 2. Used equations and their brief characterization

No.	Model	Equation	Description
(1)	**Boltzmann sigmoid** (also known as logistic curve)	$I = \frac{1}{1 + e^{\frac{EC50 - c}{m_B}}}$ c ? 0	Popular model with many applications, e.g. neural networks, autocatalytic reactions, growth of tumors, toxicology (Berkson, 1944).
(2)	**Hill equation** (also known as log-logistic1 or logit curve)	$I = \frac{c^{m_H}}{c^{m_H} + EC50^{m_H}}$ c ? 0	Popular model in cancer research, enzymology etc. (Hill, 1910; Melnick and Kohn, 2000). The biological interpretation of the m_H parameter is the rate of the cooperativity of the low-weighed molecules binding on a cooperative polymer.
(3)	"**Log-Hill**" equation (Hill equation with a logarithmic transformation of the concentration)	$I = \frac{\log(c)^{m_{LH}}}{\log(c)^{m_{LH}} + \log(EC50)^{m_{LH}}}$ c > 1	Proposed modification of Equation (2) attempting to improve the fit.
(4)	**Weibüll equation**	$I = 1 - e^{\frac{-c^{mW} \ln 2}{EC50^{m_W}}}$ c ? 0	The Weibüll distribution has many applications (Weibüll, 1951), e.g. failure analyses, survival analyses, or particle-size distribution. The toxicological applications include developmental toxicology (e.g. Santojanni *et al*. 1995) and mixture toxicology (Christensen, 1984; Christensen and Chen, 1985). The parameter m_W influences the shape of the curve (m<1 hyperbolic saturation shape, m>1 sigmoid shape). The biological interpretation of the m_W parameter is the average number of molecules of toxicant per receptor.
(5)	"**Log-Weibüll**" **equation** (the Weibüll equation with a logarithmic transformation of the concentration)	$I = 1 - e^{\frac{-\log(c)^{mLW} \ln 2}{\log(EC50)^{mLW}}}$ c>1	Proposed modification of the Weibüll equation (4) attempting to improve the fit.
(6)	Modified **Gompertz curve**	$I = 2^{-e^{-m_G(c - EC50)}}$ c?0	The original Gompertz curve (Gompertz, 1825) was derived for the distribution of mortality in time and finds related applications e.g. modeling of growth curves (Winsor, 1932). As compared to the original Gompertz curve, our modified curve (6) uses 2 as the first base instead of Euler's e in order to set I(EC50) = 0.5 at the inflection point.
(7)	"**Log-Gompertz**" curve (a modified Gompertz curve) with logarithmic transformation of the concentration)	$I = 2^{-e^{-m_{LG}[\log(c) - \log(EC50)]}}$ c>0	Proposed modification of Equation (6) attempting to improve the fit.

Despite the fact that Eq. (2) does not involve log(c) at first sight, it can be derived from the logarithmic form of Eq. (1) ($I = \frac{1}{1 + e^{\frac{\log(EC50) - \log(c)}{m_B}}}$) after the transformation of $m_H = \log e / m_B$. Its alternative name "log-logistic curve" is therefore deserved, just like its inclusion into the group of equations involving a logarithmic transformation of the concentration.

Table 3. The final comparison of models (average ranks ± standard deviations). The Bold-face indicates the minimal (i.e. the best) values. The lower-case letters indicate equal-quality groups obtained by clustering through employing a matrix of the pair dissimilarity probabilities from the Wilcoxon test. For each criterion, the equations are sorted in ascending order according to the average ranks of the four-assay comparison

Criterion	n							
Overall fit (AIC)		**Weibüll (4)**	**Boltzmann (1)**	**Gompertz (6)**	**Log-Weibüll (7)**	**Hill (2)**	**Log-Hill (3)**	**Log-Gompertz (7)**
All assays	32	**3.03±1.99a**	3.22±2.34a	3.44±1.66a	3.56±1.41a	4.16±0.88b	5.20±1.68c	5.39±2.30c
A. salina	7	2.71±1.70a	**2.43±2.23a**	3.71±1.70a	3.57±1.27a	4.14±0.69a	5.29±1.50b	6.14±2.26c
D. magna	8	3.50±2.45a	4.74±2.32a	3.63±1.06a	**3.00±1.60a**	4.25±1.04a	4.50±2.07a	4.38±2.97a
D. subspicatus	9	3.00±2.15a	**2.87±2.40a**	3.50±1.64a	3.63±0.88a	4.22±1.30a	5.31±1.68b	5.44±2.03b
P. reticulata	8	2.89±1.85a	**2.78±2.07a**	3.00±2.14a	4.00±1.81a	4.00±0.00a	5.68±1.49b	5.67±1.92b
Low-effect fit (the average square up to 20% of experimental inhibition		**Gompertz (6)**	**Hill (2)**	**Boltzmann (1)**	**Log-Gompertz (7)**	**Weibüll (4)**	**Log-Hill (3)**	**Log-Weibüll (5)**
All assays	32	**3.19±1.91a**	3.75±1.08b	3.91±2.53b	3.93±2.48b	4.28±2.07b	4.34±1.89b	4.59±1.46b
A. salina	7	2.86±1.86a	4.29±0.95a	**2.71±2.92a**	4.86±2.27a	3.29±1.38a	5.71±1.80a	4.29±0.95a
D. magna	8	3.50±1.60a	3.50±0.93a	5.25±2.71b	**2.88±2.36a**	5.13±1.64b	3.00±2.00a	4.75±1.28b
D. subspicatus	9	4.00±2.33a	3.44±1.51a	**3.00±2.35a**	5.89±1.24b	3.20±2.80a	4.67±1.66a	3.78±1.30a
P. reticulata	8	2.25±1.54a	3.88±0.64b	4.63±1.51b	**2.00±2.04a**	5.50±1.93c	4.13±1.36b	5.63±1.69c
Mid-range fit (the average square with 20–80% of experimental inhibition)		**Gompertz (6)**	**Weibüll (4)**	**Log-Weibüll (5)**	**Hill (2)**	**Boltzmann (1)**	**Log-Hill (3)**	**Log-Gompertz (7)**
All assays	32	**3.36±1.72a**	3.58±2.35a	3.58±2.35a	4.00±1.13a	4.03±2.35a	4.52±1.81a	4.55±2.64a
A. salina	7	3.71±1.70a	4.14±0.69a	3.57±0.98a	4.14±0.69a	**2.57±2.15a**	5.57±1.62b	6.00±2.23b
D. magna	8	4.13±1.25a	3.38±2.56a	**3.38±1.51a**	4.00±0.93a	4.63±2.39a	4.38±2.00a	4.13±3.09a
D. subspicatus	9	**3.11±1.62a**	4.22±2.45a	4.33±1.12a	3.67±1.73a	4.22±2.59a	4.00±1.94a	4.44±2.60a
P. reticulata	8	**2.43±2.15a**	4.14±2.55a	4.57±2.15a	4.29±0.76a	4.57±1.99a	4.29±1.50a	3.71±2.50a
Three-criteria comparison		**Gompertz (6)**	**Weibüll (4)**	**Boltzmann (1)**	**Hill (2)**	**Log-Weibüll (5)**	**Log-Gompertz (7)**	**Log-Hill (3)**
All assays	96	**3.33±1.75a**	3.63±2.18a	3.72±2.41a	3.97±1.04a	4.04±1.50a	4.63±2.52b	4.69±1.82b
A. salina	21	3.43±1.72a	2.81±1.57a	**2.57±2.34a**	4.19±0.75	3.81±1.08a	5.67±2.22c	5.52±1.57b
D. magna	24	3.75±1.29a	4.00±2.30a	4.88±2.38b	3.92±0.97a	**3.71±1.60a**	3.79±2.78a	3.96±2.05a
D. subspicatus	27	3.63±1.86b	3.44±2.28b	**3.30±2.45a**	3.78±1.50b	3.89±1.12b	5.31±2.06b	4.65±1.77b
P. reticulata	24	**2.43±1.87a**	4.22±2.28b	4.04±1.94b	4.04±0.56b	4.78±1.91b	3.74±2.54b	4.74±1.57b

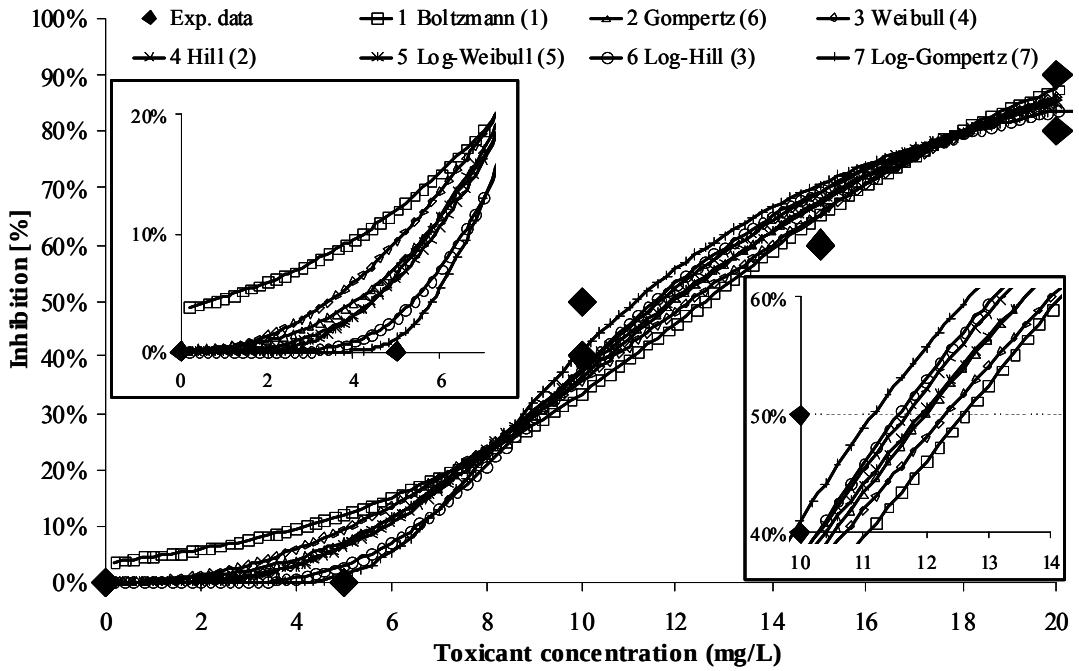

Fig. 1. A visual comparison of all seven curves fitting the primary data (N=10) from the *D. magna* assay for Sample 2 (mixture detergent). The inserts show the details for low-effects and around the EC50 value

equation was comparable to the fit provided by all of the other assays except for the log-Gompertz equation (7). For *P. reticulata*, the differences between the equations were more significant and resulted in their division into three groups. The a-group consisted of the Gompertz (6) and log-Gompertz (7) equations, the b-group of the Hill (2), log-Hill (3) and Boltzmann (1) equations, and the worst group, the c-group, remained for the Weibüll (4) and log-Weibüll (5) equations.
Mid-range fit. The ranks of the mid-range fit of all the tested equations resulted in the mutually most comparable results. The only slight exception was the *A. salina* assay where the Log-Hill (3) and Log-Gompertz (7) equations separated into a significantly worse-fitting group.

Three-criteria comparison. A comparison of the equations based on the aggregated ranks of all of the three criteria mentioned above showed that the best results were obtained using the modified Gompertz curve (6). This is the result of a good overall fit (the third best in the a-group), combined with an excellent low-effect fit (the best and significantly better than the other equations) and midrange-fit (the best, but statistically comparable to the other equations). Similar results were obtained also if assays were assessed individually. The only exception was the *D. subspicatus* assay, for which the ranks of the Boltzmann equation (1) were the best and significantly better than the ranks of the other equations. Nevertheless, even for *D. subspicatus*, the proposed modified Gompertz curve performed well.

The results obtained are in general agreement with the published literature describing a good fit especially by a logistic function (i.e. the Boltzmann equation) and the Weibülll model (Christensen, 1984; Scholze *et al.*, 2001; Gendig *et al.*, 2003). The almost comparable ranks of the mid-range fit suggest that the main differences between the equations are found especially in the low-effect area (comparable to Scholze *et al.*, 2001). The good reported low-effect fit of the Weibüll model (Isnard *et al.*, 2001; Moore and Caux, 1997) was not unambiguously confirmed and varied among the test organisms.

The results of the comparison are summarized in Table 4. When comparing all four assays together, lower average ranks were achieved by those equations not involving a logarithmic transformation of the concentration (the Boltzmann equation (1), Weibüll equation (4) and modified Gompertz equation (6)) than by those involving it (the Hill equation (2), log-Weibüll equation (5), and log-Gompertz equation (7)). Nevertheless, only the comparison based on the overall-fit (AIC ranks) was significant (α=0.05). Similar results were obtained if the data from the *D.* subspicatus, *P. reticulata* and *A. salina* assays were

Table 4. An aggregate Wicoxon comparison of the fit of the models utilizing log c transformation (the Hill's equation, log-Weibüll equation and log-Gompetz equation) to models without such a transformation (the Boltzmann equation, Weibüll equation and log-Gompertz equation). The probabilities in bold-face are significant at α=0.05. The average rank in bold-face is lower (i.e. better fit)

			Average rank ± std. deviation	
Criterion	**n**	**Probability**	**Log c**	**c**
AIC all assays	96	**0.0001**	4.35±1.80	**3.25±2.00**
AIC *A. salina*	21	**0.0457**	4.62±1.86	**2.95±1.88**
AIC *D. magna*	24	0.9772	**3.88±2.05**	3.96±2.03
AIC *D. subspicatus*	27	**0.0475**	4.46±1.67	**3.11±2.06**
AIC *P. reticulata*	24	**0.0177**	4.54±1.61	**2.88±1.94**
Low effect fit all assays	96	0.4214	4.09±1.79	**3.79±2.21**
Low effect fit *A. salina*	21	0.0537	4.48±1.47	**2.95±2.06**
Low effect fit *D. magna*	24	0.2192	**3.71±1.76**	4.63±2.12
Low effect fit *D. subspicatus*	27	0.1529	4.37±1.71	**3.41±2.27**
Low effect fit *P. reticulata*	24	0.8864	**3.83±2.13**	4.13±2.12
Mid-range fit all assays	96	0.1643	4.17±1.87	**3.66±2.15**
Mid range fit *A. salina*	21	**0.0355**	4.57±1.75	**2.90±1.87**
Mid-range fit *D. magna*	24	0.7861	**3.83±1.99**	4.04±2.12
Mid-range fit *D. subspicatus*	27	0.6742	4.15±1.88	**3.85±2.23**
Mid-range fit *P. reticulata*	21	0.4979	4.19±1.89	**3.71±2.33**

Table 5. A comparison of the EC values calculated by the probit model and the tested equations. Two variants (utilizing log c and utilizing c) were compared

Equation	Boltzman n (1)	Hill (2)	Log-Hill (3)	Wibüll (4)	Log-Weibüll (5)	Gompertz (6)	Log-Gompertz (7)
Number of EC50 values lying outside of the 95% confidence interval as calculated by the probit method							
Probit model (log c)	2	1	1	1	1	1	1
Probit model (c)	0	0	1	0	0	0	1
Number of non-overlapping 95% confidence interval							
Probit model (log c)	1	1	1	1	1	1	1
Probit model (c)	0	0	0	0	0	0	0

compared individually. For the *A. salina* assay, the better fit of the non-log equations was most pronounced and significant also in the mid-range fit category. For *D. magna*, on the other hand, the average ranks of the non-log equations in all three categories were higher than those of the log equations, but none of them was significantly higher.

Comparison to probit model. The comparison of the EC50 values calculated by the tested models and the Probit model is summarized in Table 5. With only a few exceptions, the EC50 values calculated by all the tested equations lay within the confidence intervals calculated by the probit models, both with and without a logaritmic transformation of the concentration. The latter cover non-probit EC50 values slightly better than those with log (c) included. A comparison of the overlap of the 95% confidence provided a similar coverage pattern.

Application in routine testing. The presented comparison was aimed especially at an evaluation of the ecotoxicity of the mixture data especially for legislative purposes. Based on the presented results, none of tested equations can be recommended generally. The model choice should take into consideration the assay used and also the desired toxicological value (ECXX). For the calculation of the EC50 values, any of those tested might be generally used. The values calculated by all of the tested equations were comparable to those calculated by the probit models. In addition, the mid-range fit of all the equations was comparable. If the data are poor in the partial-effect experimental points, one of the equations with a good overall fit might be recommended (the Weibüll (4), Bolztmann (1), modified Gompertz (6) or log-Weibüll equation (5)). At low effects, more significant differences between equations were found.

Mathematical remark. A slight problem might occur when using the log-Hill (3) and log-Weibüll (5) equations, as they require c>1. This problem might be simply solved by a transformation of the concentration units, e.g. from mg/L to μg/L.

Susceptibility distribution and toxic action mechanism. The shape of the CR curve depends mainly on the distribution of the susceptibility of the individuals and the toxic action mechanism. Despite the fact that with such undefined mixtures, relatively poor data, and simple equations, the deduction of any of those would be foolhardy, the results obtained can provide some hints or hypotheses.

In the Weibüll (4) and Hill (2) equations, the m-parameter is linked to the steepness of the curve. A simple comparison using a one-factor ANOVA revealed that the m_W and m_H values for the data from the *P. reticulata* assay were significantly higher as compared to the data from the other three assays. However, this difference is likely to have nothing to do with the toxic action mechanisms but is most likely related to the lower number of experimental points, especially those with partial effect. This is a result of a very common minimization of the individuals used in routine tests, especially fish tests (Jeram *et al.* 2005). The m_W-parameter in the Weibüll equation (4) is considered to be an average number of toxicant molecules per receptor (Christensen and Chen, 1985). For *A. salina* and *D. magna*, 80% of these values lie between 1 and 4; i.e. they are biologically possible.

Through a derivation of the CR curve, the susceptibility distribution is obtained. The distributions based on the tested equation resemble the normal distribution; nevertheless, they are all slightly skewed and more or less platykurtic (i.e. negative kurtosis). The log-Hill (3) and log-Gompertz (7) equations, which fitted the data significantly worse than the other equations, have positive skewness as well as significantly negative kurtosis. The better fitting curves have a less negative skewness and less significant kurtosis. Also a comparison of the log c and c-utilizing equations is interesting. From the mathematical point of view, the logarithmic transformation of the concentration blunts the high concentrations. This might be related to the transport, distribution or metabolism of a toxicant in the organism. A better fit of the non-log equations for *A. salina*, *D. subspicatus* and *P. reticulata* therefore suggests that these factors do not play such an important role and the majority of the chemicals in the tested mixtures act immediately. However, a detailed investigation of the susceptibility distribution as well as the toxic action mechanisms is beyond the scope of this work and would require significantly more abundant data.

CONCLUSION

The results show that regardless of the knowledge of the susceptibility distribution or the toxic action mechanisms simple two-parameter equations fit the concentration-response curves of both the quantal data and continuous data well. Such an approach can hence be used both for EC50 calculations and for estimations of low-effects. The quality of fit analyses can also provide hints about the toxic action mechanisms or the susceptibility distribution.

Abbreviations:

ECXX – Concentration of the toxicant affecting XX% of the tested population

AIC – Akaike information criterion

CR – Concentration-response

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