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Quantifying animal size effects on toxicity: a general approach

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Abstract

A general approach is described for quantifying size-dependent, toxicant impact. This approach is less restrictive than the commonly used approach developed by Bliss (1936), and its generality allows more effective model generation. A range of response metameters (rate of toxic action, time-to-death, ln time-to-death) and covariate (body size and toxicant concentration) transformations are assessed using an aquatic species. The best model for the error term (ϵ) can be chosen from the normal, log normal, log logistic, Weibull, and gamma distributions. Exposure survivors and assignment of times-to-death to intervals are accommodated easily with this maximum likelihood method.

Key words: Toxicity; Body size; Modeling; Statistics; Fish body size

1. Introduction

Animal size can have a significant effect on toxicant impact. Consequently, quantification of size effects was an important, early goal in toxicology. Indeed, the approach used today was formulated in the 1930s by Bliss (1936).

To develop his approach, Bliss (1936) reanalyzed Campbell's (1926) times-to-death (TTD) data for silkworm larvae given various dosages (mg/g body wt.) of arsenate. Times-to-death were transformed to rates of toxic action (1000/TTD) to equalize variances between groups of individuals receiving different dosages, thus permitting groups of individuals receiving different dosages to be pooled in subsequent analyses. Next, linearizing functions of dose and animal weight were sought so that multiple, least-squares regression methods could be applied. The log dose (log mg/individual) was selected as the dose metameter. The log body weight was used as the simplest, linearizing transformation of size. At each step in this process, data were plotted and their patterns judged visually to be normally distributed about the predicted lines.

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More explicitly, the method of Bliss involves transformation of TTD, dose, and animal size prior to least-squares, linear regression (Eq. 1):

$$\hat{y} = a + b_1 \log m - b_2 \log w + \varepsilon \quad (1)$$

where \hat{y} = rate of toxic action (1000/TTD),

m = mass of toxicant administered (mg),

w = larval wt. (g),

a , b_1 , b_2 = regression constants, and

ε = the model error term (normal distribution assumed).

Although this is the currently accepted model (e.g., Hedtke et al., 1982), other size-toxic effect relationships exist (Lamanna et al., 1955) and it is unlikely that all data sets are best fit with Eq. 1. In fact, Pallotta et al. (1962), and Lamanna and Hart (1968) cautioned against general application of any model such as Eq. 1.

There are sound reasons for exploring other data transformations. Bliss's original transformation of TTD to rates was based on the variance structure of a specific data set and may not be generally warranted. Equally plausible transformations should be assessed such as TTD vs. the probit (of cumulative mortality), and log TTD vs. the probit (Bliss, 1937). Indeed, the log TTD vs. the probit was used in the classic method of Litchfield (1949) for analyzing time-mortality data. Further, there is no a priori reason to reject other metameters such as the logit (Berkson, 1951) or Weibull (Pinder et al., 1978) transformations of cumulative mortality.

There are also statistical concerns that argue for a more general approach. Firstly, a major objective of Bliss's transformations was to produce an easily-computed, linear regression model with a normal distribution of errors. Although an important goal in 1936, it is trivial given the present ease of executing more involved computations. Secondly, there were no survivors in the silkworm data used by Bliss to develop his approach. To be of general use, the methods advocated by Bliss must be modified to accommodate survivors (right-censoring). This can be accomplished by using appropriate, maximum likelihood methods instead of least-squares regression methods. Finally, the raw data used by Bliss (1936) were originally collected by Campbell (1926) who monitored each larva continuously and recorded its moment of death. In contrast, many TTD data sets record the interval within which death occurred, e.g., between 24 and 30 h of exposure. Such interval-censored data are not optimally modeled using Bliss's approach. Use of Bliss's approach would bias such data upward by assigning the time at the end of the sampling interval as the TTD for all individuals dying within the interval.

For the reasons described above, the present reliance on least-squares fitting to Eq. 1 for modeling size-dependent mortality can result in suboptimal utilization of data. Our purpose herein is to provide a more general, statistically-sound approach

for quantifying size-dependence of toxic impact which includes Bliss's model as a special case. This maximum likelihood approach accommodates survivors and interval censoring if present. Times-to-death, ln TTD or rate of toxic action can be examined as potential response metameters. A variety of transformations of body size and exposure concentration (or dose) can also be examined. Finally, description of the model error term (ϵ) is not restricted to the normal distribution as done in the Bliss's approach. The normal, log normal, log logistic, Weibull and gamma functions can be examined for optimal description of ϵ .

2. Materials and Methods

Fish collection and maintenance

Eastern mosquitofish (*Gambusia holbrooki*, Girard 1859) were collected in June and July 1992 from Risher Pond, an abandoned farm pond on the US Department of Energy's Savannah River Site near Aiken, SC. This population of mosquitofish has been used in past toxicological studies (Diamond et al., 1989; Newman et al., 1989; Newman and Aplin, 1992) and, to our knowledge, has never experienced significant exposure to contaminants. Water temperatures ranged from 28 to 30°C during the sampling period.

Fish were maintained at 22°C in a 520 l Living Stream™ tank (Model LSW-700, Frigid Units, Inc., Toledo, OH) containing water from the nearby Upper Three Runs Creek (UTR). During the first week of captivity, fish were treated daily with Maracyn-Two™ (Mardel Laboratories, Inc., Glendale Heights, IL) and methylene blue to prevent bacterial and fungal infections. Methylene blue treatments were continued throughout the duration of captivity. Fish were fed twice daily with Tetramin® tropical fish food.

Sodium chloride exposure

An Enviro-tox™ proportional dilutor (Specialized Environmental Equipment, Inc., Easley, SC) was used for exposures. Following 1 to 3 weeks of captivity, 15 to 20 fish were randomly assigned to each of 14, 10.0-l tanks filled with UTR water. Water temperature was maintained at 21°C. Fish were exposed to nominal concentrations of 0.0, 9.7, 9.9, 10.1, 10.6, 11.5, and 13.0 g NaCl/l (Fisher Chemical, Fair Lawn, NJ) after a 24 h acclimation period. The salt solutions were delivered to seven pairs of tanks at 24 l/tank/day. Measured concentrations of sodium and chloride for each tank were summed to define sodium chloride concentrations in the survival time models.

Tanks were checked for mortality at 4 to 8 h intervals. A fish was scored as dead if no signs of ventilation or other movement were discernable after gentle prodding. Wet weight was taken for each fish at death or at the end of the exposure period. These procedures were repeated for three exposures, each using 208 to 280 fish of an appropriate size range (Table 1). To minimize cannibalism, fish from either size extreme were not exposed together. Only juveniles and females were tested.

In addition to the three data sets generated as described above, the data of Newman and Aplin (1992) were also reanalyzed. The exposure conditions were generally the

Table 1
Summary of size (g wet weight) data for mosquitofish

Exposure	<i>n</i>	Mean (SD)	Minimum	Maximum
Newman and Aplin	401	0.135 (0.156)	0.024	1.489
1	280	0.016 (0.006)	0.005	0.047
2	278	0.091 (0.039)	0.033	0.223
3	208	0.516 (0.330)	0.108	1.985

Data for mosquitofish similarly exposed by Newman and Aplin (1992) are also provided. All fish were juveniles or females except for those exposed by Newman and Aplin (1992). Exposure 1 included only juveniles and exposure 3 included only mature females. Newman and Aplin (1992) included males in their exposure.

same as those described here except a wider range of salt concentrations was used (10.3 to 20.1 g NaCl/l). Fish sizes used in this earlier exposure are summarized in Table 1.

Water chemistry

Daily measurements of temperature and dissolved oxygen were made with a Hydrolab Surveyor II™ (Hydrolab Corporation, Austin, TX) (Table 2). Samples for pH, total alkalinity, sulfate, chloride, magnesium, calcium, potassium, and sodium were taken daily. Except for procedures for pH and total alkalinity, details of water quality methods are outlined in Diamond et al. (1989) and Newman et al. (1989). Total alkalinity (potentiometric titration) and pH were measured with a Radiometer ABU91 autoburette (Radiometer America, Inc., Westlake, OH) immediately after sampling.

Modeling

Survival time models were fit by maximum likelihood methods using the SAS procedure LIFEREG (SAS, 1985; 1988). Detailed presentations of these methods can be found in Miller (1981), Cox and Oakes (1984), and Harrell (1988). Dixon and Newman (1991), and Newman and Aplin (1992) have recently advocated their use in aquatic toxicology. These models have two common forms: proportional hazards and accelerated failure time. The proportional hazards model is a type of accelerated failure time model (Dixon and Newman, 1991). The general form of an accelerated failure time model is the following:

$$\ln \text{TTD} = f(x) + \varepsilon \quad (2)$$

where ε = an error term with an assumed distribution that describes the variability

Table 2
Water quality in experimental exposures

Variable/exposure	Mean	Standard deviation	n
Temperature (°C)			
Exposure 1	21.0	0.4	56
Exposure 2	21.0	0.5	56
Exposure 3	21.4	0.8	56
Newman and Aplin	20.4	0.8	138
Dissolved oxygen (mg/l)			
Exposure 1	8.4	0.3	56
Exposure 2	8.0	0.2	56
Exposure 3	7.0	0.9	56
Newman and Aplin	6.1	0.3	136
pH (median, range)			
Exposure 1	6.38	5.96-6.81	56
Exposure 2	6.35	6.19-6.66	56
Exposure 3	6.28	5.84-6.52	56
Newman and Aplin	6.21	6.07-6.43	28
Total alkalinity (mg CaCO₃/l)			
Exposure 1	5.8	1.0	56
Exposure 2	8.0	0.4	56
Exposure 3	7.9	1.0	56
Newman and Aplin	11.0	0.9	28
Calcium (mg/l)			
Exposure 1	2.9	0.3	56
Exposure 2	2.3	0.4	56
Exposure 3	2.5	0.2	56
Newman and Aplin	3.5	1.2	27
Magnesium (mg/l)			
Exposure 1	0.3	< 0.1	56
Exposure 2	0.3	< 0.1	56
Exposure 3	0.3	< 0.1	56
Newman and Aplin	0.6	0.1	28
Potassium (mg/l)			
Exposure 1	0.6	0.1	56
Exposure 2	0.6	0.1	56
Exposure 3	0.6	0.1	56
Newman and Aplin	0.8	0.2	27
Sulfate (mg/l)			
Exposure 1	2.0	< 1.0	8
Exposure 2	1.4	0.5	8
Exposure 3	1.8	1.4	8
Newman and Aplin	-	-	-

See Materials and Methods for details. Because the chloride peak obscured that for sulfate during chromatography, sulfate concentrations were measured only in control tanks.

between individuals (not associated with the covariate effects), and $f(x)$ = a function describing the effect of covariate x on \ln TTD.

An accelerated failure model (Eq. 2) describes the influence of a covariate such as salt concentration or size on the \ln TTD. The term, $f(x)$ adjusts ('accelerates') \ln TTD among individuals associated with different levels of x . A variety of functions can be used for $f(x)$. The following linear models (Eqs. 3 to 6) were used, although more complex models can readily be assessed with this approach (Dixon and Newman, 1991).

$$f(\text{NaCl, wt.}) = a + b_s[\text{NaCl}] + b_w \text{wt.} \quad (3)$$

$$f(\text{NaCl, wt.}) = a + b_s \log [\text{NaCl}] + b_w \log \text{wt.} \quad (4)$$

$$f(\text{NaCl, wt.}) = a + b_s \log [\text{NaCl}] + b_w \text{wt.} \quad (5)$$

$$f(\text{NaCl, wt.}) = a + b_s[\text{NaCl}] + b_w \log \text{wt.} \quad (6)$$

Consistent with Bliss's approach, the $f(\text{NaCl, wt.})$ described by Eq. 4 fits most of these data best. Consequently, only models generated with \log transformed covariates (Eq. 7) will be discussed in detail. (To be consistent with Bliss (1936), logarithms for the base 10 were used to transform body weight and salt concentration. However, TTD were transformed in model development using the natural logarithm.) Combining Eqs. 2 and 4:

$$\ln \text{TTD} = a + b_s \log [\text{NaCl}] + b_w \log \text{wt.} + \varepsilon \quad (7)$$

Five distributions (normal, log normal, Weibull, gamma, and log logistic) were used to describe the error term, ε . Use of the normal distribution was consistent with Bliss's (1937) suggested use of TTD vs. the probit of cumulative mortality for time-response curves. (When the normal distribution was used, \ln TTD was replaced by TTD in Eq. 7). Similarly, use of the log normal distribution was consistent with Bliss's (1937) suggested use of the log of TTD vs. the probit metameter. The Weibull distribution was recommended by Pinder et al. (1978) for general description of survival curves. The gamma function is another generalized exponential function similar to the Weibull. The comparison of the log logistic distribution to the log normal distribution is analogous to the comparison of the probit and logit transformations for dose-response curves. These five distributions were examined for optimal fit to data from the three exposures and to data from Newman and Aplin (1992). These data differed from each other in the ranges of animal sizes and toxicant concentrations used.

Rates of toxic action were also fit by maximum likelihood methods with SAS LIFEREG. The normal distribution was assumed for ε in this model as per Bliss (1936).

$$\text{Rate} = a + b_s \log [\text{NaCl}] + b_w \log \text{wt.} + \varepsilon \quad (8)$$

Because time intervals were large (4 to 8 h) relative to the total exposure duration (96 h), the times at the beginning and end of each sampling interval were used as the lower and upper limits for TTD of any individual dying within that interval (Dixon and Newman, 1991). Survivors were assigned lower limits of TTD equal to 96 h but no upper limit for TTD. Newman and Aplin (1992) did not use interval censoring methods to analyze these data in their original article.

The relative effectiveness of candidate models for fitting these data was compared with the log likelihood statistic. However, it was not used directly because the number of estimated parameters differed between models. (Five parameters for the gamma model and four for the other models.) Instead, Akaike's Information Criterion (AIC) (Atkinson, 1980; Harrell, 1988) was used to adjust the log likelihood statistics to account for differences in model complexity. The model best fitting the data had the smallest AIC:

$$\text{AIC} = -2(\log \text{likelihood}) + 2P \quad (9)$$

where P = the number of estimated parameters.

3. Results

Log transformations of wet weight and salt concentration provided the best fit to the model. Consequently, Eqs. 7 and 8 were used to examine the candidate distributions for ϵ . However, adequate fits were obtained with other combinations of the covariates and their transformations. For example, the Weibull-TTD model fit to the Newman and Aplin data without covariate transformations yielded an AIC (1024) almost as small as those of the best models using transformed covariates (log normal, log logistic, and gamma models, Fig. 1D).

The AIC values (Fig. 1A and B) indicated that the maximum likelihood models for rate of toxic action (model 6 in Fig. 1) best fit the data from exposures 1 and 2, exposures with the narrowest ranges of fish weight. However, the TTD models with $\epsilon = \log$ normal or gamma had AIC values only slightly higher than that of the rate of toxic action model for exposure 2. These exposures included very small (mean wet wt. = 0.016 g for exposure 1) or medium fish (mean wt. = 0.091) varying in size by approx. 7 (exposure 2) to 9 fold (exposure 1). In contrast, the AIC values for models of exposure 3 (Fig. 1C) and the Newman and Aplin (Fig. 1D) data indicated that the rate of toxic action model provided the worst fit. The log normal-, log logistic- and gamma-TTD models fit the Newman and Aplin data best. These data were generated with mosquitofish exposed to a much wider range of salinities (10.3 to 20.1 g NaCl/l) than those used in the present study (9.7 to 13.0 g NaCl/l). Also, the range in body weights (approx. 60-fold) was much larger than those for exposures 1, 2 and 3. The Weibull, log logistic, and gamma models had the lowest AIC values for exposure 3 data (Fig. 1C). However, the associated b_w values for these models had relatively large P values of 0.01, 0.02, and 0.02, respectively. Although the b_w values were significant ($\alpha = 0.05$), the levels of significance were inferior to those obtained with the other

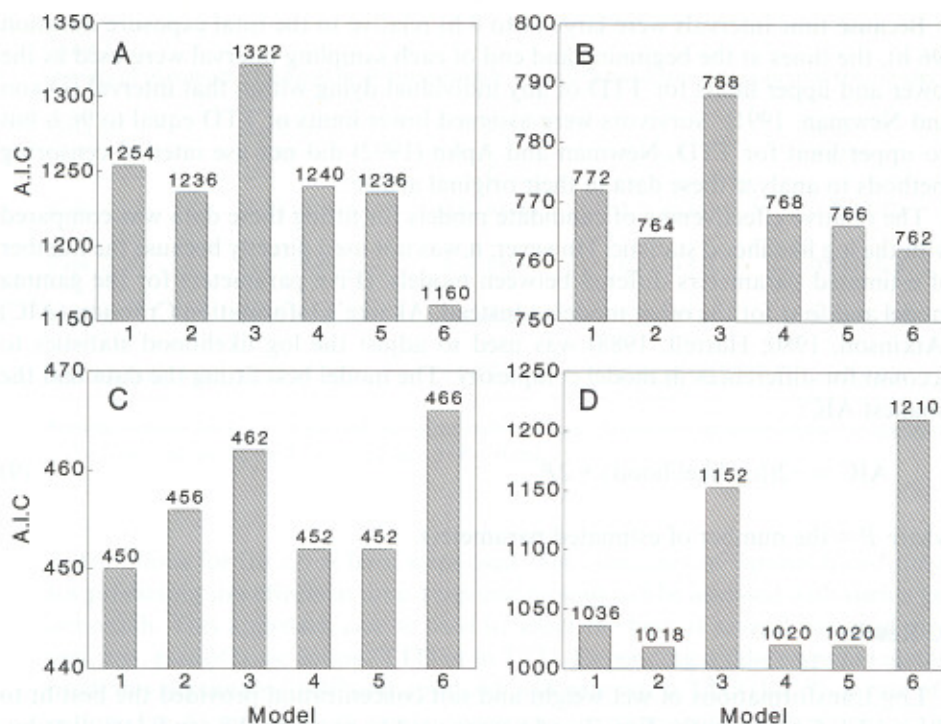


Fig. 1. Akaike's Information Criterion (AIC) values for exposures 1 (panel A), 2 (panel B) and 3 (panel C), and data from Newman and Aplin (1992) (panel D). Model 1: TTD, ϵ = Weibull; Model 2: TTD, ϵ = log normal; Model 3: TTD, ϵ = normal; Model 4: TTD, ϵ = log logistic; Model 5: TTD, ϵ = gamma; Model 6: rate of toxic action, ϵ = normal. AIC value is given above the bar for each model.

three data sets ($P < 0.001$). The relatively large mosquitofish in exposure 3 varied in size by approx. 18-fold.

Of the TTD models, the log normal model produced the lowest AIC for all data sets except exposure 3 data (Fig. 1C). Consequently, regression estimates for the log normal-TTD models are provided in Table 3 as illustrative of TTD model results. Alternatively, rate of toxic action models (Eq. 8, Table 4) generated with censored data sets could be used to quantify size effects. These rate of toxic action models had AIC values lower than the best TTD models for two (exposures 1 and 2) of the four data sets.

4. Discussion

4.1. Time-to-death and rate of toxic action models

We compared the fit to a time-response model having log transformed weight and toxicant concentrations when different transformations of the response variable and

Table 3
Results of maximum likelihood fit of mosquitofish TTD data to a log normal, survival time model

Data set	Intercept a (SE)	b_s (SE)	b_w (SE)	Scale σ (SE)
Expt. 1	10.74 (0.56)	-3.97 (0.46)	1.51 (0.14)	0.35 (0.02)
Expt. 2	11.33 (0.44)	-6.26 (0.39)	0.37 (0.11)	0.24 (0.02)
Expt. 3	12.61 (0.90)	-7.52 (0.84)	0.25 (0.14)	0.38 (0.04)
Newman and Aplin	19.28 (0.58)	-13.02 (0.46)	0.99 (0.14)	0.50 (0.03)

Standard errors are given in parentheses after each estimate. All parameter estimates were significant with a $P > 0.001$ except for the b_w for exposure 3 ($P = 0.06$).

distributions for the error term were used. The adequacy of various transformations for predicting size effects in time-response models can be assessed with such a general approach. This approach can be used to select the best of several response metameters, e.g., rate of toxic action, TTD or \ln TTD. Depending on the response metameter used, this maximum likelihood approach will generate a TTD (Eq. 7) or rate of toxic action (Eq. 8) model. Both model forms can accommodate death scored within an interval (interval censoring) and survivors (right censoring). This approach also facilitates selection of the optimal transformations of animal size and toxicant concentration. Finally, the best fit for ϵ may be chosen from several common distributions.

Although a variety of functions may be examined for ϵ in either model type, only the normal distribution was used for the rate of toxic action model (Eq. 8). This is consistent with the assumption of a normal distribution in Bliss's original approach (Bliss, 1936). The rate of toxic action model used log body weight and log toxicant

Table 4
Results of maximum likelihood fit of mosquitofish rate of toxic action data to a model assuming a normal distribution for ϵ

Data set	Intercept a (SE)	b_s (SE)	b_w (SE)	Scale σ (SE)
Expt. 1	-130 (13)	90 (10)	-33.0 (3.1)	7.7 (0.4)
Expt. 2	-94 (6)	96 (6)	-5.6 (1.7)	3.7 (0.3)
Expt. 3	-121 (15)	122 (14)	-4.7 (2.3)	6.4 (0.6)
Newman and Aplin	-697 (28)	610 (21)	-48.1 (7.2)	24.5 (1.3)

Standard errors are given in parentheses after each estimate. All estimates were significant with a $P > 0.001$ except for the b_w for exposure 3 ($P = 0.04$).

concentration as per Bliss (1936). The predicted median rate of toxic action may be estimated as illustrated below with the exposure 2 model assuming an exposure concentration of 12 g NaCl/l and an average fish size of 0.1 g wet weight.

$$\begin{aligned} \text{Median rate} &= -94 + 96 * (\log 12 \text{ g/l}) - 5.6 * (\log 0.1 \text{ g}) + 3.7 * 0 \\ &= 15.2 \text{ in units of } 1000/\text{TTD}. \end{aligned}$$

The TTD corresponding with this rate would be approx. 67 h. The second model form uses \ln TTD (or TTD) as the effect metameter, log transformations of the covariates, and one of several candidate distributions for ϵ (Eq. 7). A median TTD (MTTD) can be estimated using the model assuming a log normal distribution for ϵ generated for the exposure 2 data (Table 3). (See Dixon and Newman, 1991; Newman and Aplin 1992 for details.)

$$\begin{aligned} \text{MTTD} &= e^{11.33} e^{-6.26(\log 12 \text{ g/l}) + 0.37(\log 0.1 \text{ g})} e^{0.24 * 0} \\ &= 67 \text{ h} \end{aligned}$$

This estimated MTTD is the same as that generated with the rate of toxic action model.

Alternative transformations may be explored using the general approach described above. Interval censoring can be ignored if sampling intervals are sufficiently short relative to the exposure duration. Indeed, this was the approach taken by Newman and Aplin (1992) to illustrate the use of the survival time model in aquatic toxicology. They also selected a Weibull distribution for ϵ and did not transform body size or salt concentration. Predicted MTTD for these models (12 g NaCl/l and 0.1 g wet wt.) were slightly higher than those from the log normal model (Table 3).

Log normal distribution for ϵ and log transformed covariates (Table 3):

$$\begin{aligned} \text{MTTD} &= e^{19.28} e^{-13.02 * (\log 12 \text{ g/l}) + 0.99 * (\log 0.1 \text{ g})} e^{0.50 * 0} \\ &= 69 \text{ h} \end{aligned}$$

Weibull distribution for ϵ and untransformed covariates (interval censored):

$$\begin{aligned} \text{MTTD} &= e^{9.08} e^{-0.40 * (12 \text{ g/l}) + 1.95 * (0.1 \text{ g})} e^{0.43 * (-0.3665)} \\ &= 75 \text{ h} \end{aligned}$$

Weibull distribution for ϵ and untransformed covariates (no interval censoring):

$$\begin{aligned} \text{MTTD} &= e^{7.86} e^{-0.30 * (12 \text{ g/l}) + 1.06 * (0.1 \text{ g})} e^{0.30 * (-0.3665)} \\ &= 71 \text{ h} \end{aligned}$$

4.2. Dose-response models

As detailed in Bliss (1935), time-response models can be linked directly to dose-response models. Anderson and Weber's (1975) extension of Bliss's approach is an

excellent example of such linkage. As illustrated by Dixon and Newman (1991), and Newman and Aplin (1992), 96 h LC_{50} estimates can be produced with these TTD and rate of toxic action models. The conclusions drawn here regarding time–response data may be applied to dose– or concentration–response models. Consequently, there is no a priori reason to use the log transformations of body size or concentration in concentration–response model formulation. Other covariate transformations should be examined. Further, the recommended probit model (log concentration vs. probit) should be compared to other candidate models such as the logit (Berkson, 1951) or Weibull (Christensen, 1984; Christensen and Nyholm, 1984) models prior to final model selection.

5. Summary

The currently used methods first described by Bliss (1936) are too restrictive for general application in aquatic toxicology. They limit the user to a specific response metameter, the rate of toxic action. Repeated application of Bliss's (1936) approach has also fostered the uncritical use of log transformations of dose (or concentration) and body size. Although application of the rate of toxic action and log transformed covariates is a reasonable recommendation, the resulting model should not be used without consideration of equally viable models. For example, uncritical application of Bliss's model to the Newman and Aplin data would have produced a suboptimal model. In contrast to Bliss' (1936) approach, the methods recommended here allow comparison of a wide range of models and transformations including those constituting the Bliss approach. They also permit effective incorporation of censoring during model generation.

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