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Hazard/Risk Assessment

MODELING DOSE RESPONSE USING GENERALIZED LINEAR MODELS

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Abstract—This paper describes a method to determine and model dose–response relationships from binomial response data using generalized linear models (GLM). The main advantage of this technique is that it allows LC_p or LD_p to be determined without an initial linearizing transformation. (LC_p and LD_p are the lethal concentration or dose that causes p proportion of test animals to die at a specified time period.) Thus, the method of GLM is an appropriate way to analyze a dose–response relationship because it utilizes the inherent S-shaped feature of the toxicologic response and incorporates the sample size of each trial in parameter estimation. This method is also much better behaved when the extremes of the response probability are considered because responses of 0% and 100% are included in the model. Another advantageous feature of this method is that confidence intervals (C.I.s) for both the dose estimate and response probabilities can be computed with GLM, which provides a more complete description of the estimates and their inherent uncertainty. Because C.I.s for both the dose estimate and response probabilities can be constructed, the lowest observed effect concentration (LOEC) can also be determined.

Keywords—Dose response LC50 Generalized linear models Toxicity testing LOEC

INTRODUCTION

Toxicity bioassays are commonly used in environmental studies to evaluate the toxicity of contaminants in water, food, and soil/sediment. A series of different concentrations or doses are established and a group of organisms is exposed to each level for a given time period. The number of organisms that respond is a binomial random variable with known sample size and unknown probability p. With these data an LC_p or LD_p is calculated to characterize the response (henceforth referred to as LC_p for simplicity). The standard methods used to assess the properties of the dose–response relationship are often not the best way to achieve the desired results, whereas the generalized linear models (GLM) described in this article offer an accurate representation of the data. The goal of our investigation was to find a model that more faithfully predicted p at various levels of concentration and was able to include data from the extremes (0% and 100% response). The concentration or dose level that results in a specified probability of response in toxicity testing and a confidence interval for this value were investigated as well as the confidence interval for p.

Traditional method

Many methods have been used to calculate LC_p or LD_p in toxicity testing including probit and logit transformations, graphical interpolation, moving average, Spearman–Karber, and the binomial test [1–6]. Additionally, when data are continuous, such as those found in algal bioassays, curve-fitting methods such as weighted-least squares or nonlinear regression analysis have been used to estimate LC_p [7]. These methods tend to give very similar results for the LC50 estimate; however, they tend to produce dissimilar results for more extreme values (e.g., LC10 or LC90 estimates). In addition, accurate confidence intervals (C.I.s) (which should accompany every estimate) are sometimes either difficult or impossible to create with these methods.

In order to utilize simple linear regression techniques, the standard approach entails using either a logit or probit transformation to linearize what is typically an S-shaped relationship. Hence, the first step is to transform the data.

$$\text{Logit transform of } p_{\text{obs}} = \ln\left(\frac{p_{\text{obs}}}{1 - p_{\text{obs}}}\right)$$

$$\text{Probit transform of } p_{\text{obs}} = \Phi^{-1}(p_{\text{obs}})$$

where $\ln(x)$ is the natural logarithm of x and $\Phi^{-1}(p)$ is the inverse cumulative normal distribution function (the number that a standard normal random variable has probability p of being less than). The observed probabilities (p_{obs}) are simply the number of responses divided by the number exposed at each trial.

The observed transformed probabilities are assumed to have a normally distributed error added to the linear model. This leads to the following two possible models:

Logit regression:

$$\ln\left(\frac{p_{\text{obs}}}{1 - p_{\text{obs}}}\right) \sim \beta_0 + \beta_1\text{concn.} + N(0, \sigma^2)$$

Probit regression:

$$\Phi^{-1}(p_{\text{obs}}) \sim \beta_0 + \beta_1\text{concn.} + N(0, \sigma^2)$$

in which it is impossible to know β_0 and β_1 exactly. Instead, using simple linear regression techniques, the estimates for β_0 and β_1 ($\hat{\beta}_0$ and $\hat{\beta}_1$) that produce the most accurate description of the observed transformed probabilities are determined. Once these parameters are estimated, the predicted response probabilities can be obtained at any concentration by solving for p in the above equations.

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Inverse logit transform: $$\hat{p} = \frac{e^{(\hat{\beta}_0+\hat{\beta}_1\text{concn.})}}{1 + e^{(\hat{\beta}_0+\hat{\beta}_1\text{concn.})}}$$

Inverse probit transform: $$\hat{p} = \Phi(\hat{\beta}_0 + \hat{\beta}_1\text{concn.}).$$

In the above formulae, $\Phi(x)$ is the cumulative normal distribution (probability that a standard normal random variable is less than x).

There are some drawbacks to this approach however. First, if the observed probabilities are 0 or 1, some approximation method is required because the transformed values are infinitely negative or positive. Second, simply taking the transformation of a proportion loses information about the sample size at each concentration. A trial with 1 response out of 4 is treated identically as a trial with 100 responses out of 400. In both cases, the best guess of p is 0.25; however, there is more confidence in the accuracy of this estimate in the latter case. Hence, a technique that considers sample size when computing the LC_p would be more accurate. Third, there is no reason to presume that the assumptions for simple linear regression hold; namely, that the errors are normally distributed with constant variance. The whole idea of trying different transformations to linearize the data and then performing a linear regression on the transformed data is a rather ad hoc approach. Obviously, a preferred analysis would investigate the data using all available information in its native form, without having required transformations.

Generalized linear models

Generalized linear models are a preferable alternative to the methods described above because they take into account the true nature of the data, thereby removing the aforementioned problems. The two main practical differences between GLM and standard logistic regression (i.e., transformed dependent variable) is that GLM can accept observed values of 0% and 100% and that GLM takes into consideration the sample size (number of subjects exposed at each concentration) when estimating the coefficients and their errors. This technique (GLM) can be used to describe data coming from a variety of different distributions, such as normal data (which gives results identical to least-squares linear regression), Bernoulli success/failure data, poisson count data, and others [8]. This paper will focus on data that arise from the binomial distribution because this is the appropriate model to use for quantal toxicology data.

The binomial distribution has two parameters; n, the number exposed, and p, the response probability. The observed data, y, is the number of responses. The model can be written $y \sim \text{bin}(n,p)$. The expected number of responses is np, and the probability of seeing a particular value of y is

$$P(y) = \binom{n}{y} p^y(1 - p)^{n-y}; \qquad y = 0, 1, \ldots, n.$$

Two functions are required to specify a GLM. The first is the link function. In the context of the binomial distribution, this describes the relationship between p and a linear combination of predictors. For the cases analyzed in this paper, both the logit link and probit link will be explored. These are similar in form to the logit and probit transformations presented earlier, but the usage and interpretation are very different.

Logit link: $$\ln\left(\frac{p}{1 - p}\right) = \beta_0 + \beta_1\text{concn.}$$

Probit link: $$\Phi^{-1}{}_{(p)} = \beta_0 + \beta_1\text{concn.}$$

If one of these links is assumed to accurately represent the relationship in the data, then the true value of p can be determined by inverting the equation.

Inverse logit link: $$p = \frac{e^{(\beta_0+\beta_1\text{concn.})}}{1 + e^{(\beta_0+\beta_1\text{concn.})}}$$

Inverse probit link: $$p = \Phi(\beta_0 + \beta_1\text{concn.}).$$

The second function required to specify a GLM is the variance function, which is determined from the distribution assumed for the response data. In the present case, the binomial distribution has been assumed; therefore, estimates of β_0 and β_1 must be made to optimally fit one of the two following models depending on which link function is being invoked:

$$\text{Responses} \sim \text{bin}\left[\text{number exposed}, \frac{e^{(\beta_0+\beta_1\text{concn.})}}{1 + e^{(\beta_0+\beta_1\text{concn.})}}\right]$$

$$\text{Responses} \sim \text{bin}[\text{number exposed}, \Phi(\beta_0 + \beta_1\text{concn.})].$$

Once the parameters have been estimated, the predicted response probability, $\hat{p}$, can be obtained from the above equations.

But the question remains, how are estimates ($\hat{\beta}_0$ and $\hat{\beta}_1$) made for the parameters β_0 and β_1? To accomplish this, GLM finds the estimated parameters that minimize a quantity known as *deviance*. The definition of deviance is different for each probability distribution. For example, the deviance for normal data is the sum of the squared residuals and the deviance for binomial data is defined as

$$\text{Deviance} = 2 \sum y \ln\frac{y}{n\hat{p}} + (n - y)\ln\frac{n - y}{n - n\hat{p}}$$

where y is the number of individuals responding, $\hat{p}$ is the predicted proportion responding, n is the number exposed, and the summation is done over all trials. Instances of 0 ln 0 are set equal to 0.

The GLM uses an iteratively reweighted least-squares (IRLS) algorithm to find the parameter estimates that minimize the deviance. As a matter of notation for GLM, the *null deviance* is the amount of deviance remaining after estimating $\hat{\beta}_0$ with $\hat{\beta}_1$ set equal to zero. In other words the null deviance assumes that there is a constant probability of response regardless of concentration level. (This is analogous to the total sum of squares in linear regression after correcting for the mean.) The *residual deviance* is the amount of deviance that remains after both parameters have been estimated. (This is analogous to the residual sum of squares in linear regression.) Whether the concentration effect is significant or not can be investigated by measuring the difference between the residual and null deviances. If concentration had no effect, this difference would be distributed as a χ^2 variable with one degree of freedom (*d.f.*). Therefore a difference in these deviances that is greater than 3.84 (the critical value for χ^2_1 at $\alpha = 0.05$) indicates a significant concentration effect. To test the adequacy of the model to fit the data, the residual deviance can be compared to a χ^2 with degrees of freedom equal to the number of trials in the toxicity test minus 2. If the residual deviance is less than the critical value for $\chi^2_{\text{trials}-2}$ at $\alpha = 0.05$, then the GLM would be an appropriate model. If not, then there is still variability in the data not explained by the GLM.

To help demonstrate the method of GLM, let us investigate the following set of data (Table 1). A standard statistical computer package equipped with GLM software (see Appendix) can be used to estimate the two parameters β_0 and β_1 [9]. The estimated standard errors of these two parameters and the cor-

Table 1.Concentration–response dataset for GLM example

Beaker	Concn. (ng/ml)	Responses	Exposed
1	0.0	0	20
2	0.0	1	19
3	0.0	1	20
4	0.0	0	20
5	13.5	5	23
6	13.5	2	20
7	29.0	9	20
8	29.0	6	20
9	53.0	12	20
10	53.0	15	20
11	85.0	19	20
12	85.0	18	20
13	110.0	18	18
14	110.0	18	18

This example contains five treatment concentrations and a control. Concn. is the water concentration in ng/ml, responses are the number of individuals responding to the toxicant, and exposed are the total number in that beaker.

relation coefficient between them are also standard output for most software packages, as are the null and residual deviances.

Trials with the same concentration may be ''pooled'' together because the model assumes that two trials at the same concentration will have the same *p*. It should be noted that if the data are pooled, parameter estimates and standard errors for the LC_p will remain unchanged; however, the null deviance and degrees of freedom will decrease (due to properties of the binomial distribution). At each unique concentration, the algorithm uses only the overall response probability and total number exposed at that concentration. Therefore, variability in response probabilities between trials performed at the same concentration is not factored into the calculations. However, for a given concentration–response model, the standard error and hence confidence bands will, in general, decrease if more concentrations are added or if the sample size at each trial is increased.

The estimates and standard errors for the coefficients from both the logit and probit links are shown in Table 2. For this dataset (Table 1), the null deviance for the logit link was 207.0 on 13 *d.f.* with a residual deviance of 10.72 on 12 *d.f.* For the probit link we obtained a null deviance of 207.0 on 13 *d.f.* with a residual deviance of 9.72 on 12 *d.f.*

The level of concentration is obviously a highly significant predictor, as indicated by the large drop between the null and residual deviances. Both models fit the data well, as shown by the residual deviances being less than 21.03, the critical value of a χ^2 random variable with 12 *d.f.* at $\alpha = 0.05$. The probit link fits the data slightly better, because its residual deviance is less than the residual deviance obtained using the logit link. Therefore, the probit link is the model of choice to best explain this data set.

Table 2.Estimates and standard error (SE) for coefficients from the logit and probit links of GLM

Coefficient	Estimate	SE
Logit link		
$\hat{\beta}_0$	−2.798	0.3198
$\hat{\beta}_1$	0.0682	0.0077
Probit link		
$\hat{\beta}_0$	−1.623	0.1641
$\hat{\beta}_1$	0.0389	0.00387

Correlation of $\hat{\beta}_0$ and $\hat{\beta}_1$: −0.8094 for logit and −0.7733 for probit links.

Table 3.LC_p concentrations (ng/ml) for logit and probit links

Link	$\widehat{LC}10$	$\widehat{LC}50$
Logit	8.81	41.0
Probit	8.78	41.7

The concentrations that would cause mortality for 10% and 50% of the population are shown.

Estimating LC_p

A quantity of common interest is the concentration that has a particular probability of affecting the organism. These are known as the lethal concentrations for a given probability and denoted LC_p. The most commonly investigated is the LC50, but this analysis will look also at LC10 values.

It is impossible to know the LC_p value exactly, so estimates must be made, which will be denoted $\widehat{LC}_p$. To estimate an $\widehat{LC}_p$ at some *p* of interest, the transformed value of *p* is set equal to the fit found by applying GLM:

$$\hat{\beta}_0 + \hat{\beta}_1(\widehat{LC}_p) = \text{link}(p)$$

(Here link(*p*) is shorthand to represent either the logit or probit transformation of *p* depending on which link was used for the analysis.) Solving for $\widehat{LC}_p$, the concentration affecting a population at a specified probability is determined by

$$\widehat{LC}_p = \frac{\text{link}(p) - \hat{\beta}_0}{\hat{\beta}_1}$$

This equation simplifies when looking specifically at $\widehat{LC}50$ values. Logit(0.50) equals ln(0.50/0.50), which equals zero. Probit(0.50) also equals zero, because a standard normal deviate (with mean of 0 and variance of 1) has a 0.50 probability of being less than 0. As seen below, only two coefficients are needed:

$$\widehat{LC}50 = \frac{-\hat{\beta}_0}{\hat{\beta}_1}$$

From the specific data set examined earlier, $\widehat{LC}10$ and $\widehat{LC}50$ estimates are computed from both the logit and probit link functions. (Note that for $\widehat{LC}10$, logit(0.10) = −2.20 and probit(0.10) = −1.28.)

The resulting $\widehat{LC}_p$ concentrations are shown in Table 3. For this particular set of data, the estimated $\widehat{LC}_p$ values are quite similar for both link functions. Typically, the $\widehat{LC}50$s are close, but at the more extreme probabilities, the link functions can produce different $\widehat{LC}_p$ values.

Confidence interval for p *at* $\widehat{LC}_p$

It is a basic tenet of statistics that every point estimate should have a measure of error associated with it. By using the techniques outlined by McCullagh and Nelder [8], a C.I. for both the concentration estimate and the response probability estimate can be determined. The C.I. for the range of probabilities at a particular concentration level is described first. Because variability in the right-hand side of the model (link(*p*) = $\hat{\beta}_0$ + $\hat{\beta}_1$concn.) can be computed, the variance of logit(*p*) and probit(*p*) can be estimated by the following:

Table 4.Variances for logit and probit links

Link	Var [link(p at $\widehat{\text{LC}}10$)]	Var [link(p at $\widehat{\text{LC}}50$)]
Logit	0.0718	0.0385
Probit	0.0195	0.0120

$$\begin{aligned}\text{var}[\text{link}(p)] &= \text{var}(\hat{\beta}_0 + \hat{\beta}_1\text{concn.}) \\ &= \text{var}(\hat{\beta}_0) + 2\ \text{cov}(\hat{\beta}_0, \hat{\beta}_1)\text{concn.} \\ &\quad + \text{var}(\hat{\beta}_1)\text{concn.}^2\end{aligned}$$

The variance of each $\hat{\beta}$ is simply the square of the standard errors $(\text{se}\hat{\beta})^2$ obtained earlier from the statistical software and the covariance is the product of the two standard errors and the correlation coefficient between them ($\text{se}\hat{\beta}_0 \cdot \text{se}\hat{\beta}_1 \cdot \text{corr}$). Concentration is the $\widehat{\text{LC}}_p$ value determined earlier. Using the above data set as an example:

$$\begin{aligned}\text{var}[\text{logit}(p)] &= 0.32^2 + 2(0.32 \times 0.0077 \times -0.81)\text{concn.} \\ &\quad + 0.0077^2\text{concn.}^2 \\ \text{var}[\text{probit}(p)] &= 0.16^2 + 2(0.16 \times 0.0039 \times -0.77)\text{concn.} \\ &\quad + 0.0039^2\text{concn.}^2\end{aligned}$$

The variances at the $\widehat{\text{LC}}10$ and $\widehat{\text{LC}}50$ concentration levels found earlier are computed in units of the link function squared and listed in Table 4. Advanced statistical theory states that the regression coefficients obtained in GLM models are asymptotically normally distributed. Therefore, we can create a C.I. in link units. A $1 - \alpha$ interval for link(p) is:

$$\text{link}(p) \pm z_{\alpha/2}\sqrt{\text{var}[\text{link}(p)]}.$$

For a 90% C.I., $z_{\alpha/2} = 1.645$ is used and for a 95% C.I., $z_{\alpha/2} = 1.96$. In Table 5 we show the 95% C.I.s for both links in terms of link units and proportion responding. To obtain a confidence interval on the probability scale, the bounds are transformed using the inverse link function.

As expected, the C.I.s include the stated probability (10% and 50%, respectively) and the two links give very similar results. The intervals are not terribly wide, showing that the true response probabilities at the $\widehat{\text{LC}}10$ and $\widehat{\text{LC}}50$ are near those expected.

Confidence interval for LC_p

Of course the toxicologist wants to know, with statistical confidence, what range of concentrations could explain the specified response probability. This is answered by constructing a C.I. for the LC_p values. One way to accomplish this is with Fieller's method. Intervals for the response probabilities are constructed using the above methodology for the range of concentrations used. Those concentrations whose C.I. for p include the probability of interest, will be included in this ''confidence'' interval for LC_p. For example, the 95% C.I. for p at 38 ng/ml is (0.36, 0.53), which includes 0.50. Therefore, 38 ng/ml would be included in a 95% C.I. for the LC50.

Table 5.Confidence intervals (95%) for LC_p estimates

	Link units	
Link	Link(p at $\widehat{\text{LC}}10$)	Link(p at $\widehat{\text{LC}}50$)
Logit	(−2.72, −1.67)	(−0.385, 0.385)
Probit	(−1.56, −1.01)	(−0.215, 0.215)
	Proportion	
	p at $\widehat{\text{LC}}10$	p at $\widehat{\text{LC}}50$
Logit	(0.062, 0.158)	(0.405, 0.595)
Probit	(0.060, 0.157)	(0.415, 0.585)

Top half shows C.I. in terms of link units and bottom half shows C.I. in terms of proportions of the population.

Table 6.Confidence intervals (95%) for LC_p

Link	$\widehat{\text{LC}}10$	$\widehat{\text{LC}}50$
Logit	(−0.47, 15.5)	(35.6, 47.2)
Probit	(0.60, 15.1)	(36.4, 47.7)

Values are concentrations in ng/ml.

This is accomplished analytically by finding the set of concentrations such that the following equation holds:

$$1 - \alpha = P\left[-z_{\alpha/2} \le \frac{\hat{\beta}_0 + \hat{\beta}_1\text{concn.} - \text{link}(p)}{\sqrt{\text{var}(\beta_0 + \beta_1\text{concn.})}} \le z_{\alpha/2}\right]$$

which is algebraically equivialent to

$$[\hat{\beta}_0 + \hat{\beta}_1\text{concn.} - \text{link}(p)]^2 - z^2_{\alpha/2}\text{var}(\hat{\beta}_0 + \hat{\beta}_1\text{concn.}) \le 0.$$

Expanding the first term algebraically and using the variance determined in the previous section yields:

$$\begin{aligned}&[\hat{\beta}_0 - \text{link}(p)]^2 + 2\hat{\beta}_1[\hat{\beta}_0 - \text{link}(p)]\text{concn.} \\ &\quad + \hat{\beta}_1^2\text{concn.}^2 - z^2_{\alpha/2}[\text{var}(\hat{\beta}_0) + 2\ \text{cov}(\hat{\beta}_0, \hat{\beta}_1)\text{concn.} \\ &\quad + \text{var}(\hat{\beta}_1)\text{concn.}^2] \le 0\end{aligned}$$

which can be written as a second-order polynomial in terms of concentration:

$$\begin{aligned}&[\hat{\beta}_1^2 - z^2_{\alpha/2}\text{var}(\hat{\beta}_1)]\text{concn.}^2 \\ &\quad + [2\hat{\beta}_0\hat{\beta}_1 - 2\hat{\beta}_1\text{link}(p) - 2z^2_{\alpha/2}\text{cov}(\hat{\beta}_0, \hat{\beta}_1)]\text{concn.} \\ &\quad + [\hat{\beta}_0^2 + \text{link}(p)^2 - 2\hat{\beta}_0\text{link}(p) - z^2_{\alpha/2}\text{var}(\hat{\beta}_0)] \le 0.\end{aligned}$$

The quadratic formula $[ax^2 + bx + c = 0$; where x can be solved with $(-b \pm \sqrt{b^2 - 4ac})/2a]$ produces solutions for the endpoints and therefore the C.I. of the lethal concentration. This method fails if a equals zero or if a negative occurs under the radical.

Notice that five values are required from the GLM analysis (which we obtained from software); the two parameter estimates, the standard error of these estimates, and the correlation coefficient between them. Two additional pieces of information are needed; the z-score, which is used to determine the confidence level of the intervals and the link function of the particular probability p that is of interest. For the example data set analyzed above, the 95% C.I. of the LC10 using a logit link is the set of concentrations that satisfy the following inequality: $(0.068^2 - 1.96^2 \times 0.0077^2)\text{concn.}^2 + (2 \times -2.80 \times 0.068 - 2 \times 0.068 \times -2.20 - 2 \times 1.96^2 \times 0.32 \times 0.0077 \times -0.81)\text{concn.} + (-2.80^2 + -2.20^2 - 2 \times -2.80 \times -2.20 - 1.96^2 \times 0.32^2) \le 0$, which algebraically reduces to $0.0044\text{concn.}^2 - 0.0666\text{concn.} - 0.0319 \le 0$. The confidence bounds were located using the quadratic formula and are listed in Table 6 for both the LC10 and LC50 for each link (logit and probit). The LC10s and LC50s obtained earlier are near the

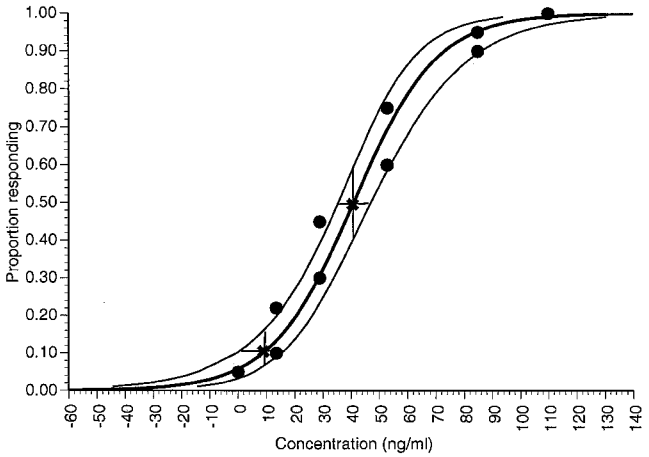

Fig. 1. Plot of concentration-response data with 95% confidence band for concentration and response. Logit link. Circles are observed data and X shows LC10 and LC50 values. Central line was fit to LC*p* values.

middle of these C.I.s, as expected. As stated earlier, it appears as though the probit link may be a better fit for these data, which is evident here by the tighter and non-negative C.I. for the LC10. This example is typical in that the intervals for LC50s are more stable than those for more extreme values of *p.*

Figure 1 displays the 95% confidence band for the response probabilities at all levels of concentration for the logit link (the confidence for the probit link is very similar). The vertical line segments in this plot represent the C.I.s calculated above for *p.* The horizontal line segments represent the C.I.s for the true concentration that would give rise to a specified response.

Technique assessment using simulations

One way to verify that the above estimation techniques pass a ''reality'' check is to perform a simulation study. This can be done by choosing a particular response curve and then randomly generating many data sets from this curve. The routines described above can be performed on each set of generated data and the results compared to the known truth.

In this case it was assumed that the true relationship is described by a logit curve that has its LC50 at 15 ng/ml and its LC10 at 5 ng/ml. By knowing these two points, we can determine the curve; $\text{logit}(p) = \ln(p/1 - p) = -3.3 + 0.22\text{concn}$. [i.e. $\ln(0.1/0.9) = a + b \cdot 5$ and $\ln(0.5/0.5) = a + b \cdot 15$ describe two equations and two unknowns whose solutions is $a = -3.3$ and $b = 0.22$.] Further, assume that 10 subjects are exposed at each concentration level of 0, 10, 20, and 30 ng/ml. For each of the 500 simulations we ran, the number ''responding'' at each concentration was randomly generated using a binomial distribution and the derived probability. These data were then used to find the $\widehat{\text{LC}}_p$ values and the C.I.s, as described earlier, for all 500 simulations. For these simulations we constructed 90% C.I.s.

The first set of 250 simulations was used to determine information relative to the LC50. The $\widehat{\text{LC}}$50s obtained appeared normally distributed with a mean of 15.2 ng/ml, which is close to the known truth of 15 ng/ml. The standard deviation of the estimates was 2.3. Of the 250 ''confidence'' intervals created for LC50, 87% contained 15 ng/ml, which is consistent with intervals made at the $\alpha = 0.90$ confidence level. (Six percent of the intervals had numerical difficulties due to either a lack of convergence by the GLM program or by attempting to take the square root of a negative number.)

The second set of 250 simulations was used to determine information relative to the LC10s. The $\widehat{\text{LC}}$10s obtained appeared normally distributed with a mean of 5.64 ng/ml, close to the known truth of 5 ng/ml. However, the variability of the estimates was quite large; the standard deviation was 4.15. Of the 250 C.I. created for the LC10, 89% contained 5 ng/ml, which again is consistent for intervals made at the $\alpha = 0.90$ level of confidence. (Five percent of the intervals had numerical difficulties.)

CONCLUSIONS

Using GLM as described in this paper is the most appropriate way to analyze the binomially distributed response data generated in toxicity tests. It allows relatively easy calculation of lethal or sublethal concentration estimates, as well as C.I.s for these and the response probabilities. Unlike simple linear regression performed on transformed data, GLM utilizes sample size information and reacts appropriately to the response probabilities of 0 and 1. Inclusion of these extreme response probabilities has no deleterious effect on the calculations or degrees of freedom and is encouraged because they help define the overall response.

In order to ensure adequate data for quantitation, the experiment should contain a range of doses wide enough to cover the full range of response probabilities. Any extrapolation beyond the range of concentration levels used is inadvisable. By increasing either the number of concentrations or the number of individuals per concentration for a given concentration–response model, the standard error will generally decrease, which will produce tighter C.I.s. When analyzing a toxicity test with this method, the test concentrations should be evenly spaced out (e.g., linear or geometric series). We have noticed that when the differences between concentrations are not reasonably uniform, the model performs poorly and can give high deviance or an error stating that the data do not fit the binomial model.

Although concentration replicates will have no effect on model estimates, independent replicates are recommended to adequately characterize a species response. These replicates may be analyzed as separate experiments and compared to each other for an estimate of precision and for confirmation of the concentration–response or dose–response function.

When examining data from a toxicity test, the analysis should be tried using more than one link and the results compared. The link that produces the smallest residual deviance will probably produce the best fit to the data. Also estimation at low and high response probabilities is generally less reliable and the estimates and their C.I.s at these points may be dependent on the link used. If there are some responses at or near the zero concentration level, then the $\widehat{\text{LC}}_p$ and related ''confidence'' interval for a small *p* could conceivably be negative as was shown in Table 6. In these cases a more refined approach would assume that concentrations must be non-negative when creating confidence intervals (i.e., zero concentration would be the lower limit).

In toxicity testing we often try to determine the lowest observed effect concentration (LOEC) of a chemical, which is often accomplished with multiple comparison procedures of treatment groups. The LOEC is the concentration at which there is an effect that is significantly greater than that observed for the control. With the dose–response model presented in this paper, it may be possible to determine the LOEC in the same fashion as comparing nonoverlapping C.I.s for two means. This

approach is conservative because two means with nonoverlapping C.I.s will always be significantly different. We propose that the LOEC would occur where the horizontal line from the upper 95% C.I. for zero concentration intersects the right 95% confidence band of the model (Fig. 1). In our example, the upper 95% C.I. for the response probability at 0 ng/ml occurs at about 0.10, while the lower 95% C.I. for the response probability for 16 ng/ml also occurs at approximately 0.10 (Fig. 1). Therefore the response probability at 16 ng/ml is significantly higher ($\alpha \leq 0.05$) than that at 0 ng/ml, which is the lowest dose for which this is true. In this particular case, we would conclude that any LC_p below the LC10 (e.g., LC05) calculated from this data set would be statistically indistinguishable from the control.

There are certain philosophical arguments about the exact nature of the C.I.s created above. In the current situation, the true LC_p is regarded as fixed. Before the data are observed, the procedure for making C.I.s is devised such that we think a high percentage of these intervals created over the long run will contain the truth. However, usually only one interval is created and it is impossible to determine whether it does or does not contain the truth. The proper interpretation of the C.I. can therefore be debated [10]. Although technically incorrect, one can simply think of the C.I. as probably covering the true value, or of the true value probably being inside the C.I.

It should be kept in mind that the duration of the $\widehat{LC}_p$ toxicity tests should always be specified. Ideally, the time interval should be sufficient to allow steady-state conditions; however, this is not always achieved. Some species may take a long time to achieve a steady-state response, which can be characterized by a decreasing LC50 over time [11]. (This is caused by a slow approach to steady-state body burdens as a result of a slow rate of toxicant elimination.) Comparability between $\widehat{LC}_p$ point estimates is ideally made when values are determined for the steady-state response or for identical time periods of exposure. Additionally, myriad changing environmental factors can also affect either the animal's performance [3,10,12] or the chemical's bioavailability and must be controlled in order to assure direct comparability.

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APPENDIX

Any general program that has algorithms for GLM such as SAS®, Systat®, and S-Plus® can be used to obtain the LC_p. For programs specifically written for GLM, we recommend GLIM (DOS, Mac, Unix) from the Numerical Algorithms Group, Inc. (Downers Grove, IL) or GLMstat (Ken Beath; CGACB@cc.newcastle.edu.au), which can be found on the Internet in the Info-Mac archives. In the Info-Mac archives, we have placed an Excel® (version 4.0) file called GLMLCp (Macintosh version) with the following formulae to calculate confidence intervals for the logit and probit links. One example of an Internet address for the Info-Mac archive is *ftp://mirrors.aol.com/pub/info-mac/sci*. A DOS Windows® version of this Excel (version 4.0) file (GLMLCp.xls) can be found in an archive of Windows-related shareware. One example of an Internet address for this archive is *ftp://ftp.cica.indiana.edu/pub/pc/win3/excel*. This file can also be obtained by sending a request via the Internet to jmeador@sci.nwfsc.noaa.gov or a blank high density diskette to J.P. Meador (please specify the Macintosh or DOS Windows format).

From the GLM program the output for $\hat{\beta}_0$, $\hat{\beta}_1$, $se\hat{\beta}_0$, $se\hat{\beta}_1$, and the correlation between $\hat{\beta}_0$ and $\hat{\beta}_1$ can be obtained. The $1 - \alpha$ C.I. is also necessary and usually set at 0.95 or 0.90. All C.I.s are constructed as two-tailed. LC_p is the response probability of choice and can be 0.5, 0.1, or any other value desired. Once the parameters are obtained from the GLM, they can be used in the following formulae and Excel spreadsheet to generate the C.I.s.

In GLMstat the coefficients and standard error are found under Estimate; $\hat{\beta}_0$ is the estimate for the grand mean (gm) and $\hat{\beta}_1$ is the estimate for concentration (concn). Correlation between concn. and gm is found under Corr. The null deviance can be obtained by fitting the model without any factors and the residual deviance can be determined when the model is fitted with concentration.

The GLM method for calculating C.I.s for LC_p is:

Proportion responding

For logit:

$$\text{lower C.I. for } p = \frac{e^{(\text{link}p - z\sqrt{\text{var}})}}{1 + e^{(\text{link}p - z\sqrt{\text{var}})}}$$

$$\text{upper C.I. for } p = \frac{e^{(\text{link}p + z\sqrt{\text{var}})}}{1 + e^{(\text{link}p + z\sqrt{\text{var}})}}$$

For probit:

$$\text{lower C.I. for } p = \text{normal distribution } (\text{link}p - z\sqrt{\text{var}})$$

$$\text{upper C.I. for } p = \text{normal distribution } (\text{link}p + z\sqrt{\text{var}})$$

where variance $= (se\hat{\beta}_0)^2 + 2 \cdot se\hat{\beta}_0 \cdot se\hat{\beta}_1 \cdot \text{corr}(\hat{\beta}_0, \hat{\beta}_1) \cdot \text{concn.} + (se\hat{\beta}_1)^2 \cdot \text{concn.}^2$, z = normal deviate for α; (if $\alpha = 0.05$, $z = 1.96$), and concn. $= LC_p$. For logit, $\text{link}p = \ln[p/(1 - p)]$. For probit, $\text{link}p = \Phi^{-1}(p)$, which can be determined with the inverse of the normal cumulative distribution (Φ^{-1}) (e.g., NORMINV in Microsoft Excel) for probability, mean, and standard deviation (p, m, s). For example, if we chose the LC50, this would give a probability 0.5. Hence, in conjunction with a normal distribution with mean of 0 and standard deviation of 1, our parameters would be (0.5, 0, 1), which would equal 0.

Upper and lower confidence intervals for p in the probit link can be determined using the normal distribution function (Φ) (e.g., NORMDIST in Microsoft Excel) for x, mean, standard deviation. For example, to compute the lower boundary of the 95% C.I. in our main example, one would evaluate (−0.215, 0, 1); x = probit units for lower C.I. for a normal distribution with mean of 0 and standard deviation of 1. The result is a probability of 0.415.

Concentration

For both links:

$$\text{lower C.I.} = \frac{-b - \sqrt{b^2 - 4ac}}{2a};$$

$$\text{upper C.I.} = \frac{-b + \equiv\phi\upsilon = "- < + \overline{\beta^- - \div\alpha\psi}}{-\alpha}$$

$$\psi o\nu\psi\nu > = \Sigma\lambda\iota\nu\kappa\,\pi - \hat{\beta}_{"}\Pi/\hat{\beta}_{+} \sim$$

$$\alpha = \hat{\beta}_{+}^{-} - \zeta^{-}\Sigma\sigma\epsilon\hat{\beta}_{+}\Pi^{-} \sim$$

$$\beta = -\hat{\beta}_{"}\hat{\beta}_{+} - -\hat{\beta}_{+}\lambda\iota\nu\kappa\,\pi - -\zeta^{-}\sigma\epsilon\hat{\beta}_{"}$$

$$\sigma\epsilon\hat{\beta}_{+}\psi o\rho\rho\Sigma\hat{\beta}_{"} < \hat{\beta}_{+}\Pi \sim$$

$$\psi = \hat{\beta}_{"}^{-} + \Sigma\lambda\iota\nu\kappa\,\pi\Pi^{-} - -\hat{\beta}_{"}\lambda\iota\nu\kappa\,\pi - \zeta^{-}\Sigma\sigma\epsilon\hat{\beta}_{"}\Pi^{-} >$$

Using the example in the manuscript, $\hat{\beta}_0 = -2.8$, $\hat{\beta}_1 = 0.068$, $se\hat{\beta}_0 = 0.32$, $se\hat{\beta}_1 = 0.0077$, and the correlation between $\hat{\beta}_0$ and $\hat{\beta}_1 = -0.8094$.