

DOSE-TIME-RESPONSE CUMULATIVE MULTINOMIAL GENERALIZED LINEAR MODEL

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In toxicological and pharmaceutical experiments, a type of quantal bioassay experiment is designed in which a response, such as mortality, in a group of animals is recorded over time points under different dose levels in the course of the experiment. The application of the typical logit and probit analyses is no longer valid in this situation because it neglects the dependency on time and also the possible interaction of time and dose concentration on the response in the experiment. In this paper, a dose-time-response model is proposed for this type of experiment and a cumulative multinomial generalized linear model that incorporates time and the other experimental conditions as covariates is developed by the theory of maximum likelihood estimation. Both the point estimator and confidence bands for $ED_{50}(t)$, the concentration of a toxicant that will kill 50% of the animals by a specific time, t ; as well as $LT_{50}(d)$, the time to 50% mortalities for a specific concentration, d , is then formulated in closed form from the newly proposed dose-time-response model. Finally, the newly proposed model is considered for a real data set to demonstrate the application.

Key Words: Dose-time-response; Generalized linear model; Iteratively reweighted least squares; $ED_{50}(t)$; $LT_{50}(d)$; Toxicity.

1. INTRODUCTION

In some toxicological and pharmaceutical bioassay experiments, a stimulus (for example, dose of a drug) is applied to n experimental units and r of them respond and $n - r$ do not respond. This type of quantal response bioassay belongs to the class of qualitative indirect bioassay. Typically, logit and probit analyses are used to estimate the dose-response relationship and then to estimate the tolerance level of the individuals in the population which is often referred to as ED_{50} , the median effective dose (ED) to produce a response in 50% of individuals on average in the population. This important characterizing parameter is also referred as LD_{50} for median lethal dose or LC_{50} for median lethal concentration or EC_{50} for the

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median effective concentration or Tlm for median tolerance limit. An extensive literature can be found from Finney (1978), Hubert (1992), and Morgan (1992).

Recently the issue of monitoring this type of experiment over time in the course of an experiment is becoming more popular (Carter and Hubert, 1984; Kim et al., 2003; Zhu, 2001, 2005; Zhu et al., 2005). In this situation, the response, such as mortality in a group of animals, is measured over time points and, therefore, is a function of time (see, for example, the data set examined in Section 4). In this data, the mortality (response) of male and female adult flour beetles (*Tribolium castaneum*) was recorded over 13 days (time) for the same individuals on different dose levels. The application of the standard logit and probit analyses is then not valid any more since it neglects the dependency on time and also the possible interaction of time and dose concentration on the response in this type of quantal bioassay experiment.

In this paper, a dose-time-response cumulative multinomial generalized linear model that incorporates time and the other experimental conditions as covariates is proposed in Section 2 for this type of experiment by using the maximum likelihood method for cumulative counts. In addition to modelling cumulative mortality as a dose-time-response function, both the point estimator and confidence bands of $ED_{50}(t)$, the concentration of a toxicant that will kill 50% of animals by a specific time, t , and $LT_{50}(t)$, the time to 50% mortality for a specific concentration, d , are then formulated in closed form in Section 3. Finally, the developed procedures are applied to a real data set under logit and probit link functions in Section 4.

2. DOSE-TIME-RESPONSE CUMULATIVE MULTINOMIAL GENERALIZED LINEAR MODEL

2.1. Model Structure

Suppose that an experiment is conducted under n dose levels. For each dose level d_j ($j = 1, \dots, n$), N_j animals are examined at discrete time points $t_1 < t_2 < \dots < t_m$. Let z_{ij} ($i = 1, \dots, m$) denote the observed cumulative mortality counts of animals responding in the i th time interval and j th dose level among the whole time course $(0, t_1]$, $(t_1, t_2]$, \dots , $(t_{m-1}, t_m]$ and (t_m, ∞) . Then, $z_{ij} - z_{(i-1)j}$ jointly follow a multinomial distribution with probability $p_{ij} - p_{(i-1)j}$, where p_{ij} is the probability that an individual animal on dose level d_j responds by time t_i . It is obvious that $0 = p_{0j} \leq p_{1j} \leq \dots \leq p_{mj} \leq p_{(m+1)j} = 1$. The log likelihood function can, therefore, be formulated as:

$$\ln(L) \propto \sum_{j=1}^n \sum_{i=1}^{m+1} (z_{ij} - z_{(i-1)j}) \ln(p_{ij} - p_{(i-1)j}) \quad (2.1)$$

where $z_{0j} = 0$ and $z_{(m+1)j} = N_j$.

To take the effects of time and time \times dose interaction into account, a generalized linear model (McCullagh and Nelder, 1989) with link function:

$$g(p) = \eta = \beta_0 + \beta_1 x + \beta_2 t^{-1} + \beta_3 x t^{-1} \quad (2.2)$$

can be employed here, where $x = \ln(\text{dose})$ and $g(\cdot)$ is the monotone differentiable link function (such as the logit and probit functions) between the linear predictor,

$\eta = \beta_0 + \beta_1 x + \beta_2 t^{-1} + \beta_3 x t^{-1}$ and the random response. In this model (2.2), the parameter β_2 and β_3 should be negative because the cumulative mortalities would increase with increasing time (and decreasing 1/time).

The model (2.2) considered only the first-order dose-time interaction because it is sufficient in most real situations. However, it can be easily extended to higher-order dose-time interactions, which might lead to overparameterization in many situations. However, this extension creates nested models and can be statistically tested by the likelihood ratio test (Stuart et al., 1999).

2.2. Parameter Estimation

To estimate the parameters from model (2.2), the iteratively reweighted least squares (Morgan, 1992) can be employed. The iterative processes can be written and updated as:

$$\Psi^{(k+1)} = \Psi^{(k)} + [I^{(k)}]^{-1} \left(\frac{\partial \ln(L)}{\partial \Psi} \right)^{(k)} \tag{2.3}$$

where $\Psi = (\beta_0, \beta_1, \beta_2, \beta_3)$ is the parameter vector. I is the expected Fisher information matrix and $\left(\frac{\partial \ln(L)}{\partial \Psi} \right)$ is the gradient vector. k denotes the k th iteration.

It is important to choose the appropriate initial values of Ψ , which can be obtained by regressing

$$g(p_{ij}) = \beta_0 + \beta_1 x_j + \beta_2 t_i^{-1} + \beta_3 x_j t_i^{-1} \tag{2.4}$$

where $p_{ij} = \frac{z_{ij}}{N_j}$ from the observed data. Therefore, the estimated dose-time-response relationship is

$$\hat{\eta} = \hat{\beta}_0 + \hat{\beta}_1 x + \hat{\beta}_2 t^{-1} + \hat{\beta}_3 x t^{-1}. \tag{2.5}$$

This model and the parameter estimation procedure are programmed in *R* (<http://www.rproject.org/>) and the *R* code can be obtained from the author.

3. THE ESTIMATION OF $ED_{100p}(t)$ AND $LT_{100p}(d)$

3.1. Estimation of $ED_{100p}(t)$

$ED_{100p}(t)$ is commonly known as the concentration level of stimulus necessary to bring about a response of a given proportion or potency, $100p\%$, of individuals in the population at time t . For example, $ED_{50}(t)$ is the concentration of a toxicant that will kill 50% of the population at time t in toxicological studies. The estimation of this quantity is one of the prime objectives for quantal bioassay.

This can be easily done with the estimated dose-time-response relationship (2.5). For a fixed potency, $100p\%$, the point estimate of the $ED_{100p}(t)$ is:

$$\widehat{ED}_{100p}(t) = \exp \left(\frac{g(p) - \hat{\beta}_0 - \hat{\beta}_2 t^{-1}}{\hat{\beta}_1 + \hat{\beta}_3 t^{-1}} \right). \tag{3.1}$$

This equation is simplified for the special case of $ED_{50}(t)$ (i.e., the median effective concentration) where $p = 50\%$ corresponding to logit and probit link functions as follows:

$$\widehat{ED}_{50}(t) = \exp\left(-\frac{\hat{\beta}_0 + \hat{\beta}_2 t^{-1}}{\hat{\beta}_1 + \hat{\beta}_3 t^{-1}}\right). \quad (3.2)$$

The confidence bands for $ED_{100p}(t)$ for time t can be obtained by the procedure from Fieller's Theorem (Fieller, 1940; Finney, 1978) as follows:

$$\widehat{ED}_{100p}(t) = \exp\left(\frac{\widehat{B}(t) \mp \sqrt{\widehat{C}(t)}}{\widehat{A}(t)}\right) \quad (3.3)$$

where

$$A(t) = b(t)^2 - z^2 c_{22}(t)$$

$$B(t) = c(t)b(t) - z^2 c_{12}(t)$$

$$C(t) = [c(t)b(t) - z^2 c_{12}(t)]^2 - [b(t)^2 - z^2 c_{22}(t)][c(t)^2 - c_{11}(t)z^2]$$

$$c(t) = g(p) - \beta_0 - \beta_2 t^{-1}$$

$$b(t) = \beta_1 + \beta_3 t^{-1}$$

$$c_{11}(t) = \text{Var}[\hat{c}(t)] = \text{Var}(\hat{\beta}_0) + \text{Var}(\hat{\beta}_2)t^{-2} + 2\text{Cov}(\hat{\beta}_0, \hat{\beta}_2)t^{-1}$$

$$c_{12}(t) = \text{Cov}[\hat{b}(t), \hat{c}(t)]$$

$$= -[\text{Cov}(\hat{\beta}_0, \hat{\beta}_1) + \text{Cov}(\hat{\beta}_2, \hat{\beta}_1)t^{-1} + \text{Cov}(\hat{\beta}_0, \hat{\beta}_3)t^{-1} + \text{Cov}(\hat{\beta}_2, \hat{\beta}_3)t^{-2}]$$

$$c_{22}(t) = \text{Var}[\hat{b}(t)] = \text{Var}(\hat{\beta}_1) + \text{Var}(\hat{\beta}_3)t^{-2} + 2\text{Cov}(\hat{\beta}_1, \hat{\beta}_3)t^{-1}$$

which can be estimated from the estimated expected Fisher information matrix in the iteratively reweighted least squares (2.3).

It is found that the structure of the confidence bands dramatically depends on the value of $\widehat{A}(t)$. Specifically, if $\widehat{A}(t) > 0$, the structure of the confidence bands for $ED_{100p}(t)$ is bounded as $(\widehat{d}_1(t), \widehat{d}_2(t))$, where

$$\begin{aligned} \widehat{d}_1(t) &= \exp\left(\frac{\widehat{B}(t) - \sqrt{\widehat{C}(t)}}{\widehat{A}(t)}\right) \\ \widehat{d}_2(t) &= \exp\left(\frac{\widehat{B}(t) + \sqrt{\widehat{C}(t)}}{\widehat{A}(t)}\right) \end{aligned} \quad (3.4)$$

and $\widehat{d}_1(t) \leq \widehat{d}_2(t)$. However, if $\widehat{A}(t) < 0$, the structure of the confidence band for $ED_{100p}(t)$ is unbounded as $(-\infty, \widehat{d}_1(t))$ or $(\widehat{d}_2(t), \infty)$.

This dependence on the quantity $\widehat{A}(t)$ can be made clear by investigating $\widehat{A}(t)$. In fact, $\widehat{A}(t)$ should be positive in a well-designed dose-response experiment. If $\widehat{A}(t) < 0$ then $\widehat{b}(t) < z\sqrt{\widehat{c}_{22}(t)}$ where $b(t)$ (i.e., the denominator in $ED_{100p}(t)$) is not statistically significantly different from zero. This only happens when the responses do not depend on the dose levels in the dose-response experiment.

Figure 2 in Section 4.3 illustrates this property with respect to the data set considered in Section 4 since $\widehat{A}(t) > 0$ for any time, t .

3.2. Estimation of Time $LT_{100p}(d)$

Another issue is to estimate the time $LT_{100p}(d)$ to 100p% mortality for a specific concentration, d . From the estimated dose-time-response relationship (2.5), we have the point estimate of the $LT_{100p}(d)$ as follows:

$$LT_{100p}\widehat{(d)} = \frac{\widehat{\beta}_2 + \widehat{\beta}_3 \ln(d)}{g(p) - \widehat{\beta}_0 - \widehat{\beta}_1 \ln(d)} \tag{3.5}$$

Similarly, Equation (3.5) can be simplified for the logit and probit link function for 50% mortality as:

$$LT_{50}\widehat{(d)} = -\frac{\widehat{\beta}_2 + \widehat{\beta}_3 \ln(d)}{\widehat{\beta}_0 + \widehat{\beta}_1 \ln(d)}$$

The confidence bands for $LT_{100p}(d)$ for fixed dose level d can be obtained by the procedure from Fieller theorem as follows:

$$LT_{100p}\widehat{(d)} = \frac{\widehat{B}(d) \mp \sqrt{\widehat{C}(d)}}{\widehat{A}(d)} \tag{3.6}$$

where

$$A(d) = b(d)^2 - z^2 c_{22}(d)$$

$$B(d) = c(d)b(d) - z^2 c_{12}(d)$$

$$C(d) = [c(d)b(d) - z^2 c_{12}(d)]^2 - [b(d)^2 - z^2 c_{22}(d)][c(d)^2 - c_{11}(d)z^2]$$

$$c(d) = \beta_2 + \beta_3 \ln(d)$$

$$b(d) = g(p) - \beta_0 - \beta_1 \ln(d)$$

$$c_{11}(d) = \text{Var}[\widehat{c}(d)] = \text{Var}(\widehat{\beta}_2) + \text{Var}(\widehat{\beta}_3)[\ln(d)]^2 + 2 \ln(d)\text{Cov}(\widehat{\beta}_2, \widehat{\beta}_3)$$

$$c_{12}(d) = \text{Cov}[\widehat{c}(d), \widehat{b}(d)]$$

$$\begin{aligned} &= -[\text{Cov}(\widehat{\beta}_0, \widehat{\beta}_2) + \text{Cov}(\widehat{\beta}_2, \widehat{\beta}_1) \ln(d) + \text{Cov}(\widehat{\beta}_0, \widehat{\beta}_3) \ln(d) \\ &\quad + \text{Cov}(\widehat{\beta}_1, \widehat{\beta}_3)[\ln(d)]^2] \end{aligned}$$

$$c_{22}(d) = \text{Var}[\widehat{b}(d)] = \text{Var}(\widehat{\beta}_0) + [\ln(d)]^2 \text{Var}(\widehat{\beta}_1) + 2 \ln(d)\text{Cov}(\widehat{\beta}_0, \widehat{\beta}_1)$$

A similar discussion about the structure of this confidence bands can be carried out according the quantity $\widehat{A}(d)$. Figure 3 in Section 4.3 illustrates this property with respect to the data set considered in Section 4 since $\widehat{A}(d) > 0$ for any dose levels, d .

4. DATA AND ANALYSIS

4.1. Data

Reproduced from Hewlett (1974) and Laurence and Morgan (1989), Table 1 provides the data from a classical quantal bioassay for pyrethrum in which the mortality of flour adults beetles was measured over time under 4 dose levels. The numbers indicate the number dead per day of adult flour beetles (*Tribolium castaneum*) exposed initially to pyrethrum, a well-known plant-based insecticide. Mixed with oil, the pyrethrum was sprayed at the given rates of application over small experimental areas in which the groups of beetles were confined but allowed to move freely. Food was provided in an attempt to prevent natural mortality.

Using a power transformation, Hewlett (1974) initially conducted an analysis of variance for this data and concluded that there was a significant sex effect, but no dose effect or any sex \times dose interaction. In his paper, Hewlett also conducted a probit analysis of the mortality at day 13. This data was further considered by Sandland (1984); Diggle and Gratton (1984); Laurence and Morgan (1989).

Pack and Morgan (1990) did a logistic analysis of the endpoint (day 13) mortality and found the following results:

$$\text{Male : } \ln(ED_{50}) = -1.361 \quad (\text{est. asymptotic s.e.} = 0.044)$$

$$\text{Female : } \ln(ED_{50}) = -0.992 \quad (\text{est. asymptotic s.e.} = 0.036)$$

Table 1 Adult flour beetles data reproduced from Pack and Morgan

Time (day)	Dose(mg/cm ²)							
	0.20		0.32		0.50		0.80	
	M	F	M	F	M	F	M	F
1	3	0	7	1	5	0	4	2
2	14	2	17	6	13	4	14	9
3	24	6	28	17	24	10	22	24
4	31	14	44	27	39	16	36	33
5	35	23	47	32	43	19	44	36
6	38	26	49	33	45	20	46	40
7	40	26	50	33	46	21	47	41
8	41	26	50	34	47	25	47	42
9	41	26	50	34	47	25	47	42
10	41	26	50	34	47	25	48	43
11	41	26	50	34	47	25	48	43
12	42	26	50	34	47	26	48	43
13	43	26	50	34	47	27	48	43
Total	144	152	69	81	54	44	50	47

In this paper, Pack and Morgan also proposed a survival analysis technique for this data, and the estimated $\ln(ED_{50})$ for day 13 were

$$\text{Male} : \ln(ED_{50}) = -1.365 \quad (\text{s.e.} = 0.034)$$

$$\text{Female} : \ln(ED_{50}) = -1.014 \quad (\text{s.e.} = 0.036)$$

From these investigations, Pack and Morgan concluded that male flour adults beetles are more susceptible than female beetles.

4.2. Model Selection

Model selection is a difficult but essential step in statistical modelling. It is usually started with visualization for the observed data to depict the latent relationship between the independent variables and the responses. Figure 1 illustrates the dose-time-response relationship for the observed data. It can be seen that the relationship between the log dose and the observed response (in logit) is relatively linear. However this linear relationship is worse between the time and the observed responses. In addition, for dose-time-response analysis, it would be more practical to consider the time effect or dose-time interaction.

Therefore, model (2.2) is fitted to this data with the logit link function for the generalized linear model, the fitted dose-time-response model for males is as follows:

$$\hat{\eta} = 3.443 + 2.376x - 7.418/t - 2.856x/t \quad (4.1)$$

and the estimated negative log-likelihood value in (2.1) is 544.86. The parameter estimates are highly statistically significant.

Figure 1 seems to suggest that there is a nonlinear quadratic relationship between the 1/time and the logit-transformed response. This can be modeled and tested with a higher-order interaction. The possible model is then a quadratic dose-time model as follows:

$$g(p) = \beta_0 + \beta_1x + \beta_2t^{-1} + \beta_3xt^{-1} + \beta_4xt^{-2} \quad (4.2)$$

With this model, the value of the fitted log likelihood (2.1) is 544.24. The likelihood ratio of this model (4.2) to the first-order interaction model (4.1) is then 0.62 ($= 544.86 - 544.24$). According to the theory of maximum likelihood estimation, this likelihood ratio is to be compared to the critical value of $0.5\chi^2$ with 1 degree of freedom, which is $0.5 \times 3.84 = 1.92$. Because this likelihood ratio is less than 1.92, it is concluded that the higher-order model (4.2) is not statistically significantly different from the first-order interaction model (4.1) and, therefore, model (2.2) is sufficient for this data to model the dose-time-response relationship.

It is possible that even the first-order dose-time interaction may not be significant and warrant further simplification to the model (2.2). Without the interaction, the model is simplified to $g(p) = \beta_0 + \beta_1x + \beta_2t^{-1}$ and the negative log-likelihood is estimated at 555.92. The log likelihood ratio is then 11.06 which is greater than 1.92, indicating that the interaction term is necessary and statistically significant.

To test whether there is a significant dose effect on the time to response, the general model (2.2) is used to test the reduced model: $g(p) = \beta_0 + \beta_2t^{-1}$.

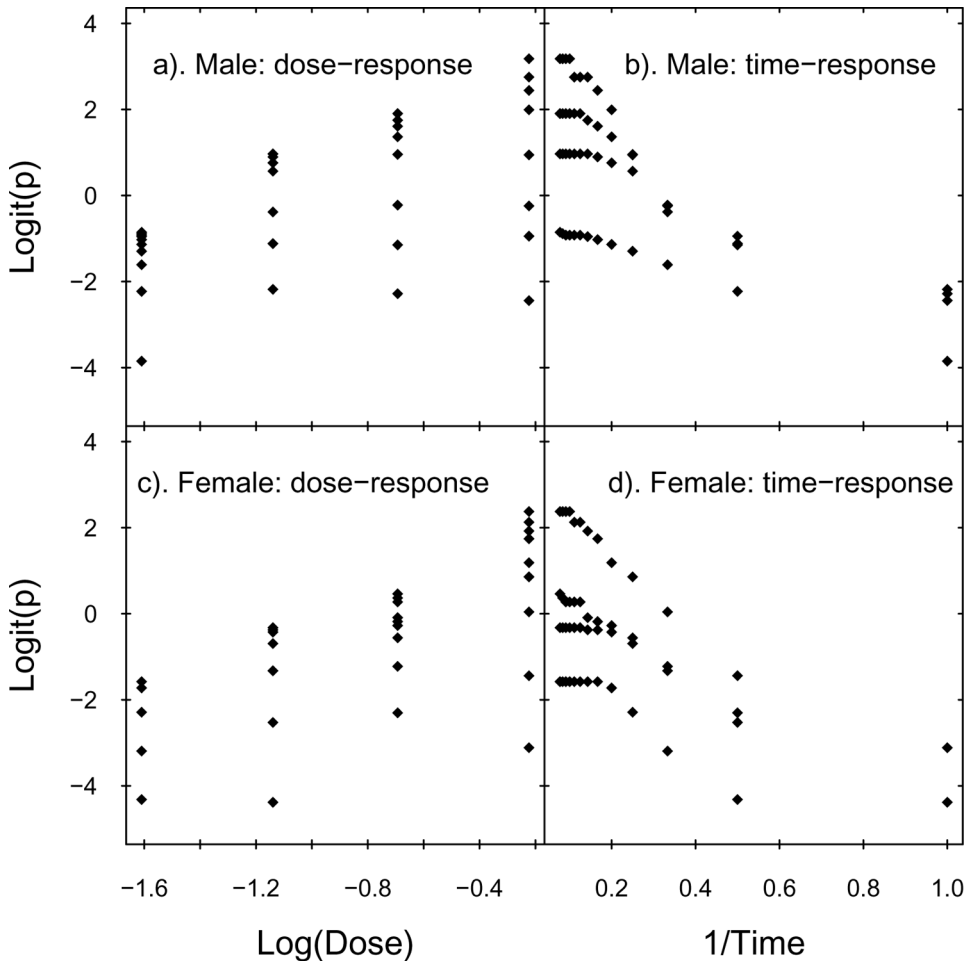


Figure 1 The dose-response and time-response relationship from the observed data.

The negative log-likelihood values for this reduced model is 589.59, which resulted the log likelihood ratio of 44.73 and indicated that the dose effect is in fact highly statistically significant. This is consistent with the conclusion from model (4.1) that the parameters are statistically significant. However, this conclusion is different from the Hewlett that there is no significant dose effect. As a referee commented intuitively that “a visual inspection of the data in Table 1 reveals that the time at which half of the beetles die is markedly different for the different doses. In fact, at the low dose (0.20 mg/cm^2), fewer than half of the insects die by the end of the experiment”, I entirely agree with his conclusion and conclude that the dose effect is statistically significant.

The same model is fitted to female beetle data and the estimated model is

$$\hat{\eta} = 3.005 + 2.594x - 7.676/t - 2.111x/t \quad (4.3)$$

Table 2 The estimated covariance matrix (square-rooted) for the parameter estimates

		Male				Female			
		$\hat{\beta}_0$	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$	$\hat{\beta}_0$	$\hat{\beta}_1$	$\hat{\beta}_2$	$\hat{\beta}_3$
Logit	$\hat{\beta}_0$	0.131	0.093	-0.222	-0.152	0.142	0.106	-0.277	-0.202
	$\hat{\beta}_1$	0.093	0.076	-0.152	-0.114	0.106	0.093	-0.203	-0.169
	$\hat{\beta}_2$	-0.222	-0.152	0.679	0.485	-0.277	-0.203	0.987	0.764
	$\hat{\beta}_3$	-0.152	-0.114	0.485	0.396	-0.202	-0.169	0.764	0.751
Probit	$\hat{\beta}_0$	0.041	0.029	-0.054	-0.038	0.046	0.034	-0.075	-0.056
	$\hat{\beta}_1$	0.029	0.025	-0.038	-0.030	0.034	0.030	-0.056	-0.048
	$\hat{\beta}_2$	-0.054	-0.038	0.143	0.105	-0.075	-0.056	0.247	0.193
	$\hat{\beta}_3$	-0.038	-0.030	0.105	0.090	-0.056	-0.048	0.193	0.193

Table 2 summarizes the estimated covariance matrix (square-rooted) for both male and female beetles. This table is provided for interested readers to reproduce the results and figures.

4.3. Estimation of $ED_{50}(t)$ and $LT_{50}(t)$

The estimated $ED_{50}(t)$ as a function of time, t , for male and female adult beetles is shown on Fig. 2. For both sexes, the $A(t)$ is positive for all time t and, therefore, the confidence intervals are meaningful.

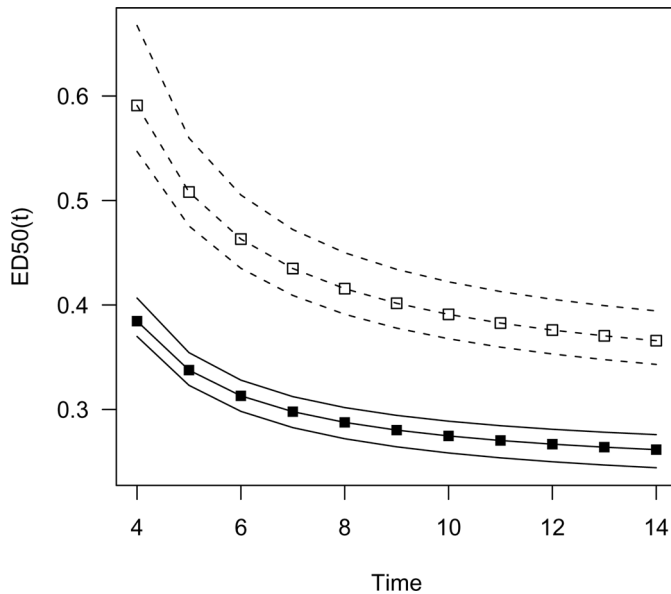


Figure 2 The estimated $ED_{50}(t)$ and the confidence bands as a function of time. The filled squares and lines are for male beetles. The open squares and dashed lines are for female beetles.

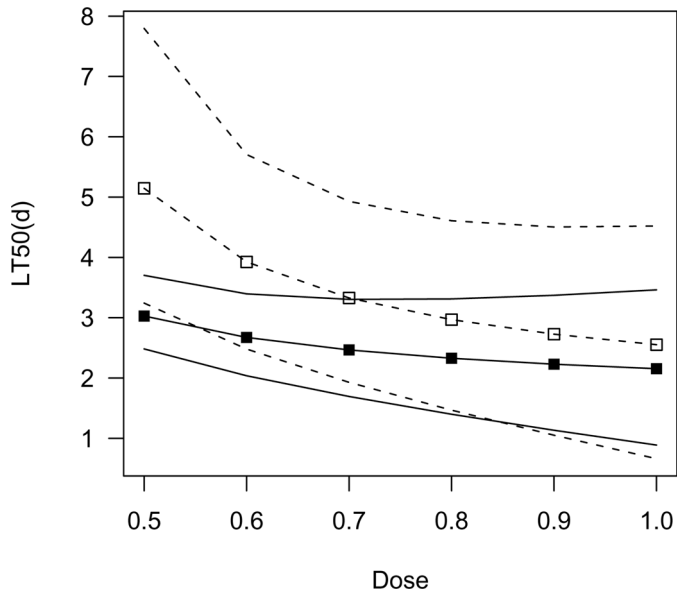


Figure 3 The estimated $LT_{50}(d)$ and the confidence bands as a function of doses. The filled squares and lines are for male beetles. The open squares and dashed lines are for female beetles.

It is evident from this figure that the estimated $ED_{50}(t)$ for males is always lower than that for females, which confirms that male flour adult beetles are more susceptible than female beetles (Pack and Morgan, 1990).

The estimation of $LT_{50}(d)$ for male adult flour beetles and female adult flour beetles can be seen from Fig. 3. Similarly, the estimated $LT_{50}(d)$ for males is lower than that for females which confirms the founding in Pack and Morgan, but the confidence intervals are overlapping.

These results also lead to the same conclusions by Hewlett that there is a significant sex effect on the time to response and that there is statistically significant evidence of differences in overall mortality between the two sexes. This is also similar to the conclusions by Diggle and Gratton (1984); Sandland (1984); Laurence and Morgan (1989).

4.4. Comparison Between the Logit and Probit Link Functions

In modelling quantal bioassay, both the logit and probit link functions are commonly used. As a comparison to the logit link function, the probit link function is, therefore, considered to fit the dose-time-response model (2.2). The estimated models are as follows:

$$\text{Male} : \hat{\eta} = 2.023 + 1.424x - 3.905/t - 1.443x/t$$

$$\text{Female} : \hat{\eta} = 1.738 + 1.539x - 4.215/t - 1.164x/t$$

with the estimated covariance matrix in Table 2.

The same discussion for the probit link function can be done for $ED_{50}(t)$ and $LT_{50}(d)$. For brevity, the endpoint estimates for $ED_{50}(13)$ and $LT_{50}(0.8)$ are presented for comparison. For logit link function, they are:

$$\text{Male : } ED_{50}(13) = 0.264 \quad (\text{with C.I. } (0.247, 0.278) \text{ and length } 0.031)$$

$$LT_{50}(0.8) = 2.328 \quad (\text{with C.I. } (1.399, 3.312) \text{ and length } 1.913)$$

$$\text{Female : } ED_{50}(13) = 0.370 \quad (\text{with C.I. } (0.348, 0.399) \text{ and length } 0.051)$$

$$LT_{50}(0.8) = 2.968 \quad (\text{with C.I. } (1.467, 4.608) \text{ and length } 3.141)$$

For probit link function, they are:

$$\text{Male : } ED_{50}(13) = 0.269 \quad (\text{with C.I. } (0.261, 0.273) \text{ and length } 0.012)$$

$$LT_{50}(0.8) = 2.101 \quad (\text{with C.I. } (1.874, 2.389) \text{ and length } 0.515)$$

$$\text{Female : } ED_{50}(13) = 0.377 \quad (\text{with C.I. } (0.370, 0.389) \text{ and length } 0.019)$$

$$LT_{50}(0.8) = 2.836 \quad (\text{with C.I. } (2.405, 3.430) \text{ and length } 1.025)$$

By using the length of confidence interval as a standard (Petkau and Sitter, 1989), we can see that the probit link function performed better than the logit link function for this data with narrower confidence intervals and, therefore, higher precisions.

As a conclusion for this data analysis, it is certain that in the course of the experiment the male adult flour beetles are more susceptible than female adult flour beetles, and the male adult flour beetles responded faster than female adult flour beetles for the insecticide, *pyrethrum*.

5. DISCUSSION

In this paper, a dose-time-response model is proposed to analyze the cumulative mortalities in the quantal bioassay experiment. With the estimated dose-time-response model, the associated estimation of $ED_{100p}(t)$ and $LT_{100p}(d)$ can be easily formulated. The approach for analyzing this type of data is carried out by a maximum likelihood method for a cumulative multinomial generalized linear model. The logistic and probit link functions are used to fit a real data set as application and illustration.

The proposed time-dose-response model incorporated the time effect and the time \times dose interaction in the model so that a more reliable and practical analysis can be carried out for real data. Different from the conventional procedures on ED_{50} , this paper formulated both the point estimator and confidence bands in closed form for $ED_{50}(t)$ as a function of time, which is more practical than the conventional analyses just on endpoint mortality. Besides, the time $LT_{100p}(d)$ to 100p% mortality for a specific dose, d , is also formulated by the estimated dose-time-response model.

These proposed procedures are applied to a real quantal bioassay data for pyrethrum in which the mortalities of flour adult beetles were measured over 13 days and four dose levels. It was found that the first-order dose-time interaction model is sufficient for this data verified by the statistical likelihood ratio test. The estimated $ED_{50}(t)$ for any time t and $LT_{50}(d)$ for any dose level d for male beetles are lower

than those for female beetles. This analysis concluded that the male adult flour beetles were more susceptible than female adult flour beetles which is consistent with other analyses for this data in Diggle and Gratton (1984); Sandland (1984); Laurence and Morgan (1989).

Other models can be easily considered in the framework presented in this paper for the dose-time-response including higher-order dose, time and dose \times time interactions, etc. It is demonstrated that the likelihood ratio test should be used for model selection and that one should statistically test the selected model in order to avoid overparameterization.

Some additional cautions might be required to deal with intracluster correlation among units residing in the same environment in this type of experimental setting. It is recognized that the study design for this type of experiment may not be robust with regard to this issue. However, the cumulative multinomial likelihood approach in this paper with the link function in time partially addresses this correlation in time.

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