Subtropical Plant Science, 51: 38-40.1999

Robustness of the Time-Dose-Mortality Model in Bioassay Data Analysis of Microbial and Chemical Agents for Insect Control

Ming-Guang Feng[1] and Tadeusz J. Poprawski[2]

[1]Department of Biological Sciences, Huajiachi Campus, Zhejiang University, Hangzhou 310029, P. R. China; and [2](corresponding author) Beneficial Insects Research Unit, USDA-ARS Subtropical Agricultural Research Center, and Texas Agricultural Experiment Station, Texas A&M University System, 2413 East Highway 83, Weslaco, Texas 78596
Correspondence to: T. J. POPRAWSKI; USDA-ARS-SARC Beneficial Insects Research Unit
2413 East Hwy. 83, Weslaco, TX 78596.
Voice: 956/969-4873; fax: 956/969-4888; e-mail: tadp@pop.tamu.edu

ABSTRACT

The time-dose-mortality modeling technique has been developed recently to process routine bioassay data for measurement of the efficacy of chemical and microbial agents for insect and mite control. This paper attempts to summarize the advantages of the modeling technique over classical probit analysis and discusses the biological implications of parameters generated by the model when microbial control agents are involved in bioassays. The modeling technique is recommended for processing time-dose-mortality data of chemical and microbial control agents against insect hosts because it allows for simultaneous measurement of the effects of time, dose, and time-dose interaction in a single model.

RESUMEN

El modelo estadístico tiempo-dosis-mortalidad ha sido desarrollado recientemente para procesar la información generada en bioensayos de rutina para medir la eficacia de los agentes químicos y microbianos en el control de insectos y ácaros. Este artículo intenta resumir las ventajas de este modelo sobre el análisis probit clásico y discute las implicaciones biológicas de los parámetros generados por el modelo cuando los agentes microbianos de control están implicados en pruebas biológicas. Esta técnica de modelos estadísticos es recomendada para procesar la información de tiempo-dosis-mortalidad de agentes de control químico y microbiano contra insectos hospederos porque permite medir simultaneamente en un solo modelo el efecto del tiempo, la dosis y la interacción de ambos.

Key Words: Time-dose-mortality model; probit analysis; microbial control agents; bioassay.

The most promising microbial species or strains for insect control can be recognized or selected largely based upon their indices of virulence (e.g., LD_{50} and LT_{50}) for the target insect species. The virulence indices are usually estimated by processing the time-dose-mortality (TDM) data that are generated in a routine bioassay. Finney's (1971) 'Probit Analysis' is used as the 'know how' in TDM analysis. Recently, however, a novel model was found to better fit the TDM data and to generate biologically sound parameters in estimating virulence indices (Robertson and Preisler, 1992; Feng *et al*., 1996; Nowierski *et al*., 1996). This model is called the time-dose-mortality model. We present an overview of the model and discuss its advantages over classical probit analysis in the processing of TDM data.

Limitations of Probit Analysis. A quantitative bioassay must include varying doses (or concentrations) of a biologically active agent (e.g., chemical toxicant or microbial agent), a target insect colony or population consisting of a reasonable number of individuals (e.g., >30) per dose and replication, and time intervals at each of which an observation is made for scoring a response. The response is 'all or none' for each individual, but is a binomial variable (a proportion ranging from 0 to 1) for the whole colony tested. Thus, the bioassay always generates data in the form:

d_1	n_{10}	n_{11}	n_{12}	...	n_{1J}
d_2	n_{20}	n_{21}	n_{22}	...	n_{2J}
...	...	...	...	...	...
d_I	n_{I0}	n_{I1}	n_{I2}	...	n_{IJ}

where d_i is the ith dose used in the bioassay (i= 1, 2, ···, I) and n_{ij} the number of target insects surviving the ith dose at the jth time interval (j= 1, 2, ···, J) during the bioassay. The cumulative mortality data at d_i can be estimated by calculating $(n_{i0}-n_{ij})/n_{i0}$.

There exists a graphic sigmoid relationship when the cumulative mortality of target insects is plotted against the values of doses used. This constitutes the biological background on which Finney (1971) developed the probit analysis. The sigmoid relationship can be linearized by simply transforming the values of doses and the cumulative mortality

to logarithms and probit units, respectively. Therefore, a linear regression model can be easily generated to describe the relationship between the probitized mortality and the log-transformed doses. Further, the estimates of the intercept and slope parameters from the regression can be used to compute the lethal dose causing an expected mortality (LD_{50}, LD_{75}, LD_{90}, etc.). Similarly, the linear regression of time (after treatment) against the probitized mortality at a given dose gives the intercept and slope for estiming the lethal time for an expected mortality at that dose (e.g., LT_{50}). This is the process of probit analysis. Probit analysis uses only a small part of the observations generated from the bioassay, e. g., mortality records only at one time interval (for lethal dose estimates) or at only one dose (for lethal time estimates). The interaction between dose and time, the most important characteristic for the biologically active agent tested, can not be depicted from probit analysis because the two variables are separately involved in the regression. Moreover, the intercept and slope parameters estimated from probit analysis simply provide a measurement of the quantitative relationship for the two variables involved, but have little biological meaning.

Time-Dose-Mortality Modeling Analysis. It is however possible to integrate into a single model time and dose, the two variables that appear to be significantly influential on mortality. The integration not only enables quantitative measurement of the time-dose interaction and allows generation of parameters useful for estiming virulence indices, but it also depicts the outlook of the bioassay in a biologically meaningful way. Preisler and Roberston (1989) described a model that satisfies these objectives and named it the time-dose-mortality model based on its function and usefulness. The model was used to successfully fit the data from bioassays of various chemical insecticides (Preisler and Roberston, 1989) and later was recommended for general use (Roberston and Preisler, 1992). When the model was recently introduced in the processing of bioassay data of entomopathogenic fungi on grasshoppers and aphids, Nowierski *et al.* (1996) further clarified the sources of variation associated with the modeling, and Feng *et al.* (1996, 1999) gave a biological interpretation to the parameters of the model. From these contributions, the TDM modeling technique for analysis of TDM data can be presented as follows.

Modeling. Assuming that a bioassay includes I doses or concentrations and J times of observation after treatment, the cumulative mortality probability, p_{ij}, caused by the dose d_i (i= 1, 2, ···, I) at the time of the jth observation t_j (j= 1, 2, ···, J) can be described as:

$$P_{ij} = 1 - \exp[-\exp(\tau_j + \beta \log_{10}(d_i))] \quad (1)$$

where β is the slope describing the dose effect, and τ_j the parameter(s) for the time effect of d_i during the period from the start to the jth observation, [t_1, t_2, ···, t_{j-1}, t_j). Equation 1 is called CLL model because it includes a linear part (i.e., $\tau_j + \beta \log_{10}(d_i)$) which is named the complementary log-log line or the linear predictor when $\ln(-\ln(1-p_{ij})$ is assumed to be linear in the covariates. However, because p_{ij} is a binomial variable dependent on time, it does not satisfy the requirement of time independence for the model, and therefore Eq. 1 can not fit directly the TDM data.

With the time independence in mind, let us consider the actual mortality, q_{ij}, caused by d_i during the time interval [t_{j-1}, t_j]:

$$q_{ij} = 1 - \exp[-\exp(\gamma_j + \beta \log_{10}(d_i))] \quad (2)$$

where β is equal to that in Eq. 1 and γ_j describes the time effect of d_i during [t_{j-1}, t_j]. Due to the independence between the time intervals, Eq. 2 is allowed to fit the TDM data by approaching the binomial response variable to the maximum likelihood equation:

$$\prod_{j=1}^{J} \prod_{i=1}^{I} q_{ij}^{r_{ij}} (1 - q_{ij})^{n_{ij} - r_{ij}} \quad (3)$$

where n_{ij} is the number of insects surviving d_i at t_{j-1} and r_{ij} (= n_{ij}-n_{ij-1}) represents the number of insects that actually die during the time interval [t_{j-1}, t_j]. The observed q_{ij} is estimated as r_{ij}/n_{ij} in the calculations. The fitting results in the parameters $\hat{\gamma}_j$, and $\hat{\beta}$ for Eq. 2. Then, the $\hat{\tau}_j$ is obtained using the formula:

$$\hat{\tau}_j = \ln\left(\sum_{k=1}^{j} e^{\hat{\gamma}_k}\right) \quad (4)$$

The fitting of Eq. 2 yields not only the estimates of $\hat{\gamma}_j$ and $\hat{\beta}$ but also their variance and covariance. The variance and covariance for $\hat{\gamma}_j$ and $\hat{\beta}$ can be estimated as:

$$\mathrm{var}(\hat{\tau}_j) = \frac{1}{e^{2\hat{\tau}_j}} \sum_{m=1}^{j} \sum_{n=1}^{j} e^{\hat{\gamma}_m + \hat{\gamma}_n} \mathrm{cov}(\hat{\gamma}_m, \hat{\gamma}_n) \quad (5)$$

$$\mathrm{cov}(\hat{\tau}_j, \hat{\beta}) = \frac{1}{e^{\hat{\tau}_j}} \sum_{n=1}^{j} e^{\hat{\gamma}_n} \mathrm{cov}(\hat{\beta}, \hat{\gamma}_n) \quad (6)$$

Goodness of Fit for the Binomial Variable. Nowierski *et al.* (1996) found that the Hosmer-Lemeshow statistic (Hosmer and Lemeshow, 1989), C, was suitable for testing the goodness of fit in the analysis of the TDM data. It is actually the modified Pearson's x^2 by grouping, i.e.,

$$\hat{C} = \sum_{k=1}^{g} \frac{(o_k - n_k \bar{\pi}_k)}{n_k \bar{\pi}_k (1 - \bar{\pi}_k)} \quad (7)$$

where o_k, n_k, and $\bar{\pi}_k$ are the number of insects that have died, the total number of insects, and the average conditional mortality probability estimated using Eq. 2, respectively, in the kth group. The C statistic follows a x^2 distribution with df=g-2 (usually g = 10).

Estimation of the Lethal Dose Effect. The estimates of $\hat{\beta}$ and $\hat{\tau}_j$ are used to calculate the logarithm of the lethal dose $\hat{\theta}_j$ responsible for p_{ij} at t_j

$$\hat{\theta}_j = \frac{\ln(-\ln(1 - p_{ij})) - \hat{\tau}_j}{\hat{\beta}} \quad (8)$$

and its variance

$$\mathrm{var}(\hat{\theta}_j) = \frac{1}{\hat{\beta}^2}(\hat{\theta}_j^2 \mathrm{var}(\hat{\beta}) + \mathrm{var}(\hat{\tau}_j) + 2\hat{\theta}_j \mathrm{cov}(\hat{\tau}_j, \hat{\beta}) \quad (9)$$

When p_i=0.5, Eq. 8 gives an estimate of $\log_{10}(LD_{50})$, i.e., $\hat{\theta}_j = (-0.3665 - \hat{\tau}_j) / \hat{\beta}$.

Estimation of the Lethal Time Effect. For a given d_i, the time it takes to cause an expected mortality, p_e, is calculated by:

$$\theta_i = \frac{t_j + (t_{j+1} - t_j)(p_e - \hat{p}_{ij})}{(\hat{p}_{ij+1} - \hat{p}_{ij})} \quad (10)$$

where $\hat{p}_i$ is estimated from Eq. 1 using $\hat{\beta}$ and $\hat{\tau}_j$. When p_e=0.5, Eq. 10 yields a solution to the LT_{50} at d_i.

Software for the Modeling. The TDM data modeling method is mathematically more robust and complicated than the probit analysis. The modeling requires the use of sophisticated software, e.g., GLIM (Payne, 1978; Roberston and Preisler, 1992), SAS Proc Logistic and Proc Genmod (SAS, 1992; Nowierski *et al.*, 1996). A new software, DPS, developed by Tang and Feng (1997), has a function that treats all the procedures outlined above by simply selecting 'Bioassay-TDM modeling' from a menu.

CONCLUSION

The TDM modeling method is significantly advantageous over probit analysis. First, the model includes the two independent variables, dose and time, and enables the generation of parameters that describe not only the separate effects of dose and time on the tested agent-insect relationship, but also the reasonable interaction between the two variables. Second, the parameters, $\hat{\beta}$, $\hat{\gamma}_j$ and $\hat{\tau}_j$, obtained from the modeling are biologically sound and can be interpreted clearly as the dose effect, and the conditional and cumulative time effects, respectively. Third, the modeling includes the use of all of the TDM data from a bioassay instead of part of them, thus yielding information that truly reflects the outcome of the experiment without misleading. Fourth, the length of time, j, corresponding to the largest value of the parameter for the conditional time effect can be used as an estimate of latent period of a microbial agent that is assayed against insects (Feng *et al.*, 1998). Finally, the model is generally useful for the analysis of data from bioassays that include a binomial variable which expresses the conditional response probability of the target organism and its dependence on the time and dose variables.

Considering the listed advantages, the TDM model is undoubtedly a very useful tool for replacement of the probit analysis method conventionally used. The model is strongly recommended for the analysis of TDM data of microbial control agents on arthropods.

ACKNOWLEDGMENTS

This work was supported by the Outstanding Young Scientists Foundation of China (grant No. 39525004) and the Natural Science Foundation of China (grant No. 39870513).

REFERENCES

Feng, M.G., Q.Y. Tang, G.C. Hu and S.W. Huang. 1996. Susceptibility of seven species of aphids to a *Beauveria bassiana* isolate: analysis of time-mortality model. J. Basic Sci. Engineer 4: 22-23.

Feng, M.G., C.L. Liu, J.H. Xu and Q. Xu. 1998. Modeling and biological implication of the time-dose-mortality data for the entomophthoralean fungus, *Zoophthora anhuiensis* on the green peach aphid, *Myzus persicae*. J. Invertebr. Pathol. 72: 246-251.

Feng, M.G., T.J. Poprawski, R.M. Nowierski and Z. Zeng. 1999. Infectivity of *Pandora neoaphidis* (Zygomycetes: Entomophthorales) to *Acyrthosiphon pisum* (Homoptera: Aphididae) in response to varying temperature and photoperiod regimes. J. Appl. Entomol. 123:29-35.

Finney, D.J. 1971. Probit Analysis, 3rd ed. Cambridge University Press, Cambridge.

Hosmer, D.W. and S. Lemeshow. 1989. Applied Logistic Regression. Wiley, New York.

Nowierski, R.M., Z. Zeng, S. Jaronski, F. Delgado and W. Swearingen. 1996. Analysis and modeling of time-dose-mortality of *Melanoplus sanguinipes*, *Locusta migratoria migratorioides*, and *Schistocerca gregaria* (Orthoptera: Acrididae) from *Beauveria*, *Metarhizium*, and *Paecilomyces* isolates from Madagascar. J. Invertebr. Pathol. 67: 236-252.

Payne, C.D., Ed. 1978. The GLIM System, release 3.77 manual. Numerical Algorithms Group, Oxford, England.

Preisler, H.K. and J.L. Roberston. 1989. Analysis of time-dose-mortality data. J. Econ. Entomol. 82: 1534-1542.

Roberston, J.L. and H.K. Preisler. 1992. Pesticide Bioassays with Arthropods. CRC Press, Boca Raton, FL.

SAS. 1992. SAS/STAT Users Guide 6.07. SAS Institute Inc., Cary, NC.

Tang, Q.Y. and M.G. Feng. 1997. Practical Statistics and Computer Processing Environment. China Agricultural Press, Beijing, P. R. of China.