A COMPARATIVE STUDY OF AN ACCELERATED LIFE-TEST MODEL AND A TOXICOKINETICS-BASED MODEL FOR THE ANALYSIS OF PORCELLIO SCABER SURVIVAL DATA

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Abstract—Statistical models have long been used for reliability analysis and risk assessment. In the present study, an accelerated life-test model was used to analyze a set of dose-time-response data obtained with the terrestrial isopod Porcellio scaber. Survival data were experimentally obtained by exposing P. scaber to diazinon (a nonpersistent insecticide) at six concentrations between 2 and 11.31 μg/g (toxicant/soil). Survival data are presented on a weekly basis. The accelerated life-test model assumed a log-normal distribution and constant variance across all diazinon concentrations. Model parameters were obtained by maximum likelihood estimation. The accelerated life-test model was compared to a toxicokinetics-based model reported in the literature. Survival predictions made by both models were compared with the observed data. Both the accelerated life-test model and the toxicokinetics-based model underestimated toxicity at a diazinon concentration of 8 μg/g. Overall, however, the accelerated life-test model outperformed the toxicokinetics-based model, with survival predictions closer to the observed data in most cases and a stronger correlation between predicted and observed survivals. However, as a statistical model, the accelerated life-test model did not reveal mechanistic information, and only statistical and distributional interpretations of its model parameters could be made.

Keywords—Accelerated life-test model Risk assessment Dose-time-response data

INTRODUCTION

Bioassays have attracted the attention of researchers in the fields of ecotoxicology and environmental safety as quick and relatively simple tools for assessing the toxicity of environmental pollutants. Some bioassay data are obtained as dose-time-response data; that is, the response of the testing organisms (death or some other endpoint) to a toxicant is monitored under different doses of the toxicant and at various inspection times. As Sun et al. [1] pointed out, dose-time-response data provide more information than the dose-response data that are more frequently seen in the literature. Toxicokinetics-based models have been continually developed for the understanding of bioassay data [2]. Of note are compartment models [3], such as the one-compartment model [4] and the multiple-compartment model [5], that are frequently used for analysis of toxicity data.

Bioassays that generate dose-time-response data resemble, in a way, accelerated life tests. Accelerated life tests are frequently conducted by industry, primarily for product reliability studies. Such tests are performed at high stressor (e.g., temperature) levels (compared to normal conditions) so that the failing process of a product is accelerated. This acceleration effect becomes more evident as the stressor level increases, and it can be modeled by the so-called failure-time regression analysis within a certain range of the stressor level. In this way, the reliability of the product under normal conditions can be estimated using the regression model built from the accelerated life tests, which can be significantly shorter in length than a test conducted under normal conditions. Similar to an accelerated life test, the toxicant under investigation in a bioassay serves as the stressor, and death (or some other endpoint) of the test organisms is expected to occur sooner as the toxicant concentration (or stressor level) increases.

Although bioassays and reliability studies are conducted for different purposes, the modeling approach used for accelerated life tests can be directly applied to the analysis of dose-time-response data obtained from bioassays. Many other statistical methods have been used for toxicity data analysis [6,7]. However, to my knowledge, accelerated life-test models have not been employed in this field. In the present study, the use of accelerated life-test models is demonstrated by analyzing a dose-time-response data set for which a mechanistically based model had been reported in the literature. The modeling performances of the accelerated life-test model and the mechanistically based model are compared, and the advantages and disadvantages of these models are discussed.

DATA DESCRIPTION

The terrestrial isopod Porcellio scaber has been used for evaluating soil toxicity. A detailed assay procedure was described in the study of Widianarko and Van Straalen [8]. Using diazinon (an organophosphorus insecticide) as a toxicant, those authors obtained the survival data of P. scaber. Note that diazinon is a nonpersistent chemical. Widianarko and Van Straalen developed a toxicokinetics-based model to describe the P. scaber survival data. Key assumptions of this model were that the external degradation of diazinon followed first-order kinetics, that the kinetics of the internal concentration of diazinon followed a one-compartment model, and that the hazard rate was proportional to the internal diazinon concentration. Widianarko and Van Straalen further obtained estimates of the model parameters by nonlinear regression.

This data set was chosen because of the nonpersistent nature of diazinon. In the case of nonpersistent toxicants, their con-
Accelerated life-test model

Table 1. Death and survival of Porcellio scaber exposed to diazinon

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*The last row shows the number of P. scaber that survived at the end of the experiment.

centrications vary with time because of evaporation, biotransformation, or other possible elimination mechanisms. However, in industrial reliability analysis, the stressor level normally does not change with time. Therefore, the existence of a nonpersistent toxicant in the present study constituted an extra challenge for the accelerated life-test model, which does not account for the change in stressor level with time.

The P. scaber data were originally presented as dose-time-response data [8]. The test organisms were exposed to six concentrations (µg/g [toxicant/soil]) of diazinon and were inspected daily for a total of 42 d [8]. However, survival data were presented on a weekly basis (i.e., at a regular time interval of 7 d) [8]. In this case, when a death occurred, it was known that the death occurred within some time interval (e.g., between 7 and 14 d). In reliability or life-test data analysis, data of this type are referred to as censored data. More specifically, left-censored data indicate that failures (or deaths) occurred at some (unknown) time before the inspection, and right-censored data indicate that failures (or deaths) were not observed before the inspection and will occur at some (unknown) time after the inspection. Interval-censored data indicate that failures (or deaths) occurred at some (unknown) time between two inspections.

In the present study, the P. scaber data were reexpressed as the number of P. scaber that died within each inspection time interval and the number of P. scaber that survived at the end of the experiments. These data are shown in Table 1. In the subsequent statistical analysis, according to the before-mentioned descriptions of censored data, data in the first row (0–7 d) of Table 1 were treated as left-censored data, data in the last row (>42 d) as right-censored data, and all other data as interval-censored data.

MAXIMUM LIKELIHOOD ESTIMATION

Maximum likelihood estimation (MLE) methods are frequently used to obtain parameter estimates for statistical models. Maximum likelihood estimation aims to find the model parameters that are the most consistent with the observed data by maximizing the likelihood function or, equivalently, the log-likelihood function. For a set of n independent observed data points, the likelihood function can be written as

\[ L(\theta; \text{data}) = C \prod_{i=1}^{n} L_i(\theta; \text{data}_i) \]  

where \( \theta \) is the model parameter or a vector containing the model parameters, \( L_i(\theta; \text{data}) \) is the likelihood of \( \theta \) given the observed data, \( C \) is a constant that is usually taken to be 1, and \( L_i(\theta; \text{data}_i) \) is the contribution to the overall likelihood of each observed data point and is calculated for left-censored, right-censored, and interval-censored data, respectively, as shown in the following equations:

\[ L_i(\theta; \text{data}_i) = \int_{0}^{t_i} f(t; \theta) \, dt = F(t_i; \theta) \]  

(2)

\[ L_i(\theta; \text{data}_i) = \int_{t_i}^{\infty} f(t; \theta) \, dt = 1 - F(t_i; \theta) \]  

(3)

\[ L_i(\theta; \text{data}_i) = \int_{t_{i-1}}^{t_i} f(t; \theta) \, dt = F(t_i; \theta) - F(t_{i-1}; \theta) \]  

(4)

where \( t \) is an inspection time, \( t_{i-1} \) is the inspection time immediately before \( t_i \), and \( f(t; \theta) \) and \( F(t_i; \theta) \) are the density and cumulative distribution function, respectively, of some assumed distribution of the observed data. For the P. scaber survival data set involved in the present study, note that several entries of zero are contained in Table 1. Because they do not contribute to the overall likelihood, they are not included in the statistical analysis that is described below.

DEVELOPMENT OF THE ACCELERATED LIFE-TEST MODEL

The statistical software SPLIDA (downloadable from the Internet at http://www.public.iastate.edu/~splida), which is based on the commercially available statistical software S-PLUS (Insightful, Seattle, WA, USA), was used for the statistical analyses in the present study. To determine the most suitable data distribution, six probability plots assuming the smallest extreme value, normal, logistic, Weibull, log-normal, and log-logistic distributions were made. The log-normal distribution was the most consistent with the observed data (see Results and Discussion) and was chosen for subsequent analyses.

An accelerated life-test data analysis model assuming log-normal distribution can be expressed as

\[ \Pr(T < t) = \Phi_{\text{normal}} \left[ \log(t) - (\beta_0 + \beta_1 x) \right] / \sigma \]  

(5)

where \( \Pr(T < t) \) is the probability of failure (or death) before time \( t \), \( \Phi \) denotes cumulative normal distribution, \( x \) is the (initial) toxicant concentration, \( \sigma \) is the standard deviation of the data, and \( \beta_0 \) and \( \beta_1 \) are constants. The parameters \( \sigma \), \( \beta_0 \), and \( \beta_1 \) can be obtained using the MLE method described previously. Note that no transformation of toxicant concentration (e.g., square root transformation, log transformation) was performed in the present study.

The model shown by Equation 5 assumes constant \( \sigma \) at all toxicant concentrations. The \( \sigma \) value of data obtained at each concentration can be estimated from the inverse of the slope of the corresponding fitted straight line in a log-normal probability plot [9]. This was conducted in the present study to determine whether this assumption was reasonable for the P. scaber data. A more rigorous examination of the constant \( \sigma \) assumption was also performed. As such, individual models in the form of

\[ \Pr(T < t) = \Phi_{\text{normal}} \left[ \log(t) - \mu \right] / \sigma \]  

(6)

were fitted to data at each toxicant concentration (note that \( \beta_0 + \beta_1 x \) is replaced by \( \mu \) when \( x \) is given), and the parameters \( \mu \) and \( \sigma \) were estimated using the MLE method. The values
of log likelihood were computed at each toxicant concentration, and the sum of the log-likelihood values ($l_i$) was recorded.

Additionally, the value of log likelihood ($l_i$) for Equation 5 was recorded when the maximum likelihood estimators were obtained. A log-likelihood ratio test was conducted by calculating $-2(l_2 - l_1)$. This value was compared to the critical $\chi^2$ value with appropriate degrees of freedom.

After obtaining model parameters, the number of deaths at each inspection time was predicted by multiplying the total number of $P. scaber$ put on test at each concentration by the probability of death at each inspection time as calculated using Equation 5 or Equation 6, depending on the results of the $\chi^2$ test. The model residuals were examined for possible departures from the log-normal distribution assumption.

**RESULTS AND DISCUSSION**

The probability plots assuming the six distributions (not shown) suggested that Weibull and log-normal distributions were likely candidates as an appropriate distribution assumption for the observed $P. scaber$ data. A closer inspection at the Weibull probability plot with MLE model fitting (Fig. 1) indicated departures from this distribution assumption at high concentrations (8 and 11.3 $\mu g/g$) and, to some degree, at low concentrations (2 and 2.83 $\mu g/g$). Because entries of zero in Table 1 did not contribute to the MLE and were not used (mentioned previously), fewer than six datum points were involved in model fitting at certain concentrations ($\geq 4.00 \mu g/g$). This can be seen in Figure 1 as well as in Figures 2 and 3. The log-normal probability plot with MLE model fitting (Fig. 2) also exhibited some departures from this distribution assumption at lower concentrations. Consequently, log-normal distribution was chosen as the underlying distribution model for further statistical analyses.

As mentioned previously, the slope of fitted line corresponds to $1/\sigma$ in a log-normal probability plot and can be used as a rough evaluation of the constant $\sigma$ assumption. The fitted lines in the log-normal probability plot shown in Figure 2 had approximately similar slopes, suggesting that $\sigma$ was approximately constant across all concentrations and that the variation exhibited in the slopes of the fitted lines in Figure 2 could be attributed to random error. For the log-likelihood ratio test, the log-likelihood values of the individual models (Eqn. 6) and of the constant-$\sigma$ model (Eqn. 5), as well as the parameter estimates for $\sigma$, are shown in Table 2. The $\chi^2$ with nine degrees of freedom (the difference between the number of parameters estimated) led to the following results: $-2(-393.4 + 384.8) = 17.2 < \chi^2_{0.01}$ (see later discussion). Thus, the assumption of constant $\sigma$ across all concentrations was acceptable.

The MLE parameter estimates for the accelerated life data model assuming log-normal distribution and constant $\sigma$ (Eqn. 5) are presented in Table 3. The standard errors and confidence intervals of the parameter estimates were obtained using the procedures described by Meeker and Escobar [9]. These values are provided in Table 3. The parameter estimate for $\beta_1$ had a value of $-0.3683$, with a 95% confidence interval of $-0.4617$ and $-0.2749$. Because this interval did not include zero, it can be concluded that the (negative) effect of toxicant concentration on $P. scaber$ survival was significant, which was expected.

Interpretations of model parameters can be made with reference to the log-normal distribution mathematically displayed by Equation 5. Equation 5 indicates that the logarithm of failure (or death) time—that is, $\log(t)$—is normally distributed with mean $\beta_0 + \beta_1 x$ and standard deviation $\sigma$. Because normal distribution is symmetric, $\beta_0 + \beta_1 x$ in fact represents $\log(t_{0.5})$, where $t_{0.5}$ is the time at which half the testing organisms die and half survive at a given toxicant concentration. To obtain $t_{0.5}$, an exponentiation of $\log(t_{0.5})$ is necessary. Therefore, $e^{\beta_0 + \beta_1 x}$ represents the half-life time of $P. scaber$ at a given toxicant concentration $x$.

Given a $\beta_0$ value of 5.4085 and a $\beta_1$ value of $-0.3683$...
The toxicokinetics-based model developed by Widianarko and Van Straalen [8] performed equally poorly for the concentration of 8 \( \mu \text{g/g} \) and generally less satisfactorily at other concentrations compared to the accelerated life-test model (Table 4). A measure of the badness-of-fit can be obtained by calculating the sum of squared differences between predicted and observed survival data. Such a measure had a numerical value of 696 for the accelerated life data model and of 983 for the toxicokinetics-based model [8]. Another comparison of model performance can be obtained by comparing model predictions with observed data and counting the frequency that one model outperforms the other. For the 36 observed datum points, the accelerated life-test model offered 25 predictions closer in absolute value to the observed data, whereas the toxicokinetics-based model offered only eight such predictions. Three ties were found between the two models. A third measure of model performance is the correlation between predicted and observed survivals. For the accelerated life-test model, the \( r^2 \) (squared correlation coefficient) was 0.90, whereas the \( r^2 \) for the toxicokinetics-based model was 0.87. Thus, it can be concluded that overall, the accelerated life-test model outperformed the toxicokinetics-based model in describing survival data of \( P. \ scaber \). This conclusion is not changed regardless of whether survival data at 8 \( \mu \text{g/g} \) obtained between 14 and 42 d were included in the comparisons.

The advantage of the toxicokinetics-based model is the physical interpretation of model parameters, as explained by Widianarko and Van Straalen [8]. This model also accounts for the elimination of nonpersistent chemicals. By contrast,
the accelerated life-test model is a statistical model, and only statistical and distributional interpretations, which do not reflect mechanistic information, can be made for its parameters. Nonetheless, as far as model performance is concerned, the toxicokinetics-based model did not seem to offer an advantage over the accelerated life data model for describing the observed *P. scaber* survival data. Results in the present study also suggested that the existence of a time-varying stressor did not seem to produce fatal flaws in the performance of the accelerated life-test model for the data set analyzed.

As mentioned previously, the accelerated life-test model (as well as the toxicokinetics-based model) failed for data obtained at 8 μg/g after 7 d of exposure (Table 4). The accelerated life-test model also provided unsatisfactory prediction for survival at 11.31 μg/g during the first inspection period (0–7 d), with an absolute difference of five between predicted and observed survivals. Despite the data reliability issues discussed previously, it is possible that toxic effect may be elicited via a different mechanism at high toxicant concentrations than at low toxicant concentrations, or that the toxicokinetics may change at high concentrations, as was observed previously [10]. A change in the failing mechanism usually leads to the breakdown of an accelerated life-test model; thus, such a model in its original form (Eqn. 5) can only be established within a certain range of the stressor level. Note that this change in toxicokinetics may also affect the performance of the toxicokinetics-based model, as was pointed out by Widianarko and Van Straalen [8]. In light of the potential change in mechanism or toxicokinetics, additional parameters may be considered to render the accelerated life-test model more flexible. The difficulty, however, may be obtaining the maximum likelihood parameter estimates for such a model given the size of the *P. scaber* survival data. Similar to the accelerated life-test model, the toxicokinetics-based model [8] may also be modified and extended. Widianarko and Van Straalen [8] also noted the potential conflict between model extensions and the size of available data.

It is well known that the variation in toxicity data of bioassays can be large. The survival data obtained at a diazinon concentration of 8 μg/g discussed previously could be caused by assay variability. This was not verified, however. Because of the potential limitation of resources, the observed survival data shown in Table 1 were obtained without replication of the experiment. Taking into account data variability, data reliability issues, and lack of replication, the significance level of the χ² test was chosen to be 0.01 instead of 0.05, which is more commonly used.

The dose-time-response data used to develop the accelerated life-test data were obtained by making repeated inspections during one experimental run for each concentration tested. The consequence of doing so is that the assumption of independent observations is violated. For example, at a given toxicant concentration, the number of *P. scaber* alive at an inspection time (except for the first inspection) is not independent from the number of *P. scaber* alive at the previous inspection time. This violation of the independence assumption impacts the accelerated life-test model as well as the toxicokinetics-based model [8], which incorporates statistical concepts. In this case, the question of interest becomes whether the accelerated life-test model can still be developed and used. As Thursby et al. [11] pointed out, scientific judgment sometimes needs to dominate over statistics. Results in the present study showed that the accelerated life-test model provided sufficient modeling power and can be used at least as an approximation.

The accelerated life-test model is simple and, therefore, cannot be used for toxicity data obtained from a complicated assay procedure. For example, in the study of Péry et al. [12], which involved the toxicity of zinc to *Daphnia magna*, the toxicant concentration was renewed at a certain point in time during the assay because of concerns regarding a too-low mortality of *D. magna*. The accelerated life-test model used in the present study cannot handle the artificial change in toxicant concentration, which limits its use in similar situations. Another limitation of the accelerated life-test model is that as a statistical method, a data set of sufficient size is required. In an attempt to model the survival data of Sheephead minnows first reported by Parrish [13], the accelerated life-test model (Eqn. 5) did not perform well because of the existence of many “zero deaths” observed at various toxicant concentrations and within different inspection intervals. By contrast, the model based on multiple-compartment theory that was developed by Sun et al. [1] seemed to give satisfactory model predictions for this particular data set.

**CONCLUSION**

An accelerated life data model assuming log-normal distribution and constant σ was applied to the survival data of *P. scaber* exposed to diazinon, a nonpersistent insecticide. The results of this model were compared with the results of a toxicokinetics-based model and the observed data. It was found that both the accelerated life data model and the toxicokinetics-based model failed to satisfactorily predict survivals at a diazinon concentration of 8 μg/g. In most cases, the accelerated life-test model yielded survival predictions closer to the observed data. Correlation between predicted and observed survivals was stronger for the accelerated life-test model than for the toxicokinetics-based model. Therefore, the accelerated life-test model outperformed the toxicokinetics-based model in terms of survival prediction. The parameters

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* Numbers without parentheses are observed data; numbers in parentheses are survivals predicted by accelerated life-test model developed in the present study (Eqn. 5); and numbers in brackets are survivals predicted by the model developed by Widianarko and Van Straalen [8].
Accelerated life-test model had statistical and distributional interpretations (e.g., half-life time of the test organisms at a given toxicant concentration) but did not contain mechanistic information.

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