United States Environmental Protection Agency | National Center for Environmental Assessment Research Triangle Park, NC 27711 | EPA/ September, 2008

Research and Development

Toxicodiffusion Model

External Draft Version 1.0

Prepared by

National Center for Environmental Assessment
Office of Research and Development
U. S. Environmental Protection Agency
Research Triangle Park, N C 27711

NOTICE

The U.S. Environmental Protection Agency, through its National Center for Environmental Assessment at Research Triangle Park, produced this report. This document is a preliminary draft. It has not been formally released by the U.S. Environmental Protection Agency and should not be construed to represent Agency policy. It is circulated as a guide for reviewers of the model.

TABLE OF CONTENTS

AUTHORS

Jeff Gift (Project Officer)
U.S. EPA National Center for Environmental Assessment
Research Triangle Park, NC

Angela Howard (Project Lead)
U.S. EPA National Center for Environmental Assessment
Research Triangle Park, NC

Yiliang Zhu
University of South Florida
Tampa, FL

Bruce Allen
Lockheed Martin Information Technology
Research Triangle Park, NC

SECTION 1: INTRODUCTION

This report is intended to provide an overview of beta version 1.0 of the implementation of a model of repeated measures data referred to as the toxicodiffusion model. The implementation described here represents the first steps towards integration of the toxicodiffusion model into the EPA benchmark dose software (BMDS). This version does run from within BMDS 2.0 using an option screen for making model selection as is done for other models in the BMDS 2.0 suite.

This introduction provides a "high level" background for the toxicodiffusion model and the current implementation. It discusses the perceived needs and requirements for this implementation. This is followed by a description of the modeling framework and criteria for implementing the toxicodiffusion model within BMDS, i.e., it is contrasted with other models that are part of the BMDS software. These discussions are intended to satisfy the reporting requirements of the Council for Regulatory Environmental Modeling (CREM, 2003) concerning the development, evaluation, and application of environmental models.

Specific toxicodiffusion model design issues (theoretical development, mathematical formulation and identification of data and parameter value inputs and constraints) and model coding are discussed in Section 2. In subsequent sections, the method of running the software is described, with some sample input and output explained as an example of its use in a risk assessment (Section 3). The user who simply wants to understand how to get the model running can skip ahead to Section 3, perhaps returning to Section 2 for information about the options that must be specified in the input file and the various choices available for those options.

Finally, in the Addendum is a report on the testing completed to date. That testing has focused on the comparison of runs of the original toxicodiffusion model (written in R and strictly within the R environment) to the BMDS-integrated version that is its current form. Thus the testing has focused on ensuring that the same results are obtained in the new version as were obtained in the previous version. One of the main purposes of the document is to provide reviewers information about the testing that was done; that information, with preliminary test results, is provided in the Addendum.

1.1 MODELING NEEDS AND REQUIREMENTS

Time-course, or repeated (response) measures, data can be utilized to characterize toxic effects that are potentially evolving along critical time points. Neurotoxicity experiments, such as neurobehavioral screening studies, often produce time-course data. It may be advantageous for dose-response assessments to consider the time impact on the potential toxic effects. Thus, a model that provides predictions of responses as a function of both dose (exposure) and time should be available in to provide relevant estimates (e.g., of BMDs) when a risk assessor must rely on such data. The software under consideration here is intended to be applied to such data to model the repeated measures (over time) of a single response metric following various levels of exposure.

Thus, the type of data that are amenable to modeling with the toxicodiffusion model have the

following characteristics:

- An outcome (response variable) measured on a continuous scale.
- Single exposure (or exposure interval), to several (4-5 recommended) "dose" levels.
- Duration of the experiment (the time component) coded between 0 and a maximum positive value, with 0 being the beginning and the maximum positive value the last time point at which data are available. The time at which exposure took place must be known and coded by a value between 0 and the maximum value.
- The outcome is observed (and recorded) repeatedly over time on each study subject; the timing of the observation is given. It is not required, however, that each subject (experimental unit) yield an equal number of observations at the same time points.
- Observations should not be aggregated over subject, and data must be identifiable with each subject.
- Multiple subjects per dose group.
- Dose effects are preferably observed at more than one dose level.
- Differences in dose effects are seen at some time points.

The modeling procedure currently allows only "dose" and "time" as covariates. The illustrations in this manual use data from neurobehavioral screening experiments; however, the methods and processes are applicable generally to other types of repeated measures data. The program currently implements the toxico-diffusion model for continuous outcomes (Zhu, 2005; Zhu et al. 2005a, b). Additional mathematical models may need to be developed to describe various shapes of the dose-time-response that cannot be well represented by the toxicodiffusion model; future versions are planned to include additional models.

1.2 MODELING FRAMEWORK -- GENERAL OVERVIEW AND COMPARISON TO BMDS CONTINUOUS MODELS

The toxicodiffusion model that is incorporated into BMDS represents a type of model unlike any other model currently in BMDS. That is because it incorporates more than one explanatory (independent) variable to define the dependent variable, which in this instance is the value of some continuous response of interest. In fact, the motivation for a model like the toxicodiffusion model is to specifically allow both dose (exposure level) and time since exposure to be determinants of the response, with the proper inter-relationships between the responses at different time points within subject.

Because the response variable is continuous, the toxicodiffusion model is most similar to the continuous models that are included in BMDS. Those models also predict response as a function of the explanatory variable (dose only in those models). However, to account for the time-course of response within individuals, the toxicodiffusion model differs substantially even from the other BMDS continuous models. Thus, no general theory presenting generic model equations, likelihood functions, and BMD computations is provided here.

Note, rather, that dose-response modeling of longitudinal (time-course; repeated measures) data is different from analysis of single time point data. Longitudinal outcomes provide vital information on the time course of the toxic effects (e.g. reversible vs. irreversible), and allow assessment and quantification of toxic effects with respect to the magnitude and direction at selected time points. In the case of the functional observational battery (FOB) or tests done for neurobehavioral studies, for instance, longitudinal data allow one to characterize the difference between behavioral alterations caused by exposure relative to natural changes over time in the absence of exposure. Therefore, dose-response relations must be viewed within the time frame. A dose-time-response may be expressed as follows

$$y(d,t) = \eta(d,t) + e = A(t) + f(d,t) + e$$

where y(d,t) is the continuous outcome taken from a subject at time t under exposure level d and e is the error term. The dose-time-response model, $\eta(d, t) = A(t) + f(d,t)$, has two additive components, the control (baseline) model A(t) that describes the time-trend of the control or reference population, and f(d,t) which describes exposure-induced changes in the outcome, i.e. the exposure-effects over time.

The specific mathematical formulation for the toxicodiffusion model is presented in Section 2.

SECTION 2: TOXICODIFFUSION MODEL: DESIGN AND CODING

This section provides information related to the technical and mathematical details underlying the toxicodiffusion model. It does provide some useful information for more casual users (e.g., with respect to the transformations of the explanatory variables) but users/reviewers may want to skip forward to Section 3, which provides information on the "how-to" of running the model. Then, if one needs more information about the details of the choices outlined there, the user can obtain them from this section.

2.1 THEORETICAL DEVELOPMENT

It is common toxicological knowledge that the level of response to an insult may depend on the time since that exposure occurred, certainly for transient or repairable effects. That is, the response levels (for any exposure level) may change as a function of time since exposure; there may be a time-course pattern that can be modeled. The toxicodiffusion model is one example of a model that provides a statistically valid approach to the representation of repeated measures data via time-course prediction. In addition to the time-course (time dependence), it also accounts for level of exposure as a determinant of response (at all time points after exposure).

2.2 MATHEMATICAL FORMULATION

The equation for the toxicodiffusion model is given as follows:

$$\eta(d,t) = A(t) + B \cdot t \cdot d \cdot \exp(-k \cdot t)/(1 + C \cdot d \cdot t \cdot \exp(-k \cdot t))$$

where t is time, d is dose, and the parameters to be estimated include B, C, and k. In addition, the function A(t) can be specified to be one of the following:

$A(t) = A_0$; or
$A(t) = A_0 + A_1 t$

so that there may be up to 2 additional parameters (A_0 and A_1). The function A(t) does not include dose; it represents the time course that would be predicted in the absence of exposure. While background changes over time might be more complex, simple polynomial functions appear adequate for describing the observed time trajectory in most neurotoxicity studies that last for up to a few weeks. In future implementations, a second degree polynomial ($A_0 + A_1 t + A_2 t^2$) will be allowed.

The equation

$$f(d,t) = B \cdot t \cdot d \cdot \exp(-k \cdot t)/(1 + C \cdot d \cdot t \cdot \exp(-k \cdot t))$$

(i.e., the toxicodiffusion equation) depicts exposure effects that are transient over time, with peak

effects

$$\frac{Bd}{Cd + k * e}$$

occurring at t=1/k for any dose level. At sufficiently large dose levels (C*d>> k*e), the quantity B/C is the peak response above the background. When first order kinetics are applicable, the parameter k can be interpreted as the elimination coefficient. The quantity B/(K*e) is the initial rate of change (dose-slope at d=0) in response at the time of peak effect (TOPE), t=1/k. The quantity B*d is the initial rate of change (time-slope at t=0) of the dose-response.

2.3 PARAMETER ESTIMATION

Individual Susceptibility and Random Effects

Individual susceptibility to environmental exposure is an important source of variation, and should be accounted for as much as possible in various stages of risk assessment, including dose-response modeling. Yet variation in susceptibility is difficult to quantify biologically (e.g., because relevant biomarker data are not available). A statistical attempt to address this issue is to use the so-called random effects model to characterize the difference in response observed among subjects. This approach leads to individual dose-time-response curves for each subject, which deviates from an "average" dose-response model for the entire population. To characterize individual models, we attach a set of distinct coefficients (random effects), one for each subject, to selected model coefficients (model parameters or fixed effects). For the toxico-diffusion model, random coefficients can be, in principle, attached to each and every model coefficient (A_0, A_1, B, C, k) although the available data may be limited and cannot accommodate some selections. Thus, biological consideration and statistical criteria should be exercised to guide a practical selection of random effects.

Attaching random coefficients to A_0 allows for baseline variation in response variable among subjects; random coefficients for B characterize variation in dose-time slope among subjects. When it is reasonable to view k as the elimination parameter, it is appealing to allow for individual variation in this toxicokinetic parameter, although substantial data are necessary in order to estimate an individual level of elimination.

The statistical approach of random effects is attractive because of several aspects (Zhu et al. 2005a). First it provides a more accurate description of the "average" as well as individual dose-time-response curve by separation of between-subjects variation from error. Secondly, it includes all data in the analysis despite incomplete data arising from, for example, death of a subject, or an unbalanced experiment in which all subjects were not equal with respect to the number of time points at which response data were observed. This is a major advantage over the traditional analysis of variance. Finally, the random effects approach can incorporate serial correlation among repeated measures of the same subject when necessary.

In the current implementation, the only parameter to which random effects can be attached is A_0.

Model Fitting

Model fitting for the toxicodiffusion model is a process in which the unknown model parameters are estimated. Estimates are made for the values that maximize the likelihood function associated with the model, the distribution assumed on the error, and the distribution assumed on the random effects. Model parameters are the coefficients in the dose-time-response model including those in the toxicodiffusion function, the control trajectory, and the standard deviation of the random effects and the error. Normal distributions are assumed for the error and the random coefficients. Maximization of the likelihood function is implemented using the EM-algorithm and the Newton-Raphson algorithm as described in Lindstrom and Bates (1988, 1990). A thorough description can be found in Pinheiro and Bates (2000, Chapters 7 and 8).

There are two steps in the iteration. The first step involves the Newton-Raphson algorithm to iteratively update the estimate for the parameter vector $\hat{\beta}$ from the previous (w-th) iteration:

$$\beta^{(w+1)} = \beta^{(w)} + \left[X^{(w)^{\mathrm{T}}} V^{(w)^{-1}} X^{(w)} \right]^{-1} X^{(w)^{\mathrm{T}}} V^{(w)^{-1}} \underbrace{\left(Y - \eta(A\beta^{(w)} + Bb^{(w)}) \right)}_{residuals}$$

$$+ \left[X^{(w)^{\mathrm{T}}} V^{(w)^{-1}} X^{(w)} \right]^{-1} X^{(w)^{\mathrm{T}}} V^{(w)^{-1}} Z^{(w)} b^{(w)}$$

where

$$X^{(w)} = \left. \frac{\partial \eta}{\partial \beta^{\mathrm{T}}} \right|_{\beta^{(w)}, b^{(w)}}, \quad Z^{(w)} = \left. \frac{\partial \eta}{\partial b^{\mathrm{T}}} \right|_{\beta^{(w)}, b^{(w)}},$$

and

$$V^{(w)} = \Lambda^{(w)} + Z^{(w)} \tilde{D}^{(w)} Z^{(w)^{\mathrm{T}}}.$$

All quantities in the formula above are evaluated at $\hat{\beta}^{(w)}$ and random effects $\hat{b}^{(w)}$. The variance parameters in $V^{(w)} = \Lambda^{(w)} + Z^{(w)} \tilde{D}^{(w)} Z^{(w)^{\mathrm{T}}}$ can be estimated using the restricted maximum likelihood method (REML), where the matrix Λ is the variance of the random effects, and D the variance of the error. The final estimate $\hat{\beta}$ is obtained when $\hat{\beta}^{(w+1)}$ and $\hat{\beta}^{(w)}$ become sufficiently close under numerical convergence criterion. The second step is to update the random effects $\hat{b}^{(w)}$ using a least square method. The procedure then iterates between these two steps. The iterative formula for $\hat{\beta}$ is given here because it is the basis for a one-step bootstrap estimate of β.

Iteratively fitting a nonlinear model requires carefully choosing appropriate starting values in order to achieve convergence. This is discussed in Section 3.2. Even when a model achieves a numerical fit, it still may not be an appropriate model. The appropriateness of the model needs to be judged on both biological and statistical grounds (see Section 3.4 below).

2.4 BMD ESTIMATION

A benchmark dose is defined as the exposure level that can induce a specified level of increase

in response. For this implementation of the toxicodiffusion model, the approach that is implemented in the toxicodiffusion model is the so-called "hybrid approach." This approach is based on the specification of a two parameters. The first parameter is the level of risk increase, γ, of interest; it is termed benchmark response level (BMR) and is typically chosen to be within the range of 1%-10% additional or extra risk, but may depend on other factors relevant to the ultimate use of the risk assessment. Given a measure of probability of adverse response, p(dose, time)=p(d,t) (see below), the BMR is expressed relative to the background probability of adverse response. That is, for additional risk,

$$\gamma = Ra(BMD\gamma, t) = p(BMD\gamma, t) - p(0, t)$$

or for extra risk

$$\gamma = Re(BMD\gamma,t) = [p(BMD\gamma, t)-p(0,t)]/[1-p(0,t)].$$

In the expressions above, p(0, t) represents the background probability of adverse response at time t.

The other parameter that needs to be specified is the background probability of adverse response. When the outcome measure is continuous, as it is for the toxicodiffusion modeling under consideration, the probability measure p(d,t) (and therefore the relative risk metrics Ra and Re) can be defined in a number of ways. The toxicodiffusion program adopts a generic approach (Gaylor and Slikker, 1990; Gaylor et al., 1998) to quantify the probability of "extreme" (adverse) values (e.g. above a higher or below a lower percentile) as predicted by the dose-response model, and uses this risk for p(d,t). The method can be applied at any fixed time t using the fitted model which interpolates between the experimental time points.

From Dose-Response Model to Probabilistic Risk

Upon fitting a dose-time-response model $\eta(d,t)$ as the mean response at dose level d and time t, a statistical distribution is first postulated to describe the variability of the measurements around the mean response, $\eta(d,t)$. From the control data, intervals of extreme values are determined in conjunction with available toxicological evidence. Such intervals of "adverse" outcome are determined by the lower or upper percentiles of each timed-sample of the control. Outcome values that are below the lower percentile or above the upper percentile constitute adverse outcomes. Percentiles as cut-off points are generally preferable when the distribution is not symmetric, and are estimated by the 100i/(n+1)-percentile from a sample of n observations. In the special case of a normal distribution around the mean $\eta(d,t)$, the lower percentile can be determined as

$$C_L = \eta(0,t) - z_{1-\alpha}\sigma_0$$

for data at time t, where $z_{1-\alpha}$ is the (1-α)100% percentile of the standard normal and σ_0 is the standard deviation of the control distribution, which is assumed to be constant over time for simplicity. Similarly, the upper percentile is given by

$$C_U = \eta(0,t) + z_{1-\alpha}\sigma_0$$

Therefore, if the toxic effects are manifested by reduced level of response (decreases in the response variable are adverse), the probabilistic risk is

$$p(d,t) = \Pr ob(y < C_L \mid mean = \eta(d,t))$$

which can easily be determined given the postulated distribution for the data at time t. Using the normal distribution approximation, we have

$$p(d,t) = \Phi(\{\eta(0,t) - \eta(d,t) - z_{1-\alpha}\sigma_0\}/\sigma_d),$$

where σ_d is the standard deviation of the distribution for data of dose group d, and Φ is the cumulative distribution function of the standard normal distribution. Currently σ_d is replaced with a pooled estimate across dose groups. Future versions of the program may allow different values.

Alternatively, if the user has a specific value in mind for the cut point, to replace either C_L or C_U, then the software allows specification of actual numeric values for such a cut point. When using this option, note that the background probability of adverse response is not known a priori. But the BMR level can be specified and the program will first estimate the background probability of response and then find the BMD such that, at that BMD, the additional or extra risk is equal to the BMR (e.g., the additional risk will be equal to the BMR, say 10% above whatever the background probability of response happens to be). The user should be aware that nonsensical answers might occur if the specification of the cut point is not "in sync" with the observations in the data set being analyzed.

BMD Estimation

If the toxic effects are manifested by reduced level of response (decreases in the response variable are adverse) then, for extra risk, BMD_γ satisfies the equation

$$\eta(BMD_\gamma,t) = \eta(0,t) - z_{1-\alpha}\sigma_0 - \Phi^{-1}[p(0,t) + (1 - p(0,t))\gamma]\sigma_d;$$

for additional risk the equation simplifies to

$$\eta(BMD_\gamma,t) = \eta(0,t) - z_{1-\alpha}\sigma_0 - \Phi^{-1}[p(0,t) + \gamma]\sigma_d.$$

If the toxic effects are manifested by increased level of response (increases in the response variable are adverse), the BMD equations can be derived similarly with '$+ z_{1-\alpha}$' in place of '$- z_{1-\alpha}$' in the preceding equations.

In order to develop a time-profile of the benchmark dose (BMDγ), a sequence of time points {t} were chosen and the corresponding sequence {BMDγ(t)} is computed. To estimate risk conservatively, the program reports the minimum value

$$BMD_\gamma(t^*) = \min_t \{BMD_\gamma(t)\}$$

within a chosen time window in which critical effects are anticipated. Where the minimum value $BMD_\gamma(t^*)$ falls depends on the dose-time-response model as well as the assumed data distribution. Under some simple models, t* is a point where the risk is the largest given BMR and dose levels (Zhu, 2005). Further, if the data distribution is normal and the background risk is constant over time, t* coincides with the TOPE where the rate of change is zero with respect to time. For these models, including the toxicodiffusion model, BMD at the estimated TOPE is the minimum BMD. In general, numerical algorithms are needed to determine t* or the minimum BMD.

BMDL: Bootstrap Lower Confidence Limits to BMD

Because the BMD derived from the data and the fitted model is an estimate, its lower (sometimes upper) confidence limit is often reported as a way to quantify the statistical uncertainty associated with the BMD estimate. The toxicodiffusion model uses a bootstrap procedure (Zhu et al., 2005b) to compute the lower confidence limit, denoted by BMDL. An upper confidence limit (BMDU) can also be computed, although it is less common within the context of risk assessment.

The bootstrap method utilizes re-sampling of residuals derived from a fitted model and repeats the sampling procedure a large number of times (e.g. 1000 – 2000). Each re-sampled residual results in a new estimate of the model parameters, from which a bootstrap BMD(t) is derived. This process produces a sample of BMD(t) of which the 5th percentile can serve as an estimate of 95% lower confidence limit or BMDL. A two-sided confidence interval is formed if the user also computes the upper confidence limit BMDU (see the options for model runs given in Section 3.3). The BMDL and BMDU reported in that case then constitute the endpoints of a (1-α/2)100% confidence interval.

While other methods (e.g. delta) can be developed to estimate BMDL, closed-form formulae are unlikely because of the random effects in the model. The bootstrap method is an effective way to circumvent this and other analytical difficulties as the bootstrap can enumerate the variation of the statistic at hand with a large number of replications made increasingly easy with today's computing power. Our recommendation is that at least 1000 bootstrap resamples be computed to obtain a stable BMDL or BMDU estimate. Further research is needed to investigate an acceptable range for the number of replications, but the user can determine empirically for any data set how many bootstrap samples appear to be giving consistent bound estimates (although this requires several reruns of the program to get several BMDL or BMDU estimates that can be compared to determine if consistency, stability, has been achieved).

The bootstrap methods (cf. Efron and Tibshirani, 1993, Chapter 13) simulate the variation of the

statistic of concern (e.g. BMD). They generally consist of a large number of replications of two steps: resampling the existing data, and deriving the statistic. The bootstrap sample of the statistics then describes the variation of the statistic. For the BMD computation, we derive an alternative approach to resampling of the original data.

First, we fit the toxicodiffusion model to a dataset and calculate the BMD based on the original sample of data. From the fitted model, we calculate the residuals. The bootstrap BMD now consists of two steps: (1) sampling residuals as well as the random effects, B times, and plugging in each bootstrap sample into an one-step formula which gives a bootstrap estimate of the model parameters; (2) using the bootstrap estimate of the parameters to derive a bootstrap BMD estimate $BMD^b(t)$ where the superscript b denotes the b^{th} bootstrap iteration of the BMD(t) estimate. The bootstrap sample $\{BMD^b(t); b = 1, 2, \ldots, B\}$ elucidates an empirical distribution that approximates the true variation of the BMD estimate if the experiment were repeated over and over again. The lower percentile (e.g. 5%) of this bootstrap sample $\{BMD^b(t); b = 1, 2, \ldots, B\}$ is used by the toxicodiffusion software as an estimate of the confidence limit or BMDL.

The bootstrap method of sampling residuals in the present context is reported by Zhu et al. (2005b), and is an extension of an earlier method by Moulton and Zeger (1991) for the case of no random effects. Let's denote the residuals vector by

$$\hat{e} = Y - \eta(A\hat{\beta} + B\hat{b})$$

and the random effects vector by $\hat{b}$. Plugging in the bootstrap sample of residuals $\hat{e}^b$ and random effects $\hat{b}^b$, into the formula below yields the bootstrap estimate of the model parameters $\hat{\beta}^b$:

$$\hat{\beta}^b = \hat{\beta} + [X^t V^{-1} X]^{-1} X^t V^{-1} \hat{e}^b + [X^t V^{-1} X]^{-1} X^t V^{-1} Z\hat{b}^b$$

where

$$X = \left.\frac{\partial \eta}{\partial \beta^{\mathrm{T}}}\right|_{\hat{\beta},\hat{b}}, \quad Z = \left.\frac{\partial \eta}{\partial b^{\mathrm{T}}}\right|_{\hat{\beta},\hat{b}}, \quad V = \hat{\Lambda} + Z\hat{\tilde{D}}Z .$$

Each bootstrap $\hat{\beta}^b$ then leads to a bootstrap estimate of BMD(t) as described above.

Bootstrap sampling of error and random effects involves three factors: (1) whether to also bootstrap sample random effects in addition to residuals; (2) whether the sampling unit is all residuals of the same subject or an individual residual; (3) whether to sample within or across dose groups. The first option is determined by the significance of random effects. If the random effects are either insignificant or of negligible size, it is less effective to bootstrap random effects and suffices to bootstrap-sample residuals only. The second option is largely determined by potential serial correlation among data from the same subject. For FOB data or other data over a relatively short time period, bootstrapping individual residuals appears practical because serial correlations are less likely to be evident. In the presence of serial correlation or clustering, the

experimental subject would be the sampling unit. The last option is relevant when, for instance, the variances are non-constant across dose (experimental) groups. In such cases, dose-stratified, bootstrapping is appropriate.

The following is a step-by-step summary of the bootstrap BMD process applied in the toxicodiffusion software (Zhu et al. 2005b):

Step 1. Upon fitting a mixed-effects toxicodiffusion model to the data, obtain the residuals $\hat{e}_{ijk}$ and estimates of random effects $\hat{b}_{ij}$, where i=1, …, M, j=1, …, N, and k=1,…, L are the indices of dose, subject within dose group, and time, respectively.

Step 2. Randomly draw, with replacement, a sample of residuals $\hat{e}^b_{ijk}$ and/or random effects $\hat{b}^b_{ij}$. The bootstrap sample of residuals must be of the same length as the original residuals, and the length of the random effects sample must be the same as the original random effects. Depending on the covariance structure specified for the data, there are several options:

- In the presence of strong evidence of random effects, bootstrapping random effects in conjunction with bootstrapping residuals;
- In the presence of strong and significant serial correlation within subject, bootstrapping residuals should take the residual vector of each subject as the sampling unit.
- In the presence of varying variance across dose groups, sampling residuals within each dose group.

The three options lead to eight possible combinations of bootstrap sampling. Analysis of neurobehavioral screening data (e.g. FOB) and a preliminary simulation study suggest bootstrapping individual residuals and random effects within or across dose groups is practical.

Step 3. Compute the bootstrap estimate of the parameter $\hat{\beta}^b$ through the equation shown above.

Step 4. Compute $BMD^b(t)$ following the BMD computation procedure at a selected sequence of time points t.

Step 5. Repeat Steps 2-4 B times to obtain the bootstrap sample BMD, $\{BMD^1(t), \ldots, BMD^B(t)\}$.

Step 6. Estimate the empirical α(100)-percentile for the bootstrap sample $\{BMD^1(t), \ldots, BMD^B(t)\}$ at every t in the sequence.

2.5 MODEL CODING

R code was developed that implements the toxicodiffusion model and computes the estimates of interest to the user. That code was developed for EPA by Dr. Y. Zhu of the University of South Florida.

An interface was added within BMDS 2.0 to access the R model code and to make it run in the background of BMDS 2.0. Thus, for the user to analyze a time-course (repeated measures) data set, s/he will have to have R installed on his/her computer. The BMDS interface uses a version of "option screen" specifications for the model parameters that is similar to that used for other BMDS models.

The interface coding was accomplished within a Windows XP environment. All tests of the code have been carried out with that operating system. Version 2.7.0 or higher of R has been installed on all computers used for the tests.

SECTION 3: RUNNING THE MODEL

The current version of the toxicodiffusion model software was built to run on a Windows platform. The code has been run and tested using the Windows XP operating system.

Prior to running the model, the user will have to ensure that s/he has a version of BMDS 2.1, Build 39 or higher, installed and operating on his/her computer. NOTE: BMDS 2.1 can be installed along side BMDS 2.0; there is no need to uninstall BMDS 2.0 when installing BMDS 2.1. Only BMDS 2.1 (or later versions) will have capability to run the toxicodiffusion model.

Moreover, the user should have downloaded (from http://www.r-project.org/) and installed the latest version of R (currently version 2.7.0). The toxicodiffusion model software within BMDS 2.1 has only been tested with version 2.7.0. It is not required that an R session be running when BMDS 2.1 is used to run the toxicodiffusion model; merely having R properly installed will allow BMDS to run the toxicodiffusion model for the user.

3.1 THE INPUT FILE FORMAT AND INITIATING A MODEL RUN

Because the toxicodiffusion model requires identification of the observations with the corresponding, specific experimental units, the input file will have several entries per experimental unit, each one corresponding to a single measurement of the response at a particular time for a single experimental unit.

The user can start the process of creating a .dax file (the standard extension for BMDS 2.0 data files) for use in BMDS in one of several ways. The data may be entered in a spreadsheet; or the data may be in a flat text file, delimited as desired (e.g., by commas or spaces). With either of these options, the user can use the BMDS data import utility to create a .dax file for analysis. Within BMDS, click on the "New Dataset" Icon and a data grid screen will appear. Then from that grid screen, select the "Import Data From …" option under the "File" menu item. That option specifies the types of files that may be imported into BMDS to create the .dax file. Refer to the BMDS Help file for further detailed assistance.

Alternatively, the user may just enter the data directly into a blank grid screen opened by selecting the "New Dataset" icon.

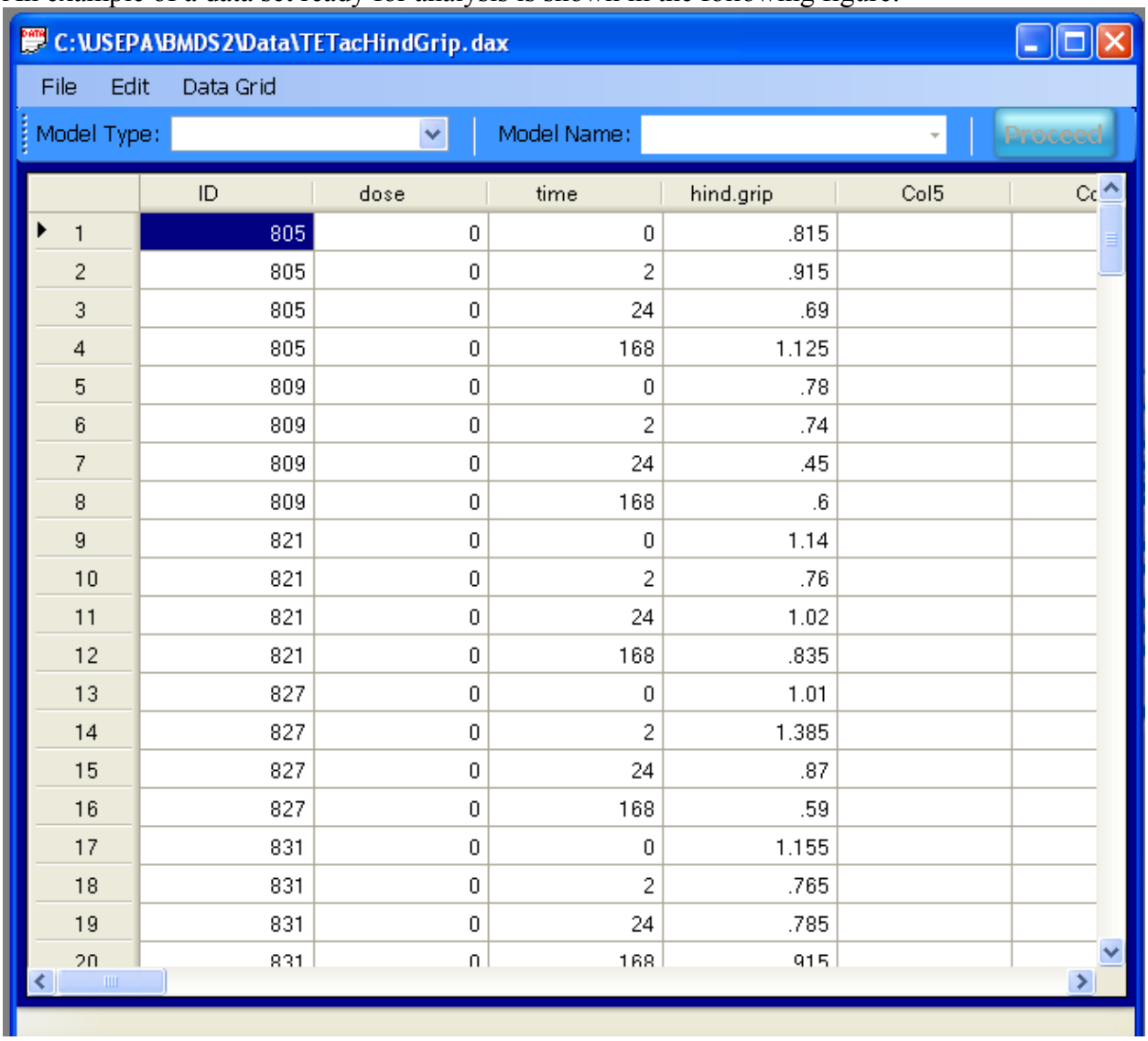
Note that, however the user chooses to create a data set for analysis, that data set will have the following characteristics:

- It will have three columns corresponding to the experimental unit identification number, dose given to that unit, and the time of observation of the response;
- multiple responses may be listed in additional, separate columns, entailing a total of at least four columns in any given data set, where the values entered are the continuous responses measured for the particular experimental unit at the particular time given in the row in question.

If, for a given row of data, a field is missing (e.g., was not recorded or was lost), then the user

should input the value -9999 as an indicator of (flag for) the missing value. This might only be applicable when the response value is missing, as a way of indicating that a data point was expected but not available. An alternative and equivalent solution for the missing data issue is just not to include the rows of data with missing values in the data file. As noted above, the random effects approach used for model fitting does not require that every experimental unit have the same number of observations or have observations all at the same time points across units.

An example of a data set ready for analysis is shown in the following figure:



	ID	dose	time	hind.grip	Col5	Co
1	805	0	0	.815		
2	805	0	2	.915		
3	805	0	24	.69		
4	805	0	168	1.125		
5	809	0	0	.78		
6	809	0	2	.74		
7	809	0	24	.45		
8	809	0	168	.6		
9	821	0	0	1.14		
10	821	0	2	.76		
11	821	0	24	1.02		
12	821	0	168	.835		
13	827	0	0	1.01		
14	827	0	2	1.385		
15	827	0	24	.87		
16	827	0	168	.59		
17	831	0	0	1.155		
18	831	0	2	.765		
19	831	0	24	.785		
20	831	0	168	.915		

In this example, "ID" is the experimental unit identification number, "dose" is the dose given to the units, and "time' is the time at which the response, "hind.grip," has been measured. This screen shot shows just the first 20 or so entries of a data file (TETacHindGrip.dax) that has a total of 199 rows of data. Additional rows are similar; they do show doses greater than 0 for other animals tested in this experiment. Appendix A shows the entire data set.

There are four repeated measures data sets (.dax files) distributed with BMDS 2.1. They are

- TETacHindGrip.dax (a portion of which is shown above)
- TETacForegrip.dax
- footsplay.dax
- gripTDMmale.dax

The last of those files has two endpoints ("forelimb" and "hindlimb") so there are a total of five sets of dose-time-response data included with BMDS 2.1 that can be analyzed. A user interested in repeated measures analysis will undoubtedly have data sets of particular interest to them that they can enter (as described above) and analyze (as described below).

Given the successful creation of the data set, the user may initiate a run by selecting the Model Type and Model Name shown at the top of the data grid screen. A Model Type set up explicitly to accommodate these time course data is "Rptd_Resp_Measures," indicating that these data are repeated (response) measures data and need to be analyzed accordingly. Find and select that option by clicking on the drop-down arrow for Model Type. When the user selects Rptd_Resp_Measures as the Model Type, the Model Name, "ToxicoDiffusion_beta," will appear automatically. Currently, the toxicodiffusion model is the only repeated response measures model incorporated into BMDS 2.0.

When the Rptd_Resp_Measures model type and ToxicoDiffusion_beta choices have been made, click on the "Proceed" button. An option screen like the following will appear:

The user will then fill in or make choices for the fields for each of the assignments. Those choices are discussed here.

Column Assignments

The drop down arrows for each of the four required fields will show the possible assignments, based on the column names in the data file. (Note that the column names can be changed in the data grid screen prior to invoking the option screen by right clicking on the column headers. See the BMDS Help for additional details.) Each of these four assignments must be made.

Plotting Assignments

The assignments here do not need to be adjusted. But if the user wants to change the appearance of the graphs produced in a run, these assignments accomplish that. A title can be assigned that

will be used to label the output plots. The user can choose to plot the results vs. time either with the time axis on the natural scaled (unscaled) or on the log-scale. The number of time points indicates at how many intermediate times (between the minimum and the maximum in the data set) the functions are evaluated for the plots (with linear interpolation being done between those time points). Finally, the user may select the x- and y-axis minimums and maximums. Here, as elsewhere, a value of "-9999" indicates a default value; any plotting assignments with -9999 will be chosen automatically by the program based on the values in the data set.

Parameter Assignments

The two options available for the parameter assignments are "Default" and "Initialized." Unlike other BMDS models, there is no "Specified" option at this time (it may be added at a later time). The "Default" choice allows the program to test and select starting values for the model parameters that it thinks are best. The user my give a set of initial values by selecting the "Initialized" option. As with other BMDS models, if the user selects "Initialized" for one parameter, s/he must do so for all the parameters.

When "Initialized" is selected, values other than -9999 should be entered. Here, as elsewhere, a value of -9999 is a flag that tells the program to use defaults.

Users who do not consider themselves experts (or who have not consulted with experts) on toxicodiffusion modeling may wish to retain the "Default" option for parameter assignments. In fact, if in the course of running the model an error occurs and is reported by BMDS (or, alternatively, if all five of the expected plots do not appear during a run and the processing terminates without appearing to give results), the user should check the parameter assignments. If they have been initialized, it is quite possible that those initial values were not adequate and the program could not find a solution from those starting points. In such an instance, it is recommended that the user either revert to using defaults for the initial values (the program will try several alternative starting points) or find better initial values (via some user-specific means of estimating such). See Section 3.2 for additional discussion of the initial values.

Other Assignments

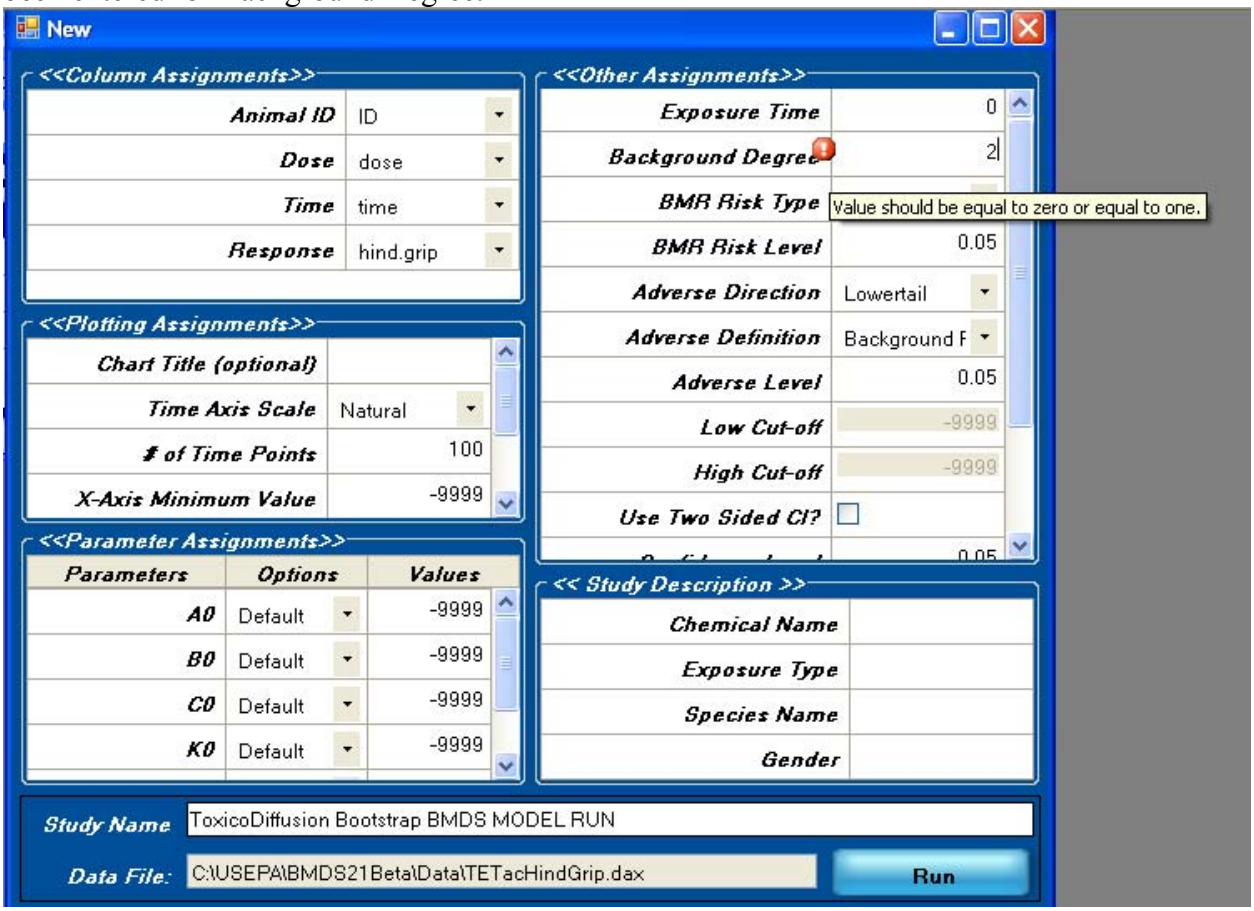
This section controls some additional specifications for the model and, primarily, provides all the additional information needed to compute the BMD and BMDL for a model run.

"Exposure Time" is the time at which the exposure occurred. IMPORTANT NOTE: The exposure time is a *fixed* feature of the data the user is analyzing. The user is expected to know at what time the exposure occurred, and to enter this data-set-specific value in this field. In many instances, the exposure time will be 0 (the start of the experiment and of the observations), and so the default value is set to 0. But be sure to verify that the exposure time is properly set according to the conditions under which the experiment was carried out. Nonsensical answers can and will be obtained (even errors might occur) if the exposure time is not specified correctly.

"Background Degree" is the degree of the polynomial describing the time-related change in response in the absence of exposure (i.e., in background). Valid values are 0 or 1, corresponding

to the polynomial $A_0 + A_1 t$ (see Section 2.2 above). If the user specifies that the degree is 0 (the default), then only the A_0 (constant) term will be included in the model. In future versions of the toxicodiffusion model, the degree will be allowed to be 0, 1, or 2.

At this point is it convenient to show an error trapping feature of the BMDS 2.1 option screen for the toxicodiffusion model. In the following figure, note that an invalid (unacceptable) value has been entered for Background Degree.



When, after entering the invalid value (2 in this case) the user tries to edit another field, or save the option file, or close the option file, the exclamation mark in the red circle will appear (flashing) next to the parameter for which there is an error. placing the cursor over the exclamation mark will show the nature of the error (in this case, a value other than 0 or 1 has been entered; unfortunately, the screen shot above does not show that the cursor was placed over the exclamation mark to elicit that explanation). The user should modify the entry as appropriate to remove the error. Several fields in this option screen have edit checks set up to help the user guarantee correct and appropriate data entry.

The "BMR Risk Type" options available in the drop down selection are "Extra" (the default) or "Added." These are the standard options for defining BMDs based on adverse rates of response (defined explicitly as in a dichotomous model or implicitly as in the hybrid approach to continuous data). Section 2.4 discusses how these choices affect the calculations.

"BMR Risk Level" specifies the actual value of extra or additional risk that is of interest to the user. This field can be changed by highlighting (e.g., by double clicking on) the value shown (the default is 0.05) and then typing in the value of interest. Values in the range of 0.01 and 0.1 (i.e., 1% to 10%) are often used in practice.

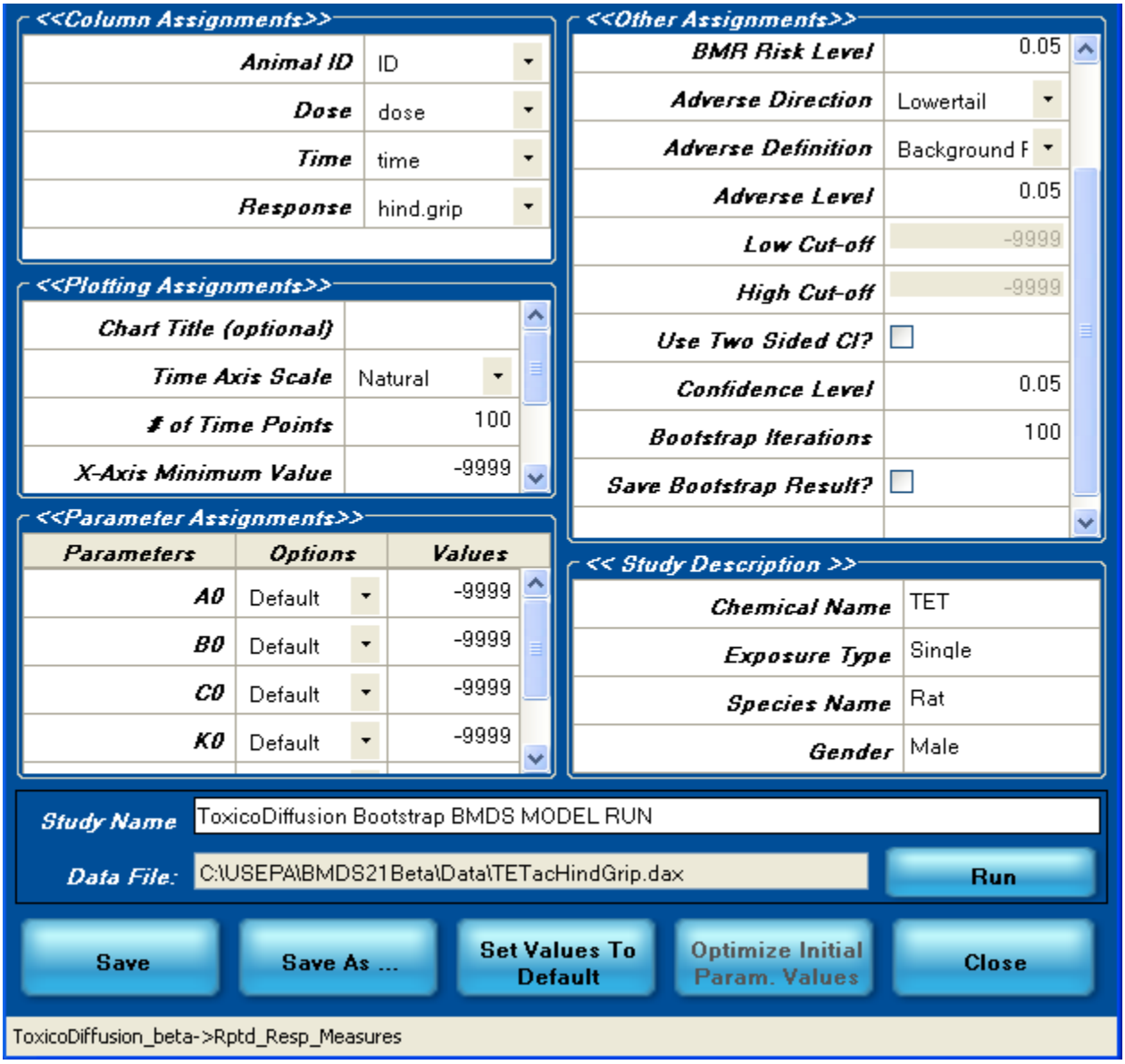
The choices of "Adverse Direction" are "Uppertail," "Lowertail," and "Bothside," indicating whether extreme values in the upper tail, lower tail, or both tails of the distribution of responses are considered adverse.

As discussed in Section 2.4 the cut point for determining where adverse responses occur (and their relative frequency) can be defined in two ways. This is reflected in the "Adverse Definition" assignment, which can take the values "Background Rate" or "Cut Point." If the "Background Rate" is selected then the user will specify a rate of response (strictly between 0 and 1) assumed for the unexposed experimental units; this rate (in conjunction with the estimated model parameters related to mean background response and variability) will be used to impute a cut point. This is the typical choice for a hybrid approach to BMD analysis of continuous data. When "Background Rate" is selected for "Adverse Definition" then the subsequent choices for "Low Cut-Off" and "High Cut-Off" will be grayed out (they no longer are relevant).

If the "Cut Point" option for "Adverse Definition" is selected, then the user is required to know, and specify, the cut-off(s) that s/he wants to use for differentiating adverse from non-adverse responses. The assignment of the "Low Cut-Off" and/or the "High Cut-Off" is done in the fields so designated. When the "Cut Point" option is selected, the "Adverse Level" field will be grayed out and the cut-off(s) relevant to the adverse direction selected earlier will be open to modification (the other one, if "Uppertail" or "Lowertail" were selected earlier will be grayed out).

The user then checks the box for "Use Two Sided CI?" if s/he wants a confidence interval showing a lower and an upper bound on the BMD. If the box is not checked, then only the lower bound (BMDL) will be estimated.

The confidence level of the BMDL (or BMDL and BMDU) calculations is specified with the "Confidence Level" assignment. IMPORTANT NOTE: with this implementation, the user is specifying the α level associated with the confidence limit. So, when one wants a 95% lower bound, for example, then the α level is 0.05, yielding a $(1-\alpha)$, or in this example a 95%, confidence level. The default choice is 0.05, corresponding to the 95% bound calculations indicated in this example (as screen shot showing the lower portion of the "Other Assignments" section is shown below). The user may change that default by picking any α strictly between 0 and 1, although choices in the range of 0.01 to 0.1 (99% to 90% confidence levels) are more typical. Note also, that if a two-sided interval is chosen (from the immediately preceding assignment) then the bounds on each side have the designated confidence level; the interval based on the estimated BMDL and BMDU will be a $(1-2\alpha)$ confidence interval for the BMD.



The number of "Bootstrap Iterations" is then entered by the user. The default is 100, which is a relatively small number of iterations. A low default was chosen because the user may want to run the model with a small number of bootstrap iterations (maybe even less than 100) to ensure that the model converges and fits, that all the parameters have been specified correctly, and/or to get a sense of the BMD and BMDL, before a larger number of bootstrap iterations are requested (increasing the value in the "Bootstrap Iterations" field). The reason for that is because the bootstrap procedure can take a fair amount of time (a minute or more perhaps) when the number of iterations is in the range of 2000 suggested in Section 2.4 as a reasonable starting point for obtaining stable BMDL (and BMDU) estimates. As discussed in Section 2.4, that believe that 2000 is a sufficiently large number of iterations can (and perhaps should) be tested by repeating the model run with the same selected number of iterations to confirm that the BMDL (and BMDU estimate if a two-sided CI has been selected) are indeed stable with that many iterations. The BMD estimate is not affected by the choice of the number of bootstrap iterations; if interest

is limited to the BMDs and not the bounds, then the user can select a very small (but positive) number of iterations. The speed of the run is directly proportional to the number of bootstrap iterations, so if run time is an issue, the smaller the number of iterations the better. To re-iterate, however, stable BMDL estimates require many bootstrap iterations and the default of 2000 is a guess of a reasonable starting point for that number.

Finally, the user can indicate whether or not s/he wants to save the bootstrap results to a file. When not absolutely required, the user might choose not to save those results, because the file can be very big depending on the number of bootstrap iterations chosen. The saved file may be used for advanced data manipulations and analyses. In either case, the standard output from a model run (discussed below) will include the BMDL (and, if a two-sided CI is selected, BMDU) estimate(s) corresponding to the confidence level requested.

Study Description

This section allows the user to provide additional details about the experiment being analyzed. Most of the individual fields (chemical name, species name and gender) are self-explanatory. The "Exposure Type" field could be filled in tot indicate route of exposure, how many exposures occurred, or other relevant information. All of these fields are optional (they may be left blank); when they are not blank, then the output file created for a model run will echo that input information.

On a related note, the use may also edit and choose a "Study Name" (the text field immediately below all of the assignments discussed above and above the non-editable "Data File" field). The text entered here will be the first "identifying" line on the output file reporting the results of a model run.

Once the option screen choices are completed, clicking on the "Run" button on that screen will initiate the model run. A series of 5 plots will appear (temporarily) on the screen to indicate that the analysis is on-going.

3.2 INITIAL VALUES

Obtaining maximum likelihood estimates through iterative numerical procedures requires good starting values for the unknown parameters, especially those that are nonlinear in the model function. In the toxicodiffusion model, for example, the parameters A_0 and A_1, of the control trajectory are linear, and the rest of the parameters are nonlinear.

The choice of initial values is crucial to achieving convergence. A number of strategies can be employed to determine appropriate initial values. Exploratory analysis of the raw data helps reveal the level and trend of outcome data with respect to the control trajectory, direction of dose-effects (e.g. increasing or decreasing trend), time of peak effect, etc. These characteristics, in turn, provide important information on the values of the model parameters. Initial values can also be estimated through either solving a set of equations or fitting a regression model without random effects. In the context of first order toxicokinetics, for example, the parameter k resembles the "elimination rate" and a possible value may exist in the scientific literature.

If the user has a set of starting values, the "Parameter Assignments" section of the option screen is the place to enter the initial values (after changing the "Default" option to "Initialized" for *every* parameter). Otherwise, the toxicodiffusion model implements an algorithm that computes starting values for the parameters by solving several sets of equations. This is repeated a number of times, each producing a set of starting values. The number of the equations varies with each data set. Under that default, the toxicodiffusion model tries one set of starting values at a time, until a numerical convergence is achieved. The starting values that are actually used to reach convergence along with the other possible starting values are included in the output file.

For most users, this automated choice of starting values is highly recommended. The unused sets of starting values can also be used manually if desired.

3.3 INTERPRETATION OF OUTPUT FILES

Appendix B contains the output file produced by running the toxicodiffusion model on the data set discussed above. That is, Appendix B is the result of hitting the "Run" button on the option screen shown in the figures above.

The output file shown in Appendix B has been color coded for ease of reference and explanation of the various sections of that output. Details related to that output are provided here, referenced by color-coded section.

Reference and Model Information: This section merely echoes user-specified information (e.g. Study Name and Study Description) as well as some information about the contents of the data set: what the dose levels are, at what times observations were available, and the sample size (i.e., the number of distinct combinations of experimental unit ID and time). The user should verify that these values are correct; if they are not, then the data file should be checked for data entry errors.

In addition, this section shows the form of the model. In this instance, note that the background "polynomial" is given by the single term "A" because the user specified a 0 degree polynomial for that background (the other alternative would be the first-degree polynomial which would show "A0 + A1*time."

Likelihood-Related Estimates: The AIC and BIC as well as the log-likelihood for the model fit to the data being analyzed are shown here. See Section 3.4 below for additional information about interpreting the AIC and BIC.

Random Effects: This section shows which parameters were selected to have random effects around the main (fixed) effect. At this time, the only parameter for which random effects are specified is the parameter A (the constant term in the background response polynomial). So, in this section there will be a standard deviation reflecting the variability of the random effects around the corresponding fixed effect. There will also be a "Residual" standard deviation reflecting the remaining variability that is not part of the random effect (reflecting the remaining

lack of fit of the model to the data and therefore associated with residuals). The distributions of the random effects and of the residuals are assumed to be independent of one another. [At a later time, when more than one random effect is allowed, the distributions of the random effects will not be assumed to be independent of one another, though they all will still be assumed independent of the residual distribution. In the case of more than one random effect, pair-wise correlation estimates will be provided as well. When those correlations are close to 1 or -1, that may be a strong indication that the data cannot support that many random effects and alternative assumptions should be tried by the user.]

Parameter Estimates: In this section, the additional results related to the model fitting are provided. Parameter estimates for the fixed effects are shown with their standard errors. In addition the degrees of freedom, t-test statistic value, and associated p-value for that test are shown, in order to facilitate evaluation of the significance of the parameters.

In addition, parameter correlations and a summary of the within-group residuals are shown. Additional considerations for interpretation of these outputs are presented in Section 3.4 below.

Initial Values: In this section one finds the set of initial values that were used when the model apparently converged to an acceptable answer. Below that is the list of the initial values that the model was scheduled to try; it starts with the first set listed and continues until it uses a set that resulted in apparent convergence. In this case, the model tried the first set of initial values and achieved success (the first set in the list is the same as the "Initial Values at the top of this section, i.e., the ones that "worked"). Possible initial sets 2 though 6 in this example were not run. See the preceding section (3.2) for additional information about initial values.

BMD Estimation: In this section the summary BMD results are presented. First, the user-specified choices for risk type, spontaneous risk level (adverse level; if a Cut Point was used instead of Background Rate, then the value of the cut point would be printed here), the area of adverse effects (Adverse Direction), and the BMR level are shown. If these are not the choices the user wants to have, the option file should be revisited and correct values entered for these fields. The same is true of the number of bootstrap iteration which is shown next.

The minimum BMD is what is shown (the BMD, as a rule, varies as a function of time). The time at which that minimum BMD was obtained is also given. The value shown for "Confidence Level" is (1-α)*100% (where α, equal to 0.05 in out example, is what was entered on the option screen). The lower limit presented is based on the user-specified number of bootstrap iterations. As discussed elsewhere, the user should test for stability of that estimate if the accuracy of the BMDL (or BMDU if that too is estimated) needs to be assured to some desired number of significant digits.

IMPORTANT NOTE: When the toxicodiffusion model is run from a data grid as shown in these examples, there is no opportunity to specify a name for the output file; it is given a default name based on the name of that dax input file. If the user is going to make additional model runs on the same data set, the previous output files will be overwritten (they are actually deleted just prior to doing another run that would create an output file with the same name). So it is

important to use the "Save As …" option under the "File" tool bar item on the output file screen to save the results, using a name that is indicative of the model run just completed (or otherwise meaningful to the user). In this way, the user may retain a set of output files showing, for example, the results of fitting different model variations (e.g., setting the background response polynomial to be constant of a first-order trend), or of calculating BMDs for different BMR choices, or the stability (or lack thereof) of the bootstrap-based BMDL calculations. Alternatively, the BMDS 2.1 session screen capability may be used to set up a session that runs the toxicodiffusion model on the same data set but with slightly different option files (and different output file names) to do this in one pass. See the BMDS Help or documentation for more about running sessions.

In addition to the text output file, five plots are produced with each run. Those are illustrated in Appendix C. A discussion of the use and significance of those plots is provided in the following section (3.4).

3.4 ADDITIONAL CONSIDERATIONS AND ISSUES FOR MODEL RUNS

The five output graphs are prepared in EMF format. The base names of the five files are

- xxxOT (observed trajectory)
- xxxFT (fitted trajectory)
- xxxRes (residuals)
- xxxRESGBD (residuals groups by dose)
- xxxBStrap (bootstrap)

where "xxx" in all of the above cases is the same as the data set (.dax file) name used as the input to the analysis.

The graph in the observed trajectory file displays each subject's responses by connecting the observed data (dots) with connected straight lines in time sequence, so as to demonstrate a time trajectory. The individual trajectories are then grouped by dose level into panels, one for each dose group. These graphs are called paneled spaghetti plots. Examination of the raw data plot is extremely helpful in determining the trajectory of the control group (e.g. is it a constant), and how exposure changes the trajectory over time (e.g. increasing or decreasing the response level; are there transient effects?).

The graph in fitted trajectory file is the same as the spaghetti plots of the raw data except that the predicted responses are used instead. This plot can reveal whether or not the predicted responses show trends resembling the observed trend – an ad hoc, but effective way to examine the appropriateness of the model. If the data are crowded at earlier time points and the plots are ineffective in showing the time trend because the extremely large time point(s) suppress the smaller time points, consider changing the plotting assigning in the option file screen so that log-scale of time (x-axis) would be used in these plots.

Two residuals plots are generated by the program for model checking. The residuals plot in "xxxRes.emf" pools residuals across all dose groups, and is an overall scatter plot of standardized residuals against the predicted responses. The plot in the residuals-grouped-by-dose file is also a scatter plot of the standardized residuals, but grouped by dose. Both plots

enable the user to check randomness of the residuals; the first plot allows one to check that the variance of the error tends to be constant with respect to the level of response. The second, grouped plot allows one to check for constant variance across dose level. The presence of any trend (increasing, decreasing, or curved) with respect to time or dose is an indication of inappropriateness of the model. The user should be aware that it is quite possible that the toxico-diffusion model may fail to describe the observed dose-effects and time trend in a particular data set.

The residual plots can also be used to detect extreme values or outliers. Generally, standardized residuals which are greater than 3 or less than -3 are extreme observations, or even outliers.

The xxxBStrap.emf file shows the time-profile of the BMDs and bootstrap BMDLs; the program produces an envelope plot in which all bootstrap replications of the BMD (grey lines) are plotted against time with the overlay of the original estimate of the BMD (dark, solid line) and the lower or upper percentile (BMDL or BMDU) of the bootstrap replications (dark, dashed lines). For example, the BMDL line is the locus of points that show the (α)100% lowest bootstrapped BMD for each time. The time associated with the minimum BMD (lowest point on the solid curve) is referred to as the TOPE (time of peak effects). It often helps to consider a log-scale of time in this graph when the maximum time is an order of magnitude bigger than the rest of the time points.

In addition to examination of the plots discussed above, additional checks should be applied to the output. As mentioned above, the algorithm that generates default initial parameter values will generate several sets of those initial values. The program takes one set of initial values at a time until it finds one that results in a numerically successful model fitting. Otherwise it will report the failure in model fitting. Careful examination of the model fitting results is necessary as an apparently successful model fit is not necessarily an appropriate model. For example, a negative value for the parameter C is usually an indication of a less desirable model on mathematical grounds; and a negative value is inappropriate for the k as the elimination parameter. It is recommended that the user make use of the automated initial value approach unless model fitting is unsuccessful. The user then can manually specify a set of initial values that might lead to a successful model fitting. The derivation of a good set of initial values is not trivial, careful examination of the data and model is required to this end. However, a technical discussion on this is beyond the scope of this manual.

Note that, if a user-specified set of starting values is invalid or fails to achieve convergence, the built-in algorithm will automatically run as if the default is entered. The algorithm will not run if the user's set of starting values works, consequently the matrix of automated starting values will not appear in the output file.

It is also recommended that if the model has a poor fit (based on the p-values, AIC, BIC, and/or log likelihood function) or the coefficients are not reasonable, then other initial values from the automatically generated list should be tried, by manually entering these values in the Parameter Assignments section of the option screen (also changing "Default" to "Initialized" for all parameters).

As discussed above, there are three model fitting criteria: the Akaike's information criterion (AIC), the Bayes information criterion (BIC), and the log-likelihood. These criteria can be used as measures of goodness-of-fit for model comparison and selection purposes. The log-likelihood function can be used to test the significance of additional terms (parameters) added to an existing model (e.g., making the background response a first degree polynomial, in time, rather than a constant). This is done by taking twice the difference between the log-likelihood function of the larger model and that of the smaller model, and comparing this so-called likelihood ratio statistic with a Chi-squared distribution. Larger differences suggest that additional parameters in the model are significant. Since the likelihood function increases with the number of parameters in the model, one often wants to add a penalty for adding parameters to the model. The AIC and BIC penalize the inclusion of excessive and unnecessary terms (parameters) in a model while at the same time evaluating the effect of additional parameters on the maximization of the likelihood function. The BIC tends to penalize the inclusion of additional parameter more than the AIC does (the "penalty" for parameters is 2k for the AIC and is k*ln(n) for the BIC, where k is the number of parameters and n is the number of observations; note that $\ln(n) > 2$ for $n > 7$). In general, smaller values of AIC and BIC and larger values of log-likelihood function are preferred.

These considerations really only apply when one is comparing different models; they do not provide a basis for evaluating the goodness-of-fit of the model to the data. The graphical comparisons suggested above are the only means currently provided for making that assessment.

ADDENDUM. CURRENT STATUS OF MODEL TESTING

This section reports on the latest set of testing that has been conducted for the software. This section can be considered a separate "module" of this documentation that is independent of the previous sections (which describe the motivation for, the background of, and the "how-to" for running and interpreting the model). Indeed, with additional testing, this section may change and be updated while the previous sections may remain the same.

4.1 CURRENT TESTING METHODS

The basis for testing the BMDS implementation of the toxicodiffusion model was to compare the model outputs obtained from that implementation to those obtained by running the original R program developed by Dr. Yiliang Zhu. The values produced by his program were used as the standard against which the BMDS implementation was compared.

Note that this test merely confirms that the new model code yields the same estimates as the old toxicodiffusion model from which it was "translated." Since the first task towards getting code that can be integrated into BMDS is to successfully translate existing working models into code that can be used for that integration, such a test is adequate for an initial check. The new program has not, however, been tested against an independent program that could be configured to run the same models. At some point, such a test might be desirable.

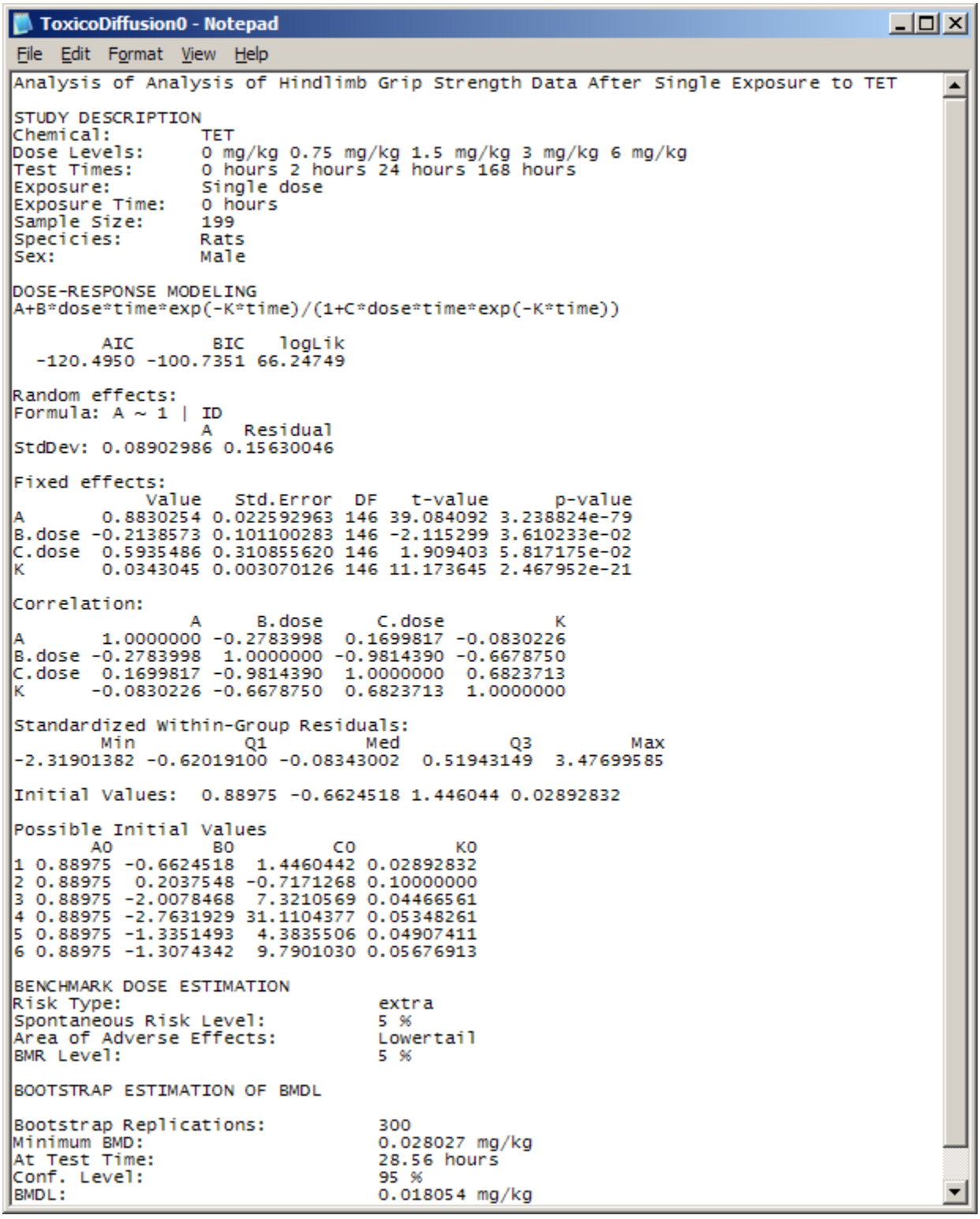
In the course of testing that the BMDS implementation responded appropriately to changes in the option screen assignments, an apparent error was uncovered. It has yet to be determined that that apparent error exists in the original R program as well; additional investigation and fixes for this error uncovered are on-going. This error is discussed in Section 4.3 below.

All runs of the original and BMDS versions of the program were done using the Windows XP operating system. R version 2.7.0 was used for all runs.

4.2 TESTING RESULTS – COMPARISON TO R VERSION

Two data sets were run for this comparison. The hind-grip data set used for the illustrations above and shown in Appendix A was one set (TETacHindGrip.dax). The other was a different hind-grip data set shown in Appendix D. It is from the data file GripTDMmale.dax.

For the TET hind-grip data set, the output produced by Dr. Zhu's R program is summarized here:



ToxicoDiffusion0 - Notepad

File Edit Format View Help

```
Analysis of Analysis of Hindlimb Grip Strength Data After Single Exposure to TET

STUDY DESCRIPTION
Chemical:         TET
Dose Levels:      0 mg/kg 0.75 mg/kg 1.5 mg/kg 3 mg/kg 6 mg/kg
Test Times:       0 hours 2 hours 24 hours 168 hours
Exposure:         Single dose
Exposure Time:    0 hours
Sample Size:      199
Specicies:        Rats
Sex:              Male

DOSE-RESPONSE MODELING
A+B*dose*time*exp(-K*time)/(1+C*dose*time*exp(-K*time))

        AIC       BIC    logLik
  -120.4950 -100.7351 66.24749

Random effects:
Formula: A ~ 1 | ID
                A   Residual
StdDev: 0.08902986 0.15630046

Fixed effects:
            Value   Std.Error  DF    t-value      p-value
A       0.8830254 0.022592963 146 39.084092 3.238824e-79
B.dose -0.2138573 0.101100283 146 -2.115299 3.610233e-02
C.dose  0.5935486 0.310855620 146  1.909403 5.817175e-02
K       0.0343045 0.003070126 146 11.173645 2.467952e-21

Correlation:
                A     B.dose     C.dose          K
A       1.0000000 -0.2783998  0.1699817 -0.0830226
B.dose -0.2783998  1.0000000 -0.9814390 -0.6678750
C.dose  0.1699817 -0.9814390  1.0000000  0.6823713
K      -0.0830226 -0.6678750  0.6823713  1.0000000

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.31901382 -0.62019100 -0.08343002  0.51943149  3.47699585

Initial Values:  0.88975 -0.6624518 1.446044 0.02892832

Possible Initial Values
        A0         B0         C0         K0
1 0.88975 -0.6624518  1.4460442 0.02892832
2 0.88975  0.2037548 -0.7171268 0.10000000
3 0.88975 -2.0078468  7.3210569 0.04466561
4 0.88975 -2.7631929 31.1104377 0.05348261
5 0.88975 -1.3351493  4.3835506 0.04907411
6 0.88975 -1.3074342  9.7901030 0.05676913

BENCHMARK DOSE ESTIMATION
Risk Type:                      extra
Spontaneous Risk Level:         5 %
Area of Adverse Effects:        Lowertail
BMR Level:                      5 %

BOOTSTRAP ESTIMATION OF BMDL

Bootstrap Replications:         300
Minimum BMD:                    0.028027 mg/kg
At Test Time:                   28.56 hours
Conf. Level:                    95 %
BMDL:                           0.018054 mg/kg
```

All the numerical values (e.g., of log-likelihood, parameter values, and BMDs) are identical to those shown in Appendix B which is the output file produced by BMDS. The BMDLs differ slightly in the second significant digit. But note that in either run, the number of bootstrap iterations is small (100 or 300) so stability of the BMDL estimate would not be expected.

For the TDM data set, the results from the original R program are shown here:

ToxicoDiffusion0 - Notepad

File Edit Format View Help

```
Analysis of Hindlimb Data

STUDY DESCRIPTION
Dose Levels:      0 2 2.5 3.5 5
Test Times:       -7 0 7 14
Exposure Time:    6
Sample Size:      300

DOSE-RESPONSE MODELING
A0+A1*time+B*dose*time*exp(-K*time)/(1+C*dose*time*exp(-K*time))

       AIC      BIC    logLik
  314.6444 340.5709 -150.3222

Random effects:
Formula: A ~ 1 | ID
                A  Residual
StdDev: 0.1863377 0.3653020

Fixed effects:
                    Value   Std.Error  DF    t-value       p-value
A.(Intercept)  2.996151303 0.046111514 221 64.9762078 5.349854e-146
A.time         0.004701099 0.002859859 221  1.6438221  1.016348e-01
B.dose        -0.175049314 0.194340258 221 -0.9007362  3.687091e-01
C.dose         0.184637396 0.648945708 221  0.2845190  7.762791e-01
K              0.580987377 0.227168116 221  2.5575217  1.121165e-02

Correlation:
             A.(Intercept)      A.time     B.dose      C.dose           K
A.(Intercept)    1.00000000 -0.63729275 -0.1723384  0.10338724 -0.04296137
A.time          -0.63729275  1.00000000  0.1410964 -0.08027947 -0.24754876
B.dose          -0.17233840  0.14109636  1.0000000 -0.97148728 -0.62323411
C.dose           0.10338724 -0.08027947 -0.9714873  1.00000000  0.55144483
K               -0.04296137 -0.24754876 -0.6232341  0.55144483  1.00000000

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.94696899 -0.55517965  0.04644483  0.62503680  2.67259949

Initial Values:  2.992632 0.005213033 -0.01183382 0.06132618 0.1413273

Possible Initial Values
        A0          A1           B0          C0        K0
1 2.992632 0.005213033 -0.011833825  0.06132618 0.1413273
2 2.992632 0.005213033 -0.002883208 -0.27023296 0.2300663
3 2.992632 0.005213033  0.130542817 -4.16955936 0.3043678
4 2.992632 0.005213033 -0.003133904  0.05766134 0.1696372
5 2.992632 0.005213033 -0.003008556 -0.10628581 0.1998518
6 2.992632 0.005213033  0.028172970 -1.08020120 0.2113497

BENCHMARK DOSE ESTIMATION
Risk Type:                      extra
Spontaneous Risk Level:         5 %
Area of Adverse Effects:        Lowertail
BMR Level:                      5 %

BOOTSTRAP ESTIMATION OF BMDL

Bootstrap Replications:         250
Minimum BMD:                    1.328973
At Test Time:                   0.68
Conf. Level:                    95 %
BMDL:                           0.684607
```

As shown in Appendix E (which contains the output file for the BMDS 2.1 run of the same data set), once again the numerical values all match. Of note here, the time of exposure time was on

day 6; the user must be sure to enter the appropriate time of exposure from knowledge of the manner in which the experiment was conducted. Also, the 250 bootstrap iterations are clearly not sufficient for stability of the BMDL estimate; the two runs shown (above and in Appendix E) both used 250 iterations and the BMDLs from those two runs differ substantially.

4.3 TESTING RESULTS – CHANGING ADVERSE DEFINITION

In testing all of the options for running the model, it was discovered that the BMDL computations are not correct for one particular set of option choices. The error occurs when the Adverse Definition is set to "Cut Point," meaning that the user will specify numerical value(s) as the cut off between adverse and non-adverse responses (as opposed to specifying a rate of adverse response). When this choice is made, the BMDL is erroneously computed for the "Adverse Level" that is specified (even though that field is grayed out on the option screen when "Cut Point" is chosen for the Adverse Definition). Unfortunately, there is no way to by-pass or avoid having an Adverse Level (it must be entered as a number and any number entered will give a wrong result for the BMDL). The error is in the logic of the program that tells it whether or not to compute BMDLs for the entered cut point or for the adverse level, both values that are read from the option file.

Although this logic error is being investigated and will be resolved, the belief is that this error should not seriously impact typical practice for doing BMD analysis of the repeated measures data under investigation. As noted in previous sections, it would be rare that a user would specify a cut point determining where adverse response lie. The approach that is customary, and in fact typical of the hybrid approach to the analysis of continuous endpoints, is to specify a background adverse rate (level) of response, and then let the model determine where the cut point is based on the observations and the fitted model parameters.

There does exist, in theory, a means by which the model as it stands now could be used to get correct BMDLs when adversity is defined by cut points. But this involves a cumbersome sequence of calculations whereby the background rate of response corresponding to the user-specified cut point is derived (after the model is fit once to the data) and then the model is rerun using that calculated background adverse level. Future iterations of the program will obviate the need for such onerous manipulations. In the mean time, it is recommended that the user specifies an adverse level (background rate of adverse response), which is generally the preferred approach anyway.

REFERENCES

CREM, 2003. Draft Guidance on the development, evaluation, and application of regulatory environmental models. Prepared by the Council for Regulatory Environmental Modeling. November, 2003.

Crump, K.S. (1984). A new method for determining allowable daily intakes. \FAT {\bf 4}, 854-871.

U.S. Environmental Protection Agency (1998) Guidelines for neurotoxicity risk assessment. *Federal Register* **63**, 26926-26954.

Gaylor, D.W., and Slikker, W. Jr. (1990). Risk assessment for neurotoxic effects. *Neurotoxicology* **11**(2): 211-218.

Gaylor, D.W., Ryan, L., Krewski, D. and Zhu, Y. (1998) Procedures for calculating benchmark doses for health risk assessment. *Regulatory Toxicology and Pharmacology* **28**, 150-164.

Moser V.C., Becking G.C., Cuomo V., Frantik, E., Kulig, B., MacPhail, R.C., Tilson, H.A., Winneke, G., Brightwell, W.S., DeSalvia, M.A., Gill, M.W., Haggerty, G.C., Hornychova, M., Lammers, J., Larsson, J., McDaniel, K.L., Nelson, B.K., Ostergaard, G. (1997) The IPCS collaborative study on neurobehavioral screening methods: results of chemical testing. *Neurotoxicology* **18**, 969-1056

Lindstrom, M. and Bates, D.(1988). Newton-Raphson and EM Algorithms for Linear Mixed-Effects Models for Repeated-Measures Data. *J. Amer Statist Asso*. 4, 1014-1022.

Lindstrom, M.J and Bates, D.M. (1990). Nonlinear mixed effects models for repeated measures data. *Biometrics* **46**, 673-687.

Pinheiro, J.C. and Bates, D.M. (2000) *Mixed-effects models in S and S-plus*. Springer-Verlag: New York.

Zhu, Y., Wessel, M.R., Liu, T., and Moser, V.C. (2005) Analyses of Neurobehavioral Screening Data: Dose-Time-Response Modeling of Continuous Outcomes. *Regulatory Toxicology and Pharmacology* **41**, pp 240-255

Zhu, Y., Jia, Z., Wang, W., Gift, J., Moser, V.C., and B.J. Pierre-Louis (2005), Data Analysis of Neurobehavioral Screening Data: Benchmark Dose Estimation. *Regulatory Toxicology and Pharmacology***,** pp 190-201

Zhu, Y. (2005) Dose-time-response modeling of longitudinal measurements for neurotoxicity risk assessment. *Environmetrics* **16**, 603-617.

Appendix A: TET Hind-Grip Data Set

```
ID dose time hind.grip
"805" 0 0 .815
"805" 0 2 .915
"805" 0 24 .69
"805" 0 168 1.125
"809" 0 0 .78
"809" 0 2 .74
"809" 0 24 .45
"809" 0 168 .6
"821" 0 0 1.14
"821" 0 2 .76
"821" 0 24 1.02
"821" 0 168 .835
"827" 0 0 1.01
"827" 0 2 1.385
"827" 0 24 .87
"827" 0 168 .59
"831" 0 0 1.155
"831" 0 2 .765
"831" 0 24 .785
"831" 0 168 .915
"835" 0 0 .805
"835" 0 2 .855
"835" 0 24 .87
"835" 0 168 .765
"839" 0 0 1.025
"839" 0 2 1.355
"839" 0 24 .96
"839" 0 168 .905
"848" 0 0 .695
"848" 0 2 1.14
"848" 0 24 1.035
"848" 0 168 1.025
"854" 0 0 .81
"854" 0 2 1.09
"854" 0 24 .885
"854" 0 168 1.255
"859" 0 0 .905
"859" 0 2 1.025
"859" 0 24 .93
"859" 0 168 .935
"807" .75 0 .83
"807" .75 2 .615
"807" .75 24 .68
"807" .75 168 .99
"812" .75 0 1.015
"812" .75 2 .505
"812" .75 24 1.05
"812" .75 168 .995
"818" .75 0 .835
"818" .75 2 .61
"818" .75 24 .76
"818" .75 168 .9
"823" .75 0 .96
"823" .75 2 1.075
"823" .75 24 .665
"823" .75 168 .745
```

```
"833" .75 0 .965
"833" .75 2 .81
"833" .75 24 .705
"833" .75 168 .73
"837" .75 0 1.27
"837" .75 2 .89
"837" .75 24 .805
"837" .75 168 1.015
"841" .75 0 1.045
"841" .75 2 .7
"841" .75 24 .685
"841" .75 168 .79
"845" .75 0 .835
"845" .75 2 .915
"845" .75 24 .88
"845" .75 168 .79
"850" .75 0 .975
"850" .75 2 .765
"850" .75 24 .815
"850" .75 168 .79
"861" .75 0 .93
"861" .75 2 1.145
"861" .75 24 .97
"861" .75 168 .86
"804" 1.5 0 .83
"804" 1.5 2 .5
"804" 1.5 24 .385
"804" 1.5 168 .94
"816" 1.5 0 .775
"816" 1.5 2 .705
"816" 1.5 24 .525
"816" 1.5 168 .78
"820" 1.5 0 .58
"820" 1.5 2 .39
"820" 1.5 24 .435
"820" 1.5 168 .63
"825" 1.5 0 .825
"825" 1.5 2 .665
"825" 1.5 24 .77
"825" 1.5 168 .845
"830" 1.5 0 .95
"830" 1.5 2 .63
"830" 1.5 24 .595
"830" 1.5 168 .735
"834" 1.5 0 .88
"834" 1.5 2 .855
"834" 1.5 24 .7
"834" 1.5 168 .665
"843" 1.5 0 1.06
"843" 1.5 2 .69
"843" 1.5 24 .82
"843" 1.5 168 .615
"847" 1.5 0 .85
"847" 1.5 2 .78
"847" 1.5 24 .775
"847" 1.5 168 1.025
"853" 1.5 0 .885
"853" 1.5 2 .33
"853" 1.5 24 .42
"853" 1.5 168 .505
"858" 1.5 0 1.01
```

```
"858" 1.5 2 .74
"858" 1.5 24 .76
"858" 1.5 168 .775
"808" 3 0 .635
"808" 3 2 .335
"808" 3 24 .355
"808" 3 168 .635
"815" 3 0 .755
"815" 3 2 .49
"815" 3 24 .53
"815" 3 168 .82
"819" 3 0 .77
"819" 3 2 .61
"819" 3 24 .555
"819" 3 168 .65
"824" 3 0 .665
"824" 3 2 .525
"824" 3 24 .47
"824" 3 168 .67
"829" 3 0 .8
"829" 3 2 .525
"829" 3 24 .625
"829" 3 168 .625
"838" 3 0 1.02
"838" 3 2 .465
"838" 3 24 .415
"838" 3 168 .87
"842" 3 0 .66
"842" 3 2 .31
"842" 3 24 .53
"842" 3 168 .765
"846" 3 0 1.045
"846" 3 2 .59
"846" 3 24 .6
"846" 3 168 1.375
"851" 3 0 .465
"851" 3 2 .445
"851" 3 24 .63
"851" 3 168 .805
"857" 3 0 .865
"857" 3 2 .635
"857" 3 24 .575
"857" 3 168 .745
"806" 6 0 .985
"806" 6 2 .4
"806" 6 24 .35
"806" 6 168 .3
"811" 6 0 .87
"811" 6 2 .445
"811" 6 24 .385
"811" 6 168 .475
"817" 6 0 .84
"817" 6 2 .335
"817" 6 24 .365
"817" 6 168 .295
"828" 6 0 .57
"828" 6 2 .35
"828" 6 24 .345
"828" 6 168 .56
"832" 6 0 .96
"832" 6 2 .395
```

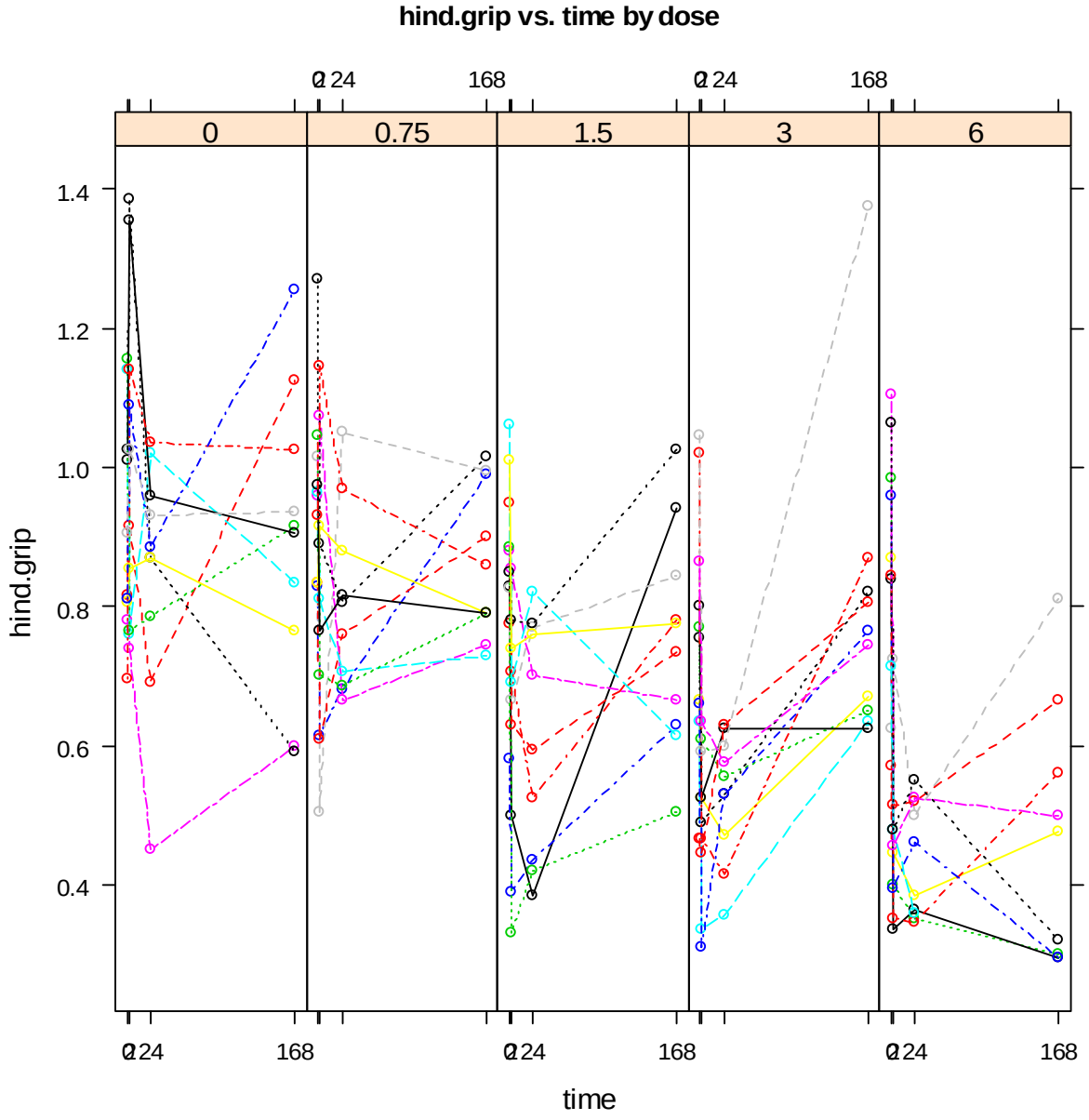
```
"832" 6 24 .46
"832" 6 168 .295
"836" 6 0 .625
"836" 6 2 .725
"836" 6 24 .5
"836" 6 168 .81
"840" 6 0 .845
"840" 6 2 .515
"840" 6 24 .52
"840" 6 168 .665
"844" 6 0 1.105
"844" 6 2 .455
"844" 6 24 .525
"844" 6 168 .5
"856" 6 0 .715
"856" 6 2 .48
"856" 6 24 .36
"860" 6 0 1.065
"860" 6 2 .48
"860" 6 24 .55
"860" 6 168 .32
```

Appendix B: Color Coded Example Output File

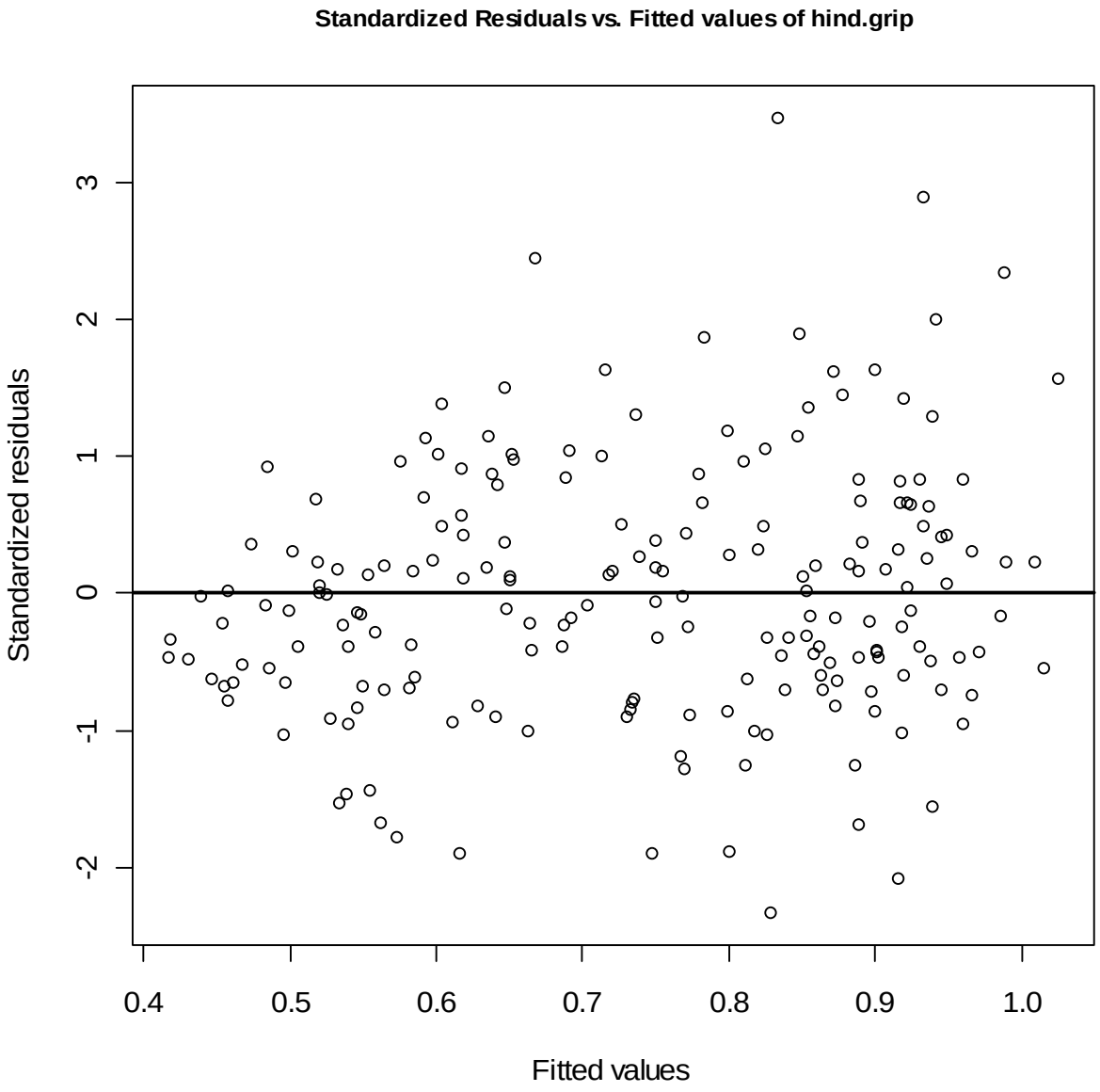
```
Analysis of  ToxicoDiffusion Bootstrap BMDS MODEL RUN
STUDY DESCRIPTION
Chemical:      TET
Dose Levels:   0 0.75 1.5 3 6
Test Times:    0 2 24 168
Exposure:      Single
Exposure Time:         0
Sample Size:   199
Species:       Rat
Sex:           Male

DOSE-RESPONSE MODELING
A+B*dose*time*exp(-K*time)/(1+C*dose*time*exp(-K*time))

        AIC       BIC   logLik
  -120.4950 -100.7351 66.24749

Random effects:
Formula: A ~ 1 | ID
                  A   Residual
StdDev: 0.08902986 0.15630046

Fixed effects:
              Value   Std.Error  DF   t-value      p-value
A       0.88302545 0.022592963 146 39.084092 3.238820e-79
B.dose -0.21385741 0.101100338 146 -2.115299 3.610236e-02
C.dose  0.59354887 0.310855798 146  1.909403 5.817178e-02
K       0.03430450 0.003070127 146 11.173645 2.467953e-21

Correlation:
                  A     B.dose     C.dose           K
A       1.00000000 -0.2783997  0.1699817 -0.08302263
B.dose -0.27839974  1.0000000 -0.9814390 -0.66787498
C.dose  0.16998165 -0.9814390  1.0000000  0.68237131
K      -0.08302263 -0.6678750  0.6823713  1.00000000

Standardized Within-Group Residuals:
       Min         Q1        Med         Q3        Max
-2.3190138 -0.6201910 -0.0834299  0.5194314  3.4769958

Initial Values:       0.88975 -0.6624518 1.446044 0.02892832

Possible Initial Values
       A0         B0         C0         K0
1 0.88975 -0.6624518  1.4460442 0.02892832
2 0.88975  0.2037548 -0.7171268 0.10000000
3 0.88975 -2.0078468  7.3210569 0.04466561
4 0.88975 -2.7631929 31.1104377 0.05348261
5 0.88975 -1.3351493  4.3835506 0.04907411
6 0.88975 -1.3074342  9.7901030 0.05676913

BENCHMARK DOSE ESTIMATION
Risk Type:                 extra
Spontaneous Risk Level:          5 %
Area of Adverse Effects:   Lowertail
BMR Level:                 5 %

BOOTSTRAP ESTIMATION OF BMDL

Bootstrap Replications:          100
Minimum BMD:               0.028027
At Test Time:              28.56
Conf. Level:               95 %
BMDL:                      0.016754
```

Appendix C: Plots Produced with Toxicodiffusion Model Runs

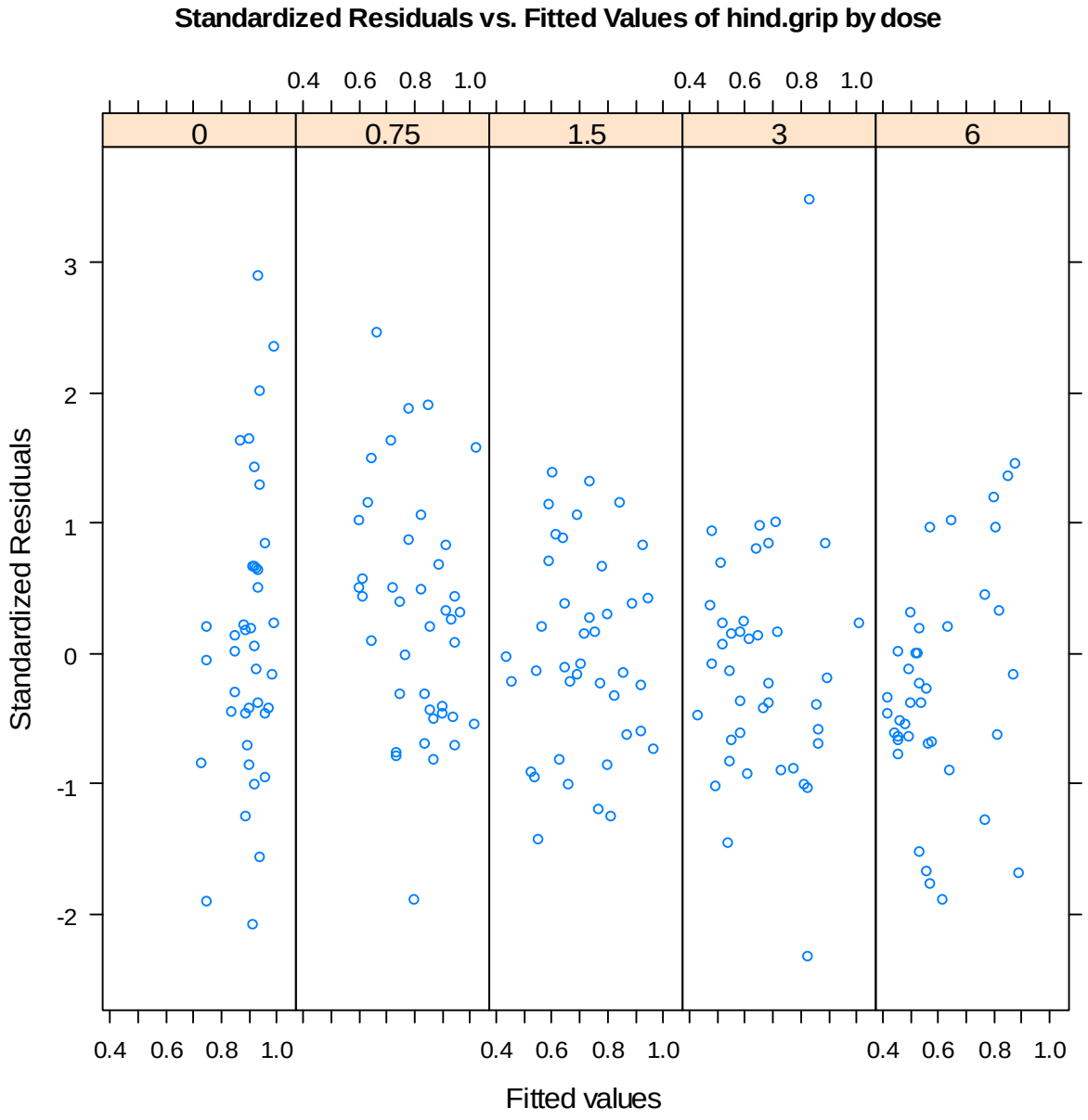
A. Spaghetti plot of observed hind limb grip strength over time by dose.



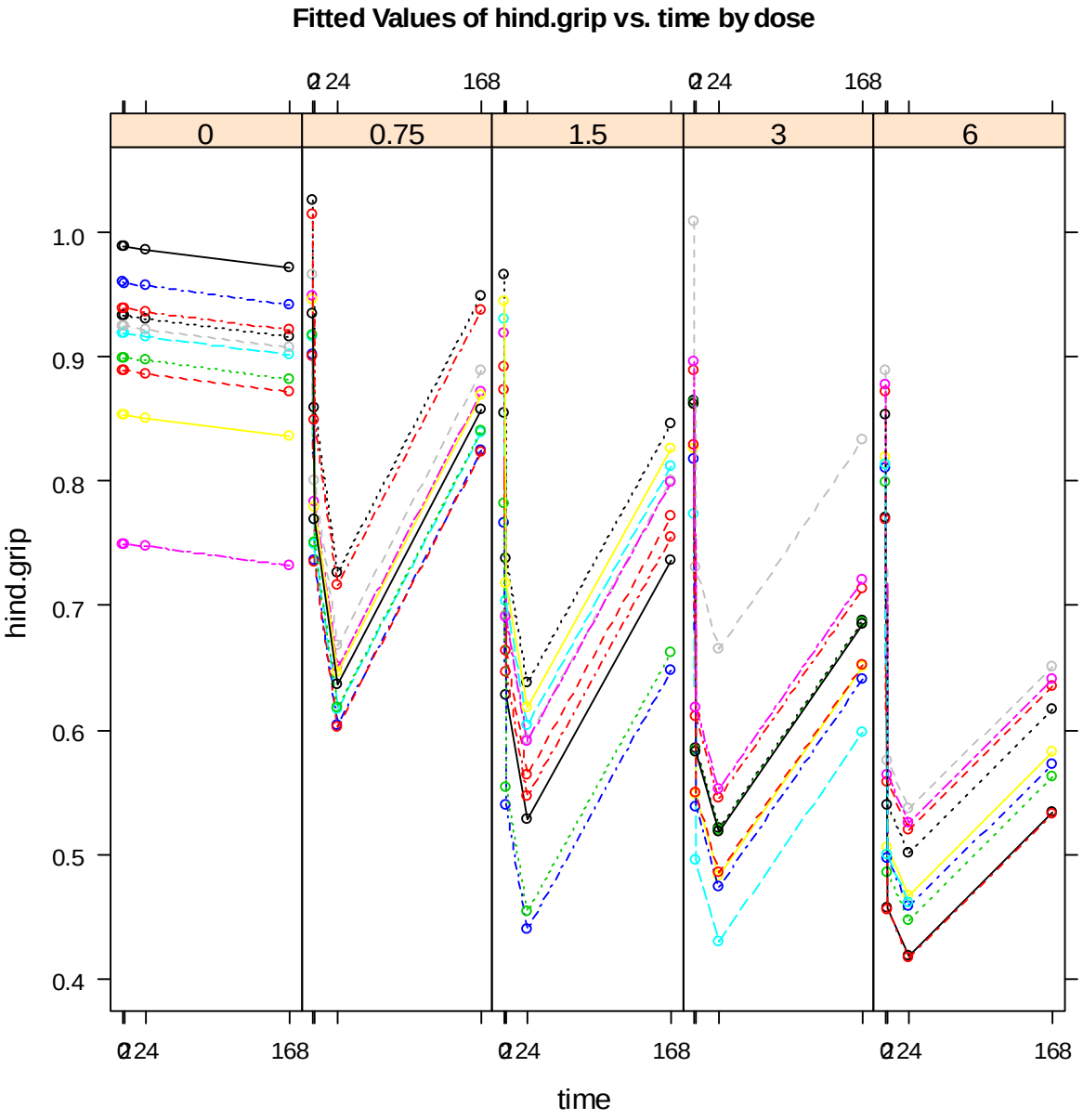
B. Standardized residual plot pooled across dose groups.



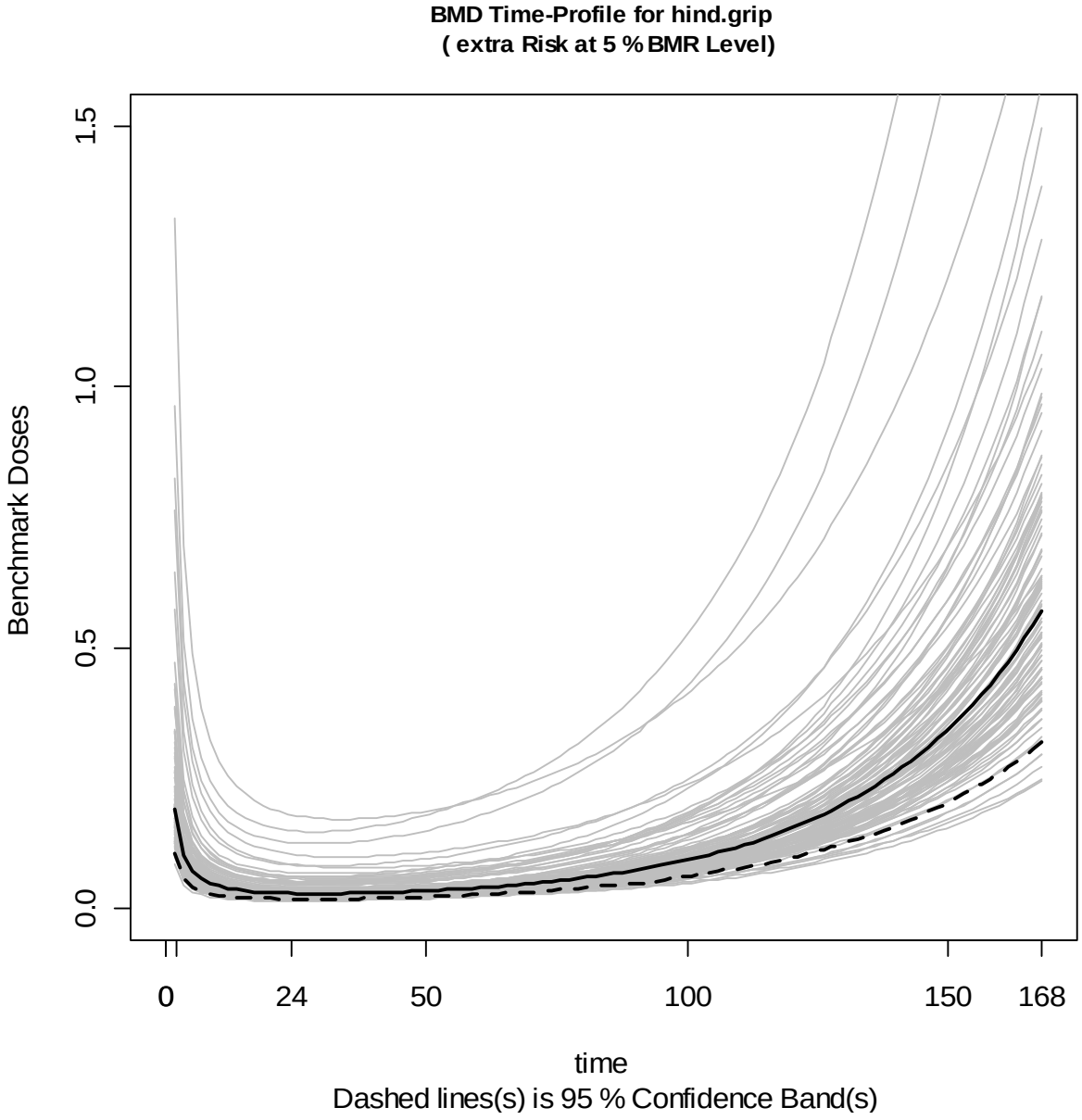
C. Standardized residual plots by dose group



D. Fitted toxicodiffusion model to responses after exposure



E. BMD and Bootstrap BMDL based on the fitted toxicodiffusion model



Appendix D: TDM Male Data Set

ID	Dose	Time	Forelimb	Hindlimb
75	0	-7	5.6	2.4
75	0	0	3	2.5
75	0	7	4.7	3.1
75	0	14	5	3.3
65	0	-7	4.8	2.4
65	0	0	4.9	3.4
65	0	7	4.1	3.3
65	0	14	4.2	3.5
70	0	-7	4.7	2.5
70	0	0	4.3	2.9
70	0	7	4.7	2.9
70	0	14	4.5	3.3
68	0	-7	3.9	2.2
68	0	0	4.7	2.5
68	0	7	4.5	3.4
68	0	14	4.2	3.5
69	0	-7	4.4	3.3
69	0	0	4.2	3.1
69	0	7	4.5	3.4
69	0	14	5.3	3.3
102	0	-7	4.4	3.3
102	0	0	4.1	3.1
102	0	7	4.7	2.9
102	0	14	5.2	3.9
105	0	-7	4.8	3.6
105	0	0	4.5	3.2
105	0	7	4.2	2.5
105	0	14	5.1	3.3
86	0	-7	4.1	3.2
86	0	0	4.5	3.5
86	0	7	4.8	3.6
86	0	14	4.5	3.1
93	0	-7	4.5	3.1
93	0	0	4.7	3
93	0	7	4.8	3.2
93	0	14	5	3.2
97	0	-7	4.3	2.9
97	0	0	4.3	3.1
97	0	7	4.2	3.4
97	0	14	4.4	3.3
239	0	-7	4.8	3.4
239	0	0	4.3	2.3
239	0	7	3.7	2.5
239	0	14	4.3	2.8
222	0	-7	4.8	2.6
222	0	0	4.4	2.7
222	0	7	4.5	3
222	0	14	5.6	3.1
226	0	-7	3.9	3.2
226	0	0	5.1	3.3
226	0	7	4.5	2.6
226	0	14	5	3.1
228	0	-7	4.1	2.5
228	0	0	4.1	3.2
228	0	7	5	3
228	0	14	5	2.6
238	0	-7	4.7	3.5

238	0	0	3.5	2.1
238	0	7	4.2	2.2
238	0	14	5.1	2.4
53	2	-7	4.9	3.6
53	2	0	4	2.5
53	2	7	4.3	3.5
53	2	14	4.4	3.1
60	2	-7	4.1	2.5
60	2	0	5	2.9
60	2	7	5.1	3.5
60	2	14	5.6	3.7
63	2	-7	5	3.2
63	2	0	3.8	2.7
63	2	7	4.3	2.5
63	2	14	4.6	3.4
79	2	-7	5.1	3.5
79	2	0	3.6	2.4
79	2	7	4.7	3.8
79	2	14	4.8	3.5
59	2	-7	5.1	3.2
59	2	0	4	2.7
59	2	7	4.1	2.8
59	2	14	4.4	3.2
95	2	-7	4.2	3.1
95	2	0	4.1	3
95	2	7	4.5	3.3
95	2	14	4.3	2.6
100	2	-7	4.1	2.9
100	2	0	4.2	2.5
100	2	7	4.4	3
100	2	14	3.9	3.1
84	2	-7	4.1	3
84	2	0	4.4	3.1
84	2	7	4.1	3.1
84	2	14	4.5	3.2
101	2	-7	4.5	3.4
101	2	0	5	3.2
101	2	7	4.3	3.5
101	2	14	5	3.9
88	2	-7	4	3.1
88	2	0	4.1	3.5
88	2	7	4.6	3.1
88	2	14	4.4	3
233	2	-7	4.2	3.3
233	2	0	4.2	2.8
233	2	7	5.2	2.3
233	2	14	5.4	2.9
216	2	-7	3.7	2.8
216	2	0	4.6	2.7
216	2	7	5	2.7
216	2	14	5.3	2.9
220	2	-7	4.4	2.2
220	2	0	4.9	2.8
220	2	7	4.4	2.9
220	2	14	4.9	2.2
231	2	-7	4.6	2.9
231	2	0	5.2	2.4
231	2	7	5.6	2.9
231	2	14	5.2	2.9
219	2	-7	4	3.1
219	2	0	4	2.6

219	2	7	4.5	2.5
219	2	14	5.4	2.9
77	2.5	-7	3.3	1.9
77	2.5	0	4.7	3
77	2.5	7	4.8	3
77	2.5	14	5.1	3.9
78	2.5	-7	3.9	2.3
78	2.5	0	4.6	3.6
78	2.5	7	4.4	3.5
78	2.5	14	4.4	3.1
55	2.5	-7	4.9	2.4
55	2.5	0	4.3	3
55	2.5	7	5.3	3.2
55	2.5	14	4.4	2.9
62	2.5	-7	4.4	3.5
62	2.5	0	4	2.9
62	2.5	7	3.8	2.9
62	2.5	14	4.1	2.8
76	2.5	-7	4.5	3.7
76	2.5	0	4.5	3.4
76	2.5	7	4.3	3
76	2.5	14	4.2	3.2
81	2.5	-7	5.5	3.6
81	2.5	0	4.3	3.2
81	2.5	7	4.4	3.5
81	2.5	14	4.4	2.7
99	2.5	-7	4.9	3.6
99	2.5	0	4.9	3.4
99	2.5	7	4	3.4
99	2.5	14	4.8	3.6
94	2.5	-7	4.2	3.3
94	2.5	0	5.1	3
94	2.5	7	4.1	2.5
94	2.5	14	5.2	3.7
90	2.5	-7	4.3	2.4
90	2.5	0	4.7	2.5
90	2.5	7	4.6	3.5
90	2.5	14	4.3	2.9
96	2.5	-7	4.2	2.9
96	2.5	0	4.2	3.3
96	2.5	7	4.7	3.7
96	2.5	14	4.6	3.1
215	2.5	-7	4.2	3.2
215	2.5	0	4.6	2.3
215	2.5	7	4.6	2.9
215	2.5	14	5.1	2.8
237	2.5	-7	3.9	2.5
237	2.5	0	4.9	2.5
237	2.5	7	4.3	2.9
237	2.5	14	5	2.8
218	2.5	-7	4.4	2.2
218	2.5	0	5.1	3.3
218	2.5	7	4.9	2.4
218	2.5	14	4.8	2.2
213	2.5	-7	4.3	3.2
213	2.5	0	4.7	2.7
213	2.5	7	4.5	2.4
213	2.5	14	5.1	2.5
224	2.5	-7	4.4	3.2
224	2.5	0	3.9	2.4
224	2.5	7	5.2	2.2

224	2.5	14	5.1	3.3
73	3.5	-7	5	3.2
73	3.5	0	4	2.8
73	3.5	7	4.1	2.7
73	3.5	14	4.8	3.4
57	3.5	-7	4.7	3.3
57	3.5	0	3.8	2.3
57	3.5	7	4.6	3.7
57	3.5	14	4.2	3.1
72	3.5	-7	4.1	3.2
72	3.5	0	3.9	2.8
72	3.5	7	4.1	2.7
72	3.5	14	3.9	3.1
56	3.5	-7	4.3	2.9
56	3.5	0	4.4	2.8
56	3.5	7	4.9	3.9
56	3.5	14	4.8	3.3
74	3.5	-7	5.1	3.3
74	3.5	0	4.2	2.7
74	3.5	7	4.5	3.5
74	3.5	14	5.1	3.7
85	3.5	-7	5.1	3.4
85	3.5	0	5.7	3
85	3.5	7	4.6	3.2
85	3.5	14	4.5	3.9
103	3.5	-7	4.9	3.2
103	3.5	0	3.9	3
103	3.5	7	5.4	3.7
103	3.5	14	4.9	3.3
83	3.5	-7	5	3.5
83	3.5	0	4.7	3
83	3.5	7	4.6	3.7
83	3.5	14	4.7	3.6
91	3.5	-7	3.9	2.9
91	3.5	0	4.5	2.3
91	3.5	7	4.6	3.3
91	3.5	14	5	2.9
106	3.5	-7	4.8	3.3
106	3.5	0	4.1	3.5
106	3.5	7	4.1	3.1
106	3.5	14	4.8	3.3
225	3.5	-7	4.3	2.3
225	3.5	0	5.1	2.7
225	3.5	7	4.3	2.5
225	3.5	14	4.8	2.5
227	3.5	-7	4.6	2.9
227	3.5	0	4.3	2.6
227	3.5	7	4.5	3
227	3.5	14	5.1	2.9
230	3.5	-7	4.5	3.1
230	3.5	0	5.3	2.7
230	3.5	7	4.9	2.8
230	3.5	14	5.6	3.3
232	3.5	-7	4.9	2.9
232	3.5	0	3.9	2.1
232	3.5	7	4.3	2.1
232	3.5	14	5.9	3
221	3.5	-7	4.1	3.1
221	3.5	0	4.1	2.7
221	3.5	7	4.9	2.6
221	3.5	14	4.7	3.1

66	5	-7	5.9	2.9
66	5	0	3.5	2.7
66	5	7	4.2	2.4
66	5	14	3.9	2.8
64	5	-7	5.1	3.5
64	5	0	4.3	2.9
64	5	7	4.7	3.3
64	5	14	5.7	3.9
54	5	-7	5.1	3
54	5	0	5.9	3.9
54	5	7	5.1	3.2
54	5	14	4.1	3.4
71	5	-7	3.9	2.7
71	5	0	3.9	2.9
71	5	7	3.7	2.6
71	5	14	3.9	2.9
58	5	-7	4.1	3.2
58	5	0	4.5	2.6
58	5	7	5.1	3.3
58	5	14	5.1	3.1
92	5	-7	4.2	3.2
92	5	0	4.6	3.4
92	5	7	4.9	3
92	5	14	4.7	3
104	5	-7	4.7	3.2
104	5	0	5	3.2
104	5	7	4.1	3.2
104	5	14	4.7	2.9
87	5	-7	4.2	3.2
87	5	0	4.4	3.1
87	5	7	4.2	3.3
87	5	14	4.4	3.2
98	5	-7	4.7	2.8
98	5	0	3.5	2.5
98	5	7	4.3	3.4
98	5	14	4.7	2.5
82	5	-7	4.6	3.2
82	5	0	4.5	2.2
82	5	7	3.9	2.5
82	5	14	4	3.1
229	5	-7	4	2.8
229	5	0	4.5	2.5
229	5	7	4.4	3.2
229	5	14	4.8	2.6
236	5	-7	4.3	2.8
236	5	0	4	2.5
236	5	7	4.6	2.1
236	5	14	5	2.9
235	5	-7	4	2.9
235	5	0	4.9	1.7
235	5	7	4.5	2.9
235	5	14	4.3	2.7
217	5	-7	4.5	3.2
217	5	0	4.3	2.6
217	5	7	5.3	3.4
217	5	14	5.1	3.1
214	5	-7	4.5	2.9
214	5	0	4	2
214	5	7	5	2.8
214	5	14	5	2.7

Appendix E: BMDS 2.1 Toxicodiffusion Model Run on TDM Male Data Set

```
Analysis of  ToxicoDiffusion Bootstrap BMDS MODEL RUN
STUDY DESCRIPTION
Dose Levels:   0 2 2.5 3.5 5
Test Times:    -7 0 7 14
Exposure Time:       6
Sample Size:  300

DOSE-RESPONSE MODELING
A0+A1*time+B*dose*time*exp(-K*time)/(1+C*dose*time*exp(-K*time))

       AIC      BIC    logLik
  314.6444 340.5709 -150.3222

Random effects:
Formula: A ~ 1 | ID
                 A  Residual
StdDev: 0.1863377 0.3653020

Fixed effects:
                     Value   Std.Error  DF    t-value       p-value
A.(Intercept)  2.996151317 0.046111514 221 64.9762079 5.349852e-146
A.time         0.004701098 0.002859858 221  1.6438219  1.016348e-01
B.dose        -0.175049877 0.194341262 221 -0.9007345  3.687100e-01
C.dose         0.184639455 0.648949657 221  0.2845205  7.762780e-01
K              0.580987806 0.227168425 221  2.5575201  1.121170e-02

Correlation:
              A.(Intercept)      A.time     B.dose      C.dose           K
A.(Intercept)    1.00000000 -0.63729277 -0.1723380  0.10338690 -0.04296145
A.time          -0.63729277  1.00000000  0.1410961 -0.08027935 -0.24754850
B.dose          -0.17233801  0.14109607  1.0000000 -0.97148741 -0.62323456
C.dose           0.10338690 -0.08027935 -0.9714874  1.00000000  0.55144569
K               -0.04296145 -0.24754850 -0.6232346  0.55144569  1.00000000

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-2.94696906 -0.55517970  0.04644479  0.62503671  2.67259878

Initial Values:      2.992632 0.005213033 -0.01183382 0.06132618 0.1413273

Possible Initial Values
        A0          A1           B0          C0        K0
1 2.992632 0.005213033 -0.011833825  0.06132618 0.1413273
2 2.992632 0.005213033 -0.002883208 -0.27023296 0.2300663
3 2.992632 0.005213033  0.130542817 -4.16955936 0.3043678
4 2.992632 0.005213033 -0.003133904  0.05766134 0.1696372
5 2.992632 0.005213033 -0.003008556 -0.10628581 0.1998518
6 2.992632 0.005213033  0.028172970 -1.08020120 0.2113497

BENCHMARK DOSE ESTIMATION
Risk Type:                  extra
Spontaneous Risk Level:           5 %
Area of Adverse Effects:    Lowertail
BMR Level:                  5 %

BOOTSTRAP ESTIMATION OF BMDL

Bootstrap Replications:           250
Minimum BMD:                1.328972
At Test Time:               0.68
Conf. Level:                95 %
```

```
BMDL:                    0.770022
```