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Non-linear Regression Analysis of Benchmark Dose for Continuous Dose Response Data

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Abstract

The benchmark dose (BMD) is a statistical estimate for the dose that induces a given response for a toxicological effect above the background incidence measured in animal experiments. In recent years, the method has been used in health risk assessment as an alternative to a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL), which are calculated by statistically testing each dose group against the controls. This work focuses on non-linear methods that are used to fit continuous dose response data, to calculate BMD and to calculate a lower statistical confidence limit of BMD, usually called BMDL. Results in this thesis show that the Hill equation fits non-linear dose responses satisfactorily. The most used methods to derive BMD for continuous dose response data are the hybrid method and relative changes in the means. Both methods still have some disadvantages. To estimate a lower statistical confidence limit of BMD, three methods are used, the delta method, the bootstrap method and the profile-likelihood method. Results show that the profile-likelihood method is the most preferable.

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Preface

This report is part of a master thesis in Mathematical Statistics at Stockholm University. The work was performed at the Institute of Environmental Medicine (IMM)[1] at Karolinska Institute (KI) in Stockholm.

I would like to thank my supervisor *Mikael Andersson* at the Department of Mathematics division Mathematical Statistics, Stockholm University, for his support and help with the mathematical part in this work.

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[1] The Institute of Environmental Medicine, a department at Karolinska Institutet, is an interdisciplinary research organization within the field of Environmental Medicine. The purpose is to carry out research and education, and perform investigations and analyses, pertaining to physical and chemical aspects of environmental medicine and health protection. Within the Institute, internationally competitive research in the fields of toxicology, environmental medicine and epidemiology is conducted. IMM works together with international health organizations, primarily within the World Health Organization´s (WHO) environmental health program as a WHO Collaboration Center for Environmental Health Effects.

1. INTRODUCTION

In environmental health risk assessment, dose-response assessment is one of four steps that are used to identify the toxic properties of a certain chemical and estimate the risk of health effects in an exposed population.Traditionally in risk assessment, analyzing dose-response data from animal studies is based on statistically testing each dose group against the controls, which results in a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) if it is not possible to determine a NOAEL [3]. Because of shortcomings in the NOAEL/LOAEL method, the benchmark dose method was introduced as an alternative [4]. The method is based on the idea to fit a mathematical model to dose-response data and thereafter to calculate BMD and its lower confidence limit (BMDL) for a dose that produces a predetermined change compared to the background.

The aim of this thesis is to analyse the use of the benchmark-dose (BMD) method for continuous data and to apply the method to data on 2,3,7,8-tetrachlorde-dibenzo-p-dioxine (TCDD) using data from animal studies.

In Chapter 2 in this work the reader is introduced to dioxins and the health effects that dioxin exposure causes. Thereafter health risk assessment and the methods that are used in dose-response assessment are presented. In Chapter 3 the design of the animal experiment is described, while Chapter 4 presents the methods that are used to fit the data, to define BMD and to calculate BMDLs. Finally in Chapter 5, the results from this work are presented and discussed. Tables including parameter estimates, BMDs and BMDLs for the fitted responses are presented in the appendices.

2. BACKGROUND

2.1 DIOXINS

Dioxin [1] is a term that refers to Polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF). PCDD and PCDF are built upon two almost planar tricyclical aromatic compounds with similar properties. There are 210 possible combinations of linking chlorine atoms to the carbon skeletons, of which 75 are variations related to PCDD and 135 to PCDF. Different dioxin compounds have different toxicities which depend on the number and positions of the chlorine atoms. The most toxic form of dioxin is 2,3,7,8-tetrachloride-dibenzo-p-dioxine (TCDD).

Figure 2.1 The structural formulas for polychlorinated dibenzo-p-dioxins (PCDD) and dibenzofurans (PCDF).

Dioxins are formed as a result of combustion processes such as commercial or municipal waste incineration and from burning fuels. Dioxins can also be formed as a result of natural processes such as forest fires or industrial processes such as chlorine bleaching of pulp and paper.

Dioxins are lipophilic and very stable, the concentrations increase with each step in the food chain. They break down so slowly that some of the dioxins from past releases will still be in the environment many years from now. Because dioxins exist throughout the environment, almost every living creature, including humans, has been exposed to dioxins. Approximately 90% of human exposure to dioxin comes from food, especially from beef, fish, and dairy products. Contamination in the food supply comes from dioxin particles that are deposited in water or soil and then proceed up the food chain through fish and livestock.

Studies have shown that exposure to dioxins at sufficiently high doses may cause a number of adverse health effects. Dioxin exposure causes chloracne, which results in small, pale yellow skin lesions that may last from weeks to years. Dioxins can cause short-term liver dysfunctions without any visible symptoms. These include changes in metabolism and enzymatic activity in the liver, which are similar to those resulting from the consumption of alcoholic beverages. In animal studies, dioxins have caused nerve damage, birth defects, increased incidence of miscarriages and significant changes to the immune system. Studies have shown that reproductive, immune and nervous systems of the developing fetus are more sensitive and susceptible to dioxin toxicity. Exposure to large amounts of dioxins over a short period of time, or continuous low-level exposure over an extended period can cause cancer and severe immune deficiency effects in animals.

2.2 RISK ASSESSMENT

In health risk assessment, focus is on the following questions:

What is the probability that a non-desired effect will occur in connection to exposure of a given dose of a certain chemical? If the non-desired effect does occur, what is the estimated severity of it?

In order to be able to make a credible risk assessment, we need information regarding toxicity of the substance and the estimated exposure.

The health risk assessment process consists of four steps: hazard identification, dose-response assessment, exposure assessment and risk characterization [2].

Hazard identification; determine damage and implications on human health a chemical could cause by reviewing studies of its effects in humans and laboratory animals.

Dose-response assessment; evaluate the information obtained during the hazard identification to estimate the amount of a chemical that is likely to result in particular health effects.

Exposure assessment; determine the size and nature of the population exposed and the route, amount and duration of the exposure.

Risk characterization; estimate the risk of health effects in an exposed population with respect to the information developed in the previous steps.

In this thesis we will focus on and analyze the methods that are used in dose-response assessment.

2.2.1 TRADITIONAL DOSE-RESPONSE ASSESSMENT

Traditionally in risk assessment, analyzing dose-response data from animal studies is based on statistically testing each dose group against the controls, which results in a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) if it is not possible to determine a NOAEL. In order to determine an acceptable daily intake (ADI), the measures are then divided with respect to uncertainty factors. These factors represent a default approach to account for uncertainties in the risk assessment, for instance difference in sensitivity between species, variation between individuals, extrapolation from LOAEL to NOAEL and difference in duration and exposure route.

Using the NOAEL in determining ADIs has many limitations. Some limitation include the following:

1. The NOAEL is limited to the doses tested experimentally.
2. Experiments involving fewer animals tend to produce larger NOAELs which may produce larger ADIs.
3. The slope of the dose response plays little role in determining the NOAEL.
4. Use of a NOAEL does not provide estimates of the potential risk associated with some exposure level.

These and other limitations have prompted a search for alternatives, and one alternative to a NOAEL is the benchmark dose (BMD) method [3].

2.2.2 THE BENCHMARK DOSE METHOD

The use of the Benchmark dose method in health risk assessment was described for the first time by Crump in 1984 [4]. He defined the BMD as the dose that induces a given response for a toxicological effect above the background incidence.

The calculation of BMD includes the following steps:

1. A response or group of responses from one or more experiments are selected.
2. Mathematical models are used to fit the dose response data.
3. The level of change from the control response, called the benchmark response (BMR) is defined.
4. The BMD, which is the dose level that produce BMR, is calculated.
5. The BMDL is calculated as a lower statistical confidence limit of BMD.

Figure 2.2 provides the graphical description of the Benchmark dose methodology.

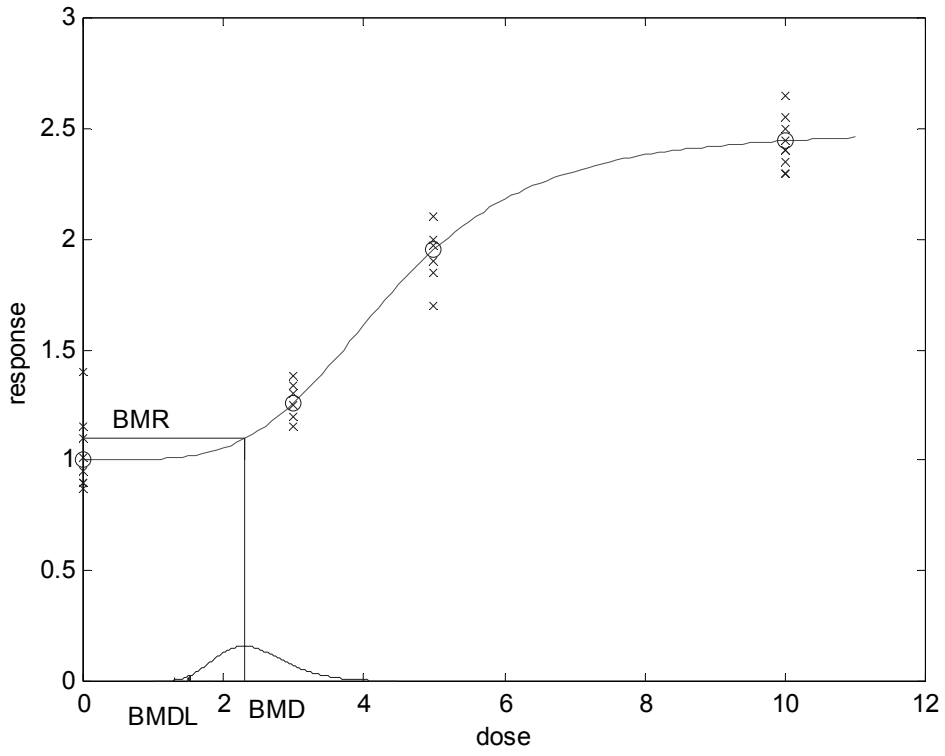

Figure 2.2 The benchmark dose method. The BMD is calculated with 10% change in the control mean.

Fitting the dose response data with a mathematical model is easy for all kinds of dose responses, but the calculation of BMD, which depends on the definition of BMR, has different difficulties depending on what kind of responses that are available.

Defining a BMD from binary (yes/no) data is relatively straightforward. Since we define the responses as relative frequencies when we fit data, it's naturally to define BMR as a change in risks. In this case BMR is defined as

$$BMR = P(d) - P(0) \qquad \text{(Additional risk)}$$

or

$$BMR = \frac{P(d) - P(0)}{1 - P(0)} \qquad \text{(Relative risk)}$$

there *P(d)* is the probability for yes in dose level *d*.

In the case of continuous data, the definition of BMR is more problematic, since we use the estimated mean and variance in the definition. A certain level of BMR will not necessarily mean the same for all toxicological endpoints. An alternative way is to transform the continuous data to binary data, but it may result in missing information, and decreased precision.

3. DATA

3.1 EXPERIMENTS DESIGN

The data used as basis for this work is a part of experiments, which studies liver tumor-promoting activity of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in two different rat strains, the sensitive Long-Evans (L-E) and the resistant Han/Wistar (H/W) [5,6]. This thesis focuses on data from the L-E strain.

Inbred female L-E rats were obtained from the breeding colony of the National Public Health Institute (Kuopio, Finland). The animals were free of typical rodent pathogens. The rats were 5 weeks of age and weighed 70.1 ± 7.8 g. The experiment was designed with a control group and 3 treatment groups. Each group consisted of 10 animals. The rats were administered TCDD with 0.007, 0.07 and 0.7 μg/kg respectively once a week for 20 weeks (1, 10 and 100 ng/kg/day). To rapidly achieve the kinetic steady state, the first dose was a loading dose, which was five times as high as the 19 consecutive maintenance doses. The total dose exposure for the animals in each group was 0.17, 1.7 and 17 mg/kg respectively. In the experiment, one of the rats in dose-group nr 4 died, and because of different reason, some of the responses could not be measured for all the animals.

3.2 EFFECTS

In the experiment, the following types of effects were studied: total organ weight, relative organ weight, clinical chemistry, tumor promotion and retinoids. The effects, which are included in each type, are the following:

Total organ weight (g):

Liver weight, thymus weight, spleen weight, kidneys weight, lungs weight and body weight gain.

Relative organ weight (g/100 g body weight):

Relative liver weight, relative thymus weight, relative spleen weight, relative kidneys weight and relative lungs weight.

The clinical chemistry:

Alanine-Aminotransferase (ALAT), Aspartate transaminase (ASAT), alkaline phosphatase (AFOS) and Gamma-glutamyl transpeptidase (GGT) with IU/liter as measure unit.

Albumin (ALB) and relative Albumin with (g/liter plasma) as measure unit.

Glucose, Triglycerides (Trigly) and Protein Free fatty acid (FFA). The measure units are (mmol/liter plasma).

Tumour promotion:

Volume fraction of foci (%), volume and number of foci/cm^3.

Retinoid:

Retinoids [7] are derived from dietary vitamin A, which is an essential nutrient derived from carotenoids in plants and retinyl esters from animal sources. By definition the term vitamin A is used not for a specific chemical but for compounds that exhibit qualitatively the biological activities of all-trans retinol. The term retinoids, on the other hand include both the natural and synthetic analogs of retinol, whether with or without biological activity (IUPAC-IUB, 1983). Vitamin A active compounds that are present in mammals include retinol, retinal, retinoic acid, retinyl esters (e.g. retinyl palmitate and retinyl stearate) and β-carotene.

In the experiment, retiniods activity was studied in liver, kidneys and plasma. The following responses were included:

- Liver (nmol/ g liver): Liver retinyl palmitate, liver retinyl stearate, liver retinol and total liver retinoids
- Kidneys (nmol/ g kidneys): Kidney retinyl palmitate, kidney retinyl stearate, kidney retinol and total kidney retinoids.
- Plasma (nmol/ g plasma): Plasma retinol, plasma ohra, plasma 13-cis retinoic acid, plasma retinoic acid, plasma linoleate, plasma oleate, plasma retinyl plamitate, plasma retinyl stearate, plasma ester

4. METHODS

4.1 MODEL SELECTION

Most of our responses have a non-linear decreasing or increasing curve. There are several ways of estimating these responses at different dose levels. In model selection, it is important to find a model that is simple, yet yields satisfactory estimates. Before we present the models that are used to fit the data, we assume that our responses are normally distributed with expected value $\mu(d)$ and variance $\sigma^2(d)$. According to the initial analysis, the normal assumption could not be rejected. However, it can be noted that the small sample size in the data reduce the power associated with this analysis.

4.1.1 SELECTION OF RESPONSES TO MODEL

One type of models that satisfy these requirements are models based on the Hill equation [8]:

$$\mu(d) = \alpha + \beta \frac{d^{\gamma}}{\kappa^{\gamma} + d^{\gamma}} \tag{4.1}$$

Where α, β and κ are unknown parameters and d is the dose level. For simplicity, we let the parameter γ be equal to 1 from now on. Then the equation follows:

$$\mu(d) = \alpha + \beta \frac{d}{\kappa + d} \tag{4.2}$$

For $d = 0$ then the ratio $\frac{d}{\kappa + d} = 0$ and for $d \to \infty$ we have $\frac{d}{\kappa + d} \to 1$.

This yields that

$\mu(0) = \alpha$.

$\mu(d) \to \alpha + \beta$ when $d \to \infty$,

where β denotes the difference between the response when d=0 and the response when d is large.

Another example following the same principle, although using an exponential transformation of the dose level, is the exponential model [9]

$$\mu(d) = \alpha + \beta(1 - e^{-d/\kappa}) \tag{4.3}$$

where d equals the dose level. In this case, we have

$(1 - e^{-d/\kappa}) = 0$ when $d = 0$

and

$(1 - e^{-d/\kappa}) \to 1$ when $d \to \infty$

which leads up to the same definitions of α and β as in the Hill model.

To get a simpler definition of the BMD, we may define β to be a ratio of $\mu(0)$ and $\mu(\infty)$. One way of modelling is

$$\mu(d) = \alpha + \alpha\beta \frac{d}{\kappa + d} \tag{4.4}$$

This model results in the same estimates as the first model but the distributions of estimates differ as the estimates are more dependent on each other here than in the first model.

The main problem with these models is that they, without restrictions, permit negative values of the responses, something that is not possible for the biological responses analyzed herein. This might result in misleading parameter estimates that imply negative responses. The problem arises when we estimate the total thymus weight from our data. When we adapt the Hill equation to observations of this response, $\mu(d)$ becomes negative when d is large (see Figure 4.1).

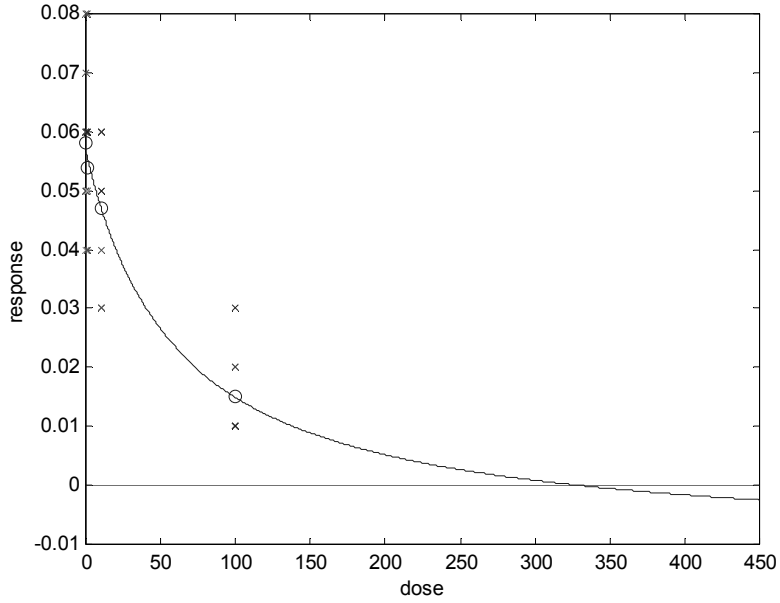

Figure 4.1 Total thymus weight, fitted by equation (4.2) with constant variance

However, this should not be possible as the response is defined as a positive weight. Table 4.1 shows parameter estimates based on our data sample. In order to avoid negative values of the response, it is necessary that $\alpha + \beta > 0$, something that is not accomplished according to Table 4.1. Hence, it is necessary to include the condition $\alpha + \beta > 0$. Therefore, we re-parameterize the model according to

$$\mu(d) = \alpha + \left(e^{\beta} - \alpha\right)\frac{d}{\kappa + d} \tag{4.5}$$

The definition remains the same for α while β is defined differently. Since the updated model yields that $\mu(\infty) \to e^{\beta}$, $\mu(d)$ will always be positive for any value of β.

Table 4.1 Parameter estimates for equation (4.2), estimated to fit total thymus weight

Model	α	κ	β	variance
$\mu(d) = \alpha + \beta\frac{d}{\kappa + d}$	0.057	55.31	-0.065	0.00012

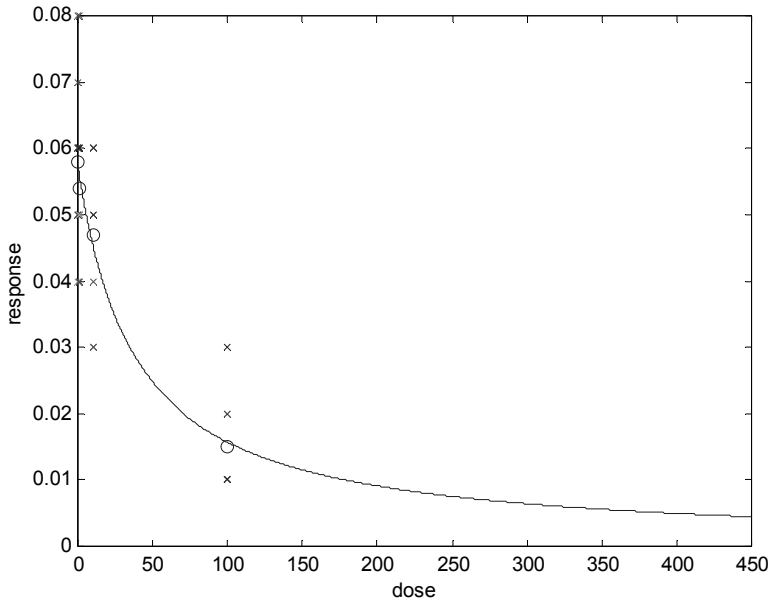

Figure 4.2 Total thymus weight, fitted by equation (4.5) with constant variance

As Figure 4.2 indicates, the estimates of the responses are positive with the new model, and the fit of the model is still good based on the deviances and the p-values in Table 4.2, which are calculated according to Section 4.1.4.

Table 4.2 Result of hypostasis tests for equations (4.2) and (4.5), selected to fit total thymus weight.

MODEL	DF	DEVIANCE	P
$\mu(d) = \alpha + \beta \frac{d}{\kappa + d}$	4	3.16	0.531
$\mu(d) = \alpha + \left(e^{\beta} - \alpha\right) \frac{d}{\kappa + d}$	4	3.39	0.495

4.1.2 VARIANCE MODEL

One important issue is to determine whether the variance is constant or not. Naturally, it would be easier to assume constant variance. However, a model with constant variance might entail estimates with lower accuracy. Below, we present two ways of modeling the variance:

- Variance dependent of the estimated response level:

$$\sigma^2(d) = \lambda\left(\mu(d)\right)^{\tau} \tag{4.6}$$

- Variance dependent of the dose level:

$$\sigma^2(d) = e^{\lambda+\tau d} \tag{4.7}$$

where λ and τ are parameters.

In both models, we end up with constant variance if τ is set to zero. When we carry out hypothesis testing we receive better deviance values when we model the variance, but since we lose one degree of freedom it is not certain that we end up with a more reliable model.

4.1.3 PARAMETER ESTIMATION

The most common way of estimating the parameters involved in the response and variance model is to use the maximum likelihood method [10]. Given continuous samples of doses and responses, which are assumed to be normally distributed with expected value $\mu(d)$ and variance $\sigma^2(d)$, for g dose levels the log-likelihood function follows:

$$\log L = -\frac{N}{2}\log(2\pi) - \sum_{i=1}^{g}\left[\frac{n_i}{2}\log\sigma^2(d_i) + \frac{(n_i-1)s_i^2}{2\sigma^2(d_i)} + \frac{n_i(\bar{y}_i - \mu(d_i))^2}{2\sigma^2(d_i)}\right], \tag{4.8}$$

where

N = total number of observations.

n_i = number of observations in sample number *i*.

$\overline{y}_i$ = the sample mean in dose group number *i*.

s_i^2 = the unbiased sample variance in dose group number *i*.

If we substitute $\mu(d_i)$ and $\sigma^2(d_i)$ according to our models, then the vector θ, that contains the unknown parameters for $\mu(d_i)$ and $\sigma^2(d_i)$, is estimated by maximizing *logL*. The maximum of *logL* is usually determined from partial derivation of *logL* with respect to the elements in θ and then setting the derivates to zero. The parameter estimates are retrieved from solving these equations numerically. There are several disadvantages of this method in our case. This method requires tedious derivation of the maximum-likelihood functions where the risk of errors should be taken into account. Solving equations numerically might also be troublesome. In practice, it is more convenient to minimize *–log L*, using an initial vector θ_0. Here we need to choose the initial vector very carefully. Otherwise we risk finding the wrong optimal θ, since *logL* might have several local maxima.

4.1.4 HYPOTHESIS TESTING

To see if a model describes the data as well as the full model does, we test the hypothesis

H_0: The selected model fits the data as well as the full model.

versus

H_1: The full model fits the data better than the selected model.

The full model is that each dose group is fitted independently, usually by making the mean and variance equal the sample mean and variance. It can be shown that the deviance, which is twice the difference of the log-likelihood associated with the "reduced" model and the full model.

$$D = -2\log(L_{\max} / L_{\text{model}})$$

follows a χ^2 distribution [11], with the number of degrees of freedom equal to the difference in the number of parameters in both models, if H_0 is correct. In this work a p-value < 0.1 is set as the rejection level for H_0, i.e. we will use the selected model to fit the data if the p-value for the test is > 0.1.

4.2 BMD

4.2.1 CONTINUOUS DOSE-RESPONSES

For continuous dose responses, the definition of the BMD is not straightforward since the definition of BMR in terms of probabilities is not obvious. Instead of utilising probabilities from the definition of the BMR, the most common way is to use expected values [8]. One way of defining the BMR is to regard it as a relative change of the expected value of the background variable (the response when *d* equal to 0), i.e.

$$BMR = \frac{\mu(BMD) - \mu(0)}{\mu(0)} \tag{4.9}$$

which yields

$$\mu(BMD) = \mu(0) + BMR\,\mu(0) \tag{4.10}$$

Another alternative is to consider the difference between the expected value of the background response versus the response when d is large, i.e.

$$BMR = \frac{\mu(BMD) - \mu(0)}{\mu(\infty) - \mu(0)} \tag{4.11}$$

which entails

$$\mu(BMD) = \mu(0) + BMR\left(\mu(\infty) - \mu(0)\right) \tag{4.12}$$

4.2.2 THE HYBRID METHOD

The previous methods for computing the BMD only consider the change in expected values. It may also be of interest to take the variance under consideration. One method that involves the variance is a method sometimes referred to as the Hybrid method [8, 12]. The idea behind the Hybrid method is to consider the probability change between the background variable and the dose response given a cut-off value c. Based on the assumption of normally distributed data, the probability that the response at dose *d* is larger than c follows:

$$P(d) = 1 - \Phi\left[\frac{c - \mu(d)}{\sigma(d)}\right],$$

where $\mu(d)$ is the expected value of the response at dose level *d*, $\sigma(d)$ is the standard deviation at dose level *d* and Φ is the distribution function of the standard normal distribution. From this definition and depending on how the BMR is defined, it is possible to derive the BMD. The two most commonly used definitions of the BMR are an absolute change and a relative change in probability.

Absolute change:

$$\begin{aligned} BMR &= P(BMD) - P(0) \\ &= 1 - \Phi\left[\frac{c - \mu(BMD)}{\sigma(BMD)}\right] - 1 + \Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right] \\ &= \Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right] - \Phi\left[\frac{c - \mu(BMD)}{\sigma(BMD)}\right] \end{aligned} \tag{4.13}$$

which yields

$$\mu(BMD) = c - \sigma(BMD)\Phi^{-1}\left(\Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right] - BMR\right) \tag{4.14}$$

Based on the expected values and the variance, it is possible to compute the BMD as a function of the model parameters, c and the BMR.

Relative change:

$$BMR = \frac{P(BMD) - P(0)}{1 - P(0)} = \frac{\Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right] - \Phi\left[\frac{c - \mu(BMD)}{\sigma(BMD)}\right]}{\Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right]} \tag{4.15}$$

that results in

$$\mu(BMD) = c - \sigma(BMD)\Phi^{-1}\left(\Phi\left[\frac{c - \mu(0)}{\sigma(0)}\right](1 - BMR)\right) \tag{4.16}$$

The disadvantage with the Hybrid method is that it is highly dependent of the definition of c. For a given value of the BMR, the method yields different BMDs if we change c.

4.3 BMDL

As there are relatively few observations in our data sample, it is important to evaluate the statistical level of uncertainty in our study. We do that through computing the lower limit of the confidence interval of the BMD, a measure called BMDL. There are several ways of computing the lower limit for BMD. In this thesis we will evaluate three different methods: the delta method, bootstrapping and the profile-likelihood method.

4.3.1 THE DELTA METHOD

In the delta method, the expected value and variance for the BMD is computed approximately by specifying a statistical distribution for each estimate. Then, the BMDL follows:

$$BMDL = \hat{BMD} - z_{1-\alpha}\hat{std}(\hat{BMD})$$

Depending on the statistical distribution used, and the definition of the BMR, we can approximately compute the expected value and the variance through Gauss' approximation formulas [13].

If

$BMD = g(X_1, X_2, ..., X_n)$ where $(X_1, X_2, ..., X_n)$ are the estimators of the parameters defining BMD.

According to Gauss' approximation formulas, we have

$$E[g(X_1, X_2, ..., X_n)] \approx g(m_1, m_2, ..., m_n)$$

where $m_i = E[X_i]$

$$V[g(X_1, X_2, ..., X_n)] \approx \sum_{i=1}^{n} V(X_i)\left(\frac{\partial g}{\partial m_i}\right)^2 + 2\sum_{i<j} C(X_i, X_j)\frac{\partial g}{\partial m_i}\frac{\partial g}{\partial m_j}$$

$E(X_i)$ is derived from the ML-method, using the parameter estimates that maximizes *logL*.

$V(X_i)$ and $C(X_i, X_j)$ are calculated from the covariance matrix, that is I^{-1} , where I is the information matrix. The estimated information matrix is derived from:

$$I(X_1, X_2, ..., X_n) = \begin{pmatrix} \frac{\partial^2 \ell}{\partial X_1^2} & \frac{\partial^2 \ell}{\partial X_1 \partial X_2} & ..... & \frac{\partial^2 \ell}{\partial X_1 \partial X_n} \\ \frac{\partial^2 \ell}{\partial X_2 \partial X_1} & \frac{\partial^2 \ell}{\partial X_2^2} & ..... & \frac{\partial^2 \ell}{\partial X_2 \partial X_n} \\ . & . & ..... & . \\ . & . & ..... & . \\ \frac{\partial^2 \ell}{\partial X_n \partial X_1} & \frac{\partial^2 \ell}{\partial X_n \partial X_2} & ..... & \frac{\partial^2 \ell}{\partial X_n^2} \end{pmatrix}$$

where ℓ is the log-likelihood function and $X_1, X_2, ..., X_n$ are the unknown variables in the selected model. The covariance matrix can be derived from

$C = I^{-1}$ where $V(X_i) = C(i,i)$ and $C(X_i, X_j) = C(i,j)$

4.3.2 THE BOOTSTRAP METHOD

The bootstrap method [14], like the delta method, is based on estimating the distributions of the parameters estimates that are used in the model. However, the advantage with bootstrapping is that it does not require any statistical assumptions for the parameter distributions. Instead, reference distributions are created by making a large number of simulations from the original data. Bootstrapping involves a three step procedure:

1. For each dose group, new samples are created with the same number of observations by random sampling with replacement from the original data.
2. Parameter estimates and BMD for the new samples are computed.
3. Step 1 and 2 are repeated thousands of times. These parameter estimates results in a reference distribution of the BMD, from which a 95% confidence interval can be derived.

4.3.3 THE PROFILE-LIKELIHOOD METHOD

The profile-likelihood method [15] calculates the BMDL asymptotically by utilizing that the deviance follows an asymptotic χ^2 distribution. The idea behind the methodology is to let one of the parameters depend on BMD, BMR and the other parameters.

Let θ be a vector consisting of the unknown parameters of the model, and let θ_i be a subvector of θ where all parameters of θ are included except for parameter number i.

Based on this model and the definition of the BMR, it is possible to estimate the BMD as a function of θ and the BMR:

BMD = g(BMR, θ) resulting in

$X_i = f(\text{BMD}, \text{BMR}, \theta_i)$

where X_i is parameter number i of the vector θ.

Let BMD1 be a lower value than BMD. If we include f(BMD1,BMR, θ_i) in our model, we have that

$D = -2\left(\log\frac{L_{\max}}{L_{bmd1}}\right)$ is χ^2 distributed with one degree of freedom, where $L_{\max}$ denotes the maximum value of the likelihood function and L_{bmd1} the maximum value of the likelihood function when we use the reduced model.

BMDL is the value of BMD1 that gives

$D \approx \chi^2_{(1-\alpha,1)}$

5. RESULTS AND DISCUSSION

Some of the responses, which are included in the data, don't have a significant effect. Other responses don't have a monotone curve. These responses were not analyzed in this work. The responses, which are analyzed, are presented in Table 5.1.

5.1 ESTIMATION

5.1.1 MODELS

In model selection, there are several issues to consider:

1. To choose models that describe our responses with accuracy but involve as few parameters as possible.
2. Test for constant variance
3. Transform the responses in order to test if the transformed data show constant variance or not.

Since there are many responses that are related to each another, our goal is to find one model that gives satisfactory estimates for all of them. We adjusted the models presented earlier in the thesis to our responses by first assuming constant variance and then testing them against the full model (which is that each dose group is fitted independently). Table 5.1 shows the results of the hypothesis testing.

Table 5.1 Results of hypotheses tests for the Hill model and the Exponential model versus the full model. Constant variance was assumed.

RESPONSE	HILL		EXP.	
	Deviance	p-value	Deviance	p-value
Body weight gain	4.39	0.356	4.30	0.368
Total liver weight	7.08	0.132	8.03	0.091
Total thymus weight*	3.16	0.532	3.17	0.530
Total spleen weight	7.18	0.127	6.60	0.158
Total lungs weight	18.31	0.0011	18.36	0.0011
Relative Liver weight	25.98	$3.2 \cdot 10^{-5}$	26.21	$2.9 \cdot 10^{-5}$
Relative thymus weight	3.58	0.466	3.58	0.465
Relative kidneys weight	17.12	0.00183	17.10	0.0018
Relative lungs weigt	19.46	0.000639	19.49	0.0006
Liver retinyl palmitate	13.91	0.0076	20.91	0.0003
Liver retinyl stearate	20.13	0.00047	27.18	$1.8 \cdot 10^{-5}$
Liver retinol	20.38	0.00042	21.18	0.0003
Liver retinoids (total)	0.0022	0.00219	25.42	$4.2 \cdot 10^{-5}$
Kidney retinol	70.17	$2.1 \cdot 10^{-14}$	70.18	$2.1 \cdot 10^{-14}$
Plasma retinol	7.86	0.0968	7.90	0.0954
Plasma retinoic acid	13.45	0.00927	2.10	0.474
Plasma 13-cis retinoic acid	7.06	0.133	7.010	0.135
Gamma-glutamyl transpeptidase	10.12	0.0384	9.97	0.0410
The number of foci / cm^3	23.18	0.00012	23.21	0.0001
Triglycerides	6.26	0.180	6.11	0.191
Volume fraction	24.38	$6.7 \cdot 10^{-5}$	23.20	0.0001
Relative albumin	4.70	0.320	5.41	0.248

For most of the total organ weights, we get acceptable p-values (p> 0.1) with the Hill model. The exception is for total lungs weight where we receive poor fit with Hill and constant variance. Estimation with the exponential model shows bad results for lungs as well. Total liver weight is below the significance level. Total and relative thymus weight, which has a very high p-value with the Hill model, has the drawback that the fitted curve assumes for negative values when the dose level is large, something that is not possible for the responses, analyzed in this work. However, the exponential model results in an acceptable curve and also a solid p-value. But if we strive to use the same model to fit the responses that are related to one another, we should utilise the modified Hill model (which is presented in Section 4.2). The other relative organ weights, liver retinoids, Gamma-glutamyl transpeptidase, the number of foci and volume fraction are poorly fitted with both models when assuming constant variance. For plasma retinoids, Plasma 13-cis retinoic acid is well estimated by the exponential model, while the Plasma retinol and plasma retinoic acid cannot be estimated by assuming constant variance. For Plasma retinol, both Hill and the exponential model are rejected on 10% significance level.

For those responses where constant variance could not be assumed, we use methods where the variance is modelled. We assumed that the variance depends on the dose level and utilised the definition:

$$\sigma^2(d) = e^{\lambda + \tau d}$$

The consequence of this is that we lose one degree of freedom compared to constant variance. Table 5.2 presents the test results against the full model.

Table 5.2 Results of hypotheses tests for the Hill model and the Exponential model versus the full model. The variance is assumed to depend on the dose level.

RESPONSE	HILL		EXP.	
	Deviance	p-value	Deviance	p-value
Total lungs weight	16.74	0.0008	16.80	0.0008
Relative Liver weight	10.57	0.014	11.15	0.011
Relative kidneys weight	2.049	0.562	2.01	0.571
Relative lungs weight	14.036	0.003	14.09	0.003
Liver retinyl palmitate	2.28	0.516	7.35	0.062
Liver retinyl stearate	16.41	0.0009	21.73	$7.4 \cdot 10^{-5}$
Liver retinol	6.62	0.085	7.18	0.066
Liver retinoids (total)	6.62	0.085	12.97	0.005
Kidney retinol	23.25	$3.6 \cdot 10^{-5}$	23.35	$3.4 \cdot 10^{-5}$
Plasma retinol	2.00	0.573	2.059	0.560
Plasma retinoic acid	13.35	0.004	2.09	0.469
Gamma-glutamyl transpeptidase	6.61	0.085	6.40	0.094
The number of foci / cm^3	2.94	0.402	3.03	0.387
Volume fraction	15.48	0.001	13.73	0.003

The results show that for relative kidneys weight, plasma retinol and the number of foci both models fit the data satisfactory when we assume that the variance depends on the dose level, while total lungs weight, Gamma-glutamyl transpeptidase, plasma retinoic acid, kidney retinol, relative liver weight and relative lungs weight are still poorly estimated. For the Liver retinoids, the results of estimating Liver retinyl palmitate are improved while the other liver redinoids responses could not be modelled. Even triglycerides is badly estimated with variance depending on the dose level but when we assume that the variance is depending on the estimated level, triglycerides become well estimated. The bad estimation performance is maintained even when we compute the logarithm of the responses. However, the models estimate

the sample means with excellent accuracy. The main reason is that the sample variance could not be assumed to be constant and is not even monotone. The problem with total lungs weight cannot be solved with our models while the Liver retinoids can be fitted if we consider the total number of retinoids in the liver instead of concentration per gram liver. Tables 5.3 and 5.4 present test results for the Hill and the exponential models with constant and modelled variance respectively. The best fit is obtained with modelled variance and we will use it from now on for these responses, i.e. liver retinoids.

Table 5.3 Results of hypotheses tests for the Hill model and the Exponential versus the full model. Constant variance is assumed. The responses are total liver retinoids.

RESPONSE	HILL		EXP.	
	Deviance	p-value	Deviance	p-value
Liver retinyl palmitate	11.45	0.022	10.08	0.039
Liver retinyl stearate	4.85	0.303	6.53	0.163
Liver retinol	9.37	0.053	9.52	0.049
Liver retinoids (total)	5.90	0.207	6.03	0.197

Table 5.4 Results of hypotheses tests for the Hill model and the Exponential model versus the full model. The variance is assumed to depend on the dose level. The responses are total liver retinoids.

RESPONSE	HILL		EXP.	
	Deviance	p-value	Deviance	p-value
Liver retinyl palmitate	1.37	0.713	0.33	0.954
Liver retinyl stearate	4.36	0.225	5.87	0.118
Liver retinol	2.57	0.464	2.68	0.443
Liver retinoids (total)	0.43	0.934	0.53	0.912

5.1.2 BMD

5.1.2.1 Relative change in the mean

The most troublesome issue with the benchmark dose method for continuous responses is the computation of the BMD. The methods that were presented in Sections 4.2.1 are the most useful ones, but they have their pros and cons. We start with computation of the BMD, derived from the definition of the BMR according to equation (4.9). We note that a response with small variances for the dose groups, results in significant difference between $\mu(0)$ and the mean for a dose level, lower than BMD, calculated with this definition. Another disadvantage is that a given change in the background mean may have a different meaning depending on the type of biological response that is investigated.

If we now consider equation (4.11). The method calculates μ(BMD) as a relative change with respect to the difference between $\mu(0)$ and $\mu(\infty)$. Because of few samples in the experiment, the estimate of $\mu(\infty)$ may be uncertain, and results in an uncertain BMD. For responses with increasing curves, which have a large ratio between $\mu(\infty)$ and $\mu(0)$, BMD becomes large even if the difference between $\mu(0)$ and $\mu(d)$ (where d is lower than BMD) is significant.

5.1.2.2 The Hybrid method

As mentioned before, the idea behind the hybrid method is to take the variance under consideration and use the difference in frequency between the control and the other dose-level, i.e. given a cut-off value, c, calculate

$$p(d) = P\left(X(d) \le c\right)$$

for decreasing responses and

$$p(d) = P\left(X(d) \ge c\right)$$

for increasing responses, where *X(d)* is the response for dose *d*.

The BMD corresponds to a satisfied absolute or relative change between P(BMD) and P(0). Before calculating BMD with this method, following needs to be determined:

1. Which cut-off value will be used? Shall we use a given c-value or shall we choose c such that we get a given P(0) value?
2. What is an appropriate BMR, i.e. how large can the acceptable change between P(0) and P(BMD) be?

If we use a fixed c, P(0) may become very low, which entails that a satisfied probability change may results in very large BMD. Another possibility is to choose c such that P(0) get a fixed value, then the BMD will be calculated with the definitions of BMR. The main problem here is, that for a given probability change, i.e. BMR, the BMD may become different depending on the value of P(0). Figure 5.1 shows BMDs for body weight gain, calculated with relative risk, i.e. according to equation (4.15), with values of c chosen such that P(0) equals 0.01, 0.05, 0.1, 0.2 and 0.5. The curves show that for a given BMR value, the BMD depends on the value of c. if we choose c, such that P(0) becomes low, BMD get a high value and vice versa.

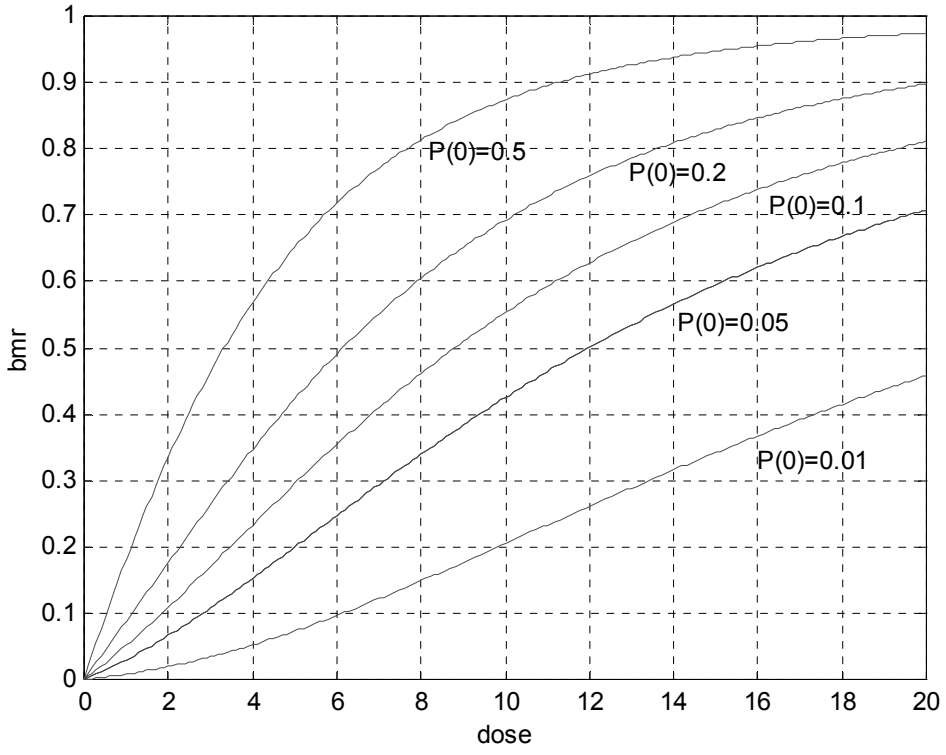

Figure 5.1 Estimated probability dose-response models for body weight gain, hybrid method with relative change. The cut-off, c, is chosen so that p(0) becomes 0.01, 0.05, 0.1, 0.2 and 0.5.

5.1.2.3 Hybrid and relative change in the control mean:

As we mentioned, the value of P(d) depend on the choice of c. For a given BMR, BMD may assume different values, which becomes low when P(0) is high. If we choose $c = \mu(0)(1+BMR)$, Which is μ(BMD) according to equation (4.10), we get

$$P(0) = 1 - \Phi\left[\frac{\mu(0) + \mu(0)BMR - \mu(0)}{\sigma(0)}\right] = 1 - \Phi\left[\frac{\mu(0)BMR}{\sigma(0)}\right]$$

$$P(BMD) = 1 - \Phi\left[\frac{\mu(0) + \mu(0)BMR - \mu(BMD)}{\sigma(0)}\right] = 1 - \Phi\left[\frac{0}{\sigma(0)}\right] = 1 - 0.5 = 0.5$$

which entails the following relations between the definitions of BMR according to the hybrid method and equation (4.9):

$$BMR_{hybrid} = \Phi\left[\frac{\mu(0)BMR_1}{\sigma(0)}\right] - 0.5 \qquad \text{(Additional risk)}$$

$$BMR_{hybrid} = \frac{\Phi\left[\frac{\mu(0)BMR_1}{\sigma(0)}\right] - 0.5}{\Phi\left[\frac{\mu(0)BMR_1}{\sigma(0)}\right]} \qquad \text{(Relative risk)}$$

Figure 5.2 shows BMD, calculated for body weight gain, liver weight and spleen weight. The curves show the change between P(0) and P(d), when c is chosen according to equation (4.10), while the vertical lines show the values of BMD, calculated according relative change in control mean. P(BMD) is equal to 0.5 for all the responses, while the probability changes between P(0) and P(BMD), i.e. BMR(hybrid), are different for different responses. As we mentioned, this depends on the ratio between the mean and the variance of the control dose.

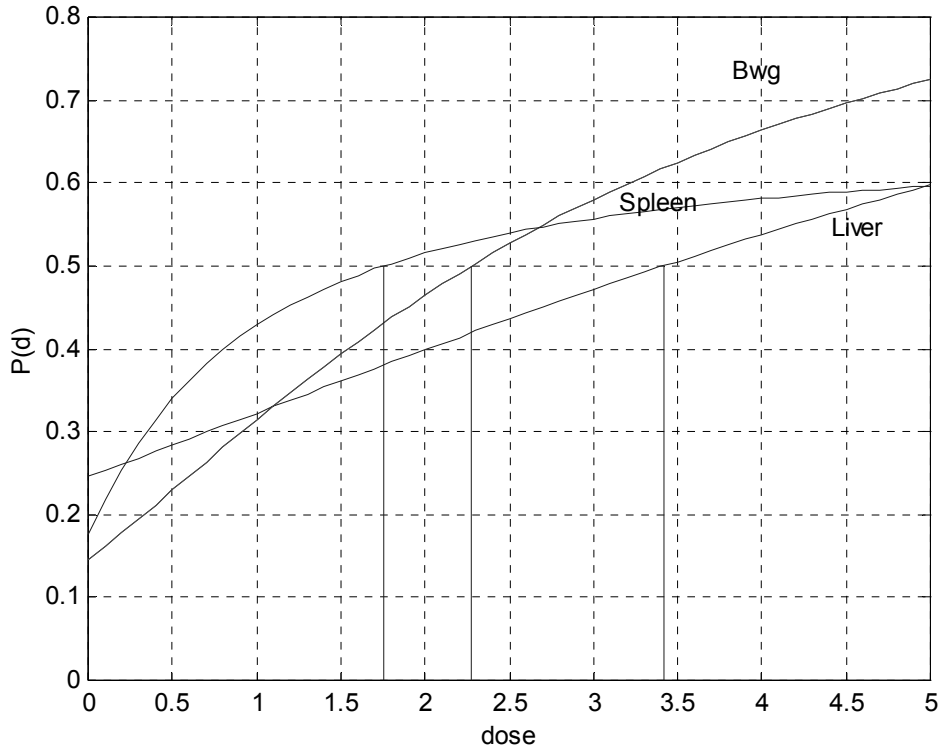

Figure 5.2 Comparisons between the hybrid method and relative change in the control mean.

5.1.3 BMDL

A comparison between the methods we presented in Section 4.3 shows that the delta method results in lower BMDL than the profile-likelihood or bootstrap methods. This does not mean that the delta method is better. On the contrary it has more disadvantages than the other methods. The delta method is based on approximately normally distributed estimates, which results in a symmetric distribution for BMD. For several responses, calculating BMDL with the delta method results in negative values, which is unrealistic. The results by bootstrap simulation show that the distribution of BMD is symmetric only for responses where the differences between low dose-level and higher dose-level are significant, while non significant differences lead to highly correlated parameters, which yields skewed distribution function for the BMD. Figures 5.3-5.12 show results from simulation for liver retinyl palmitate

and Body weight gain respectively, where the data are fitted with the Hill equation and the BMD is calculated according to equation (4.9), i.e. $BMR = \frac{\mu(BMD) - \mu(0)}{\mu(0)}$ with BMR = -0.1

The dose-response differences for liver retinyl palmitate are significant, which implies that the correlation between $\hat{\beta}$ and $\hat{\kappa}$ is low, as Figure 5.6 shows. The histograms for $\hat{\beta}$, $\hat{\kappa}$ and BMD, resulting from the simulations, show that the distribution functions for these parameters have low skewness. For Body weight gain, the differences between the dose-responses aren't significant and the estimates $\hat{\beta}$ and $\hat{\kappa}$ are dependent. The distribution functions for $\hat{\beta}$ and $\hat{\kappa}$ are skew, which yields a skewed distribution function for BMD.

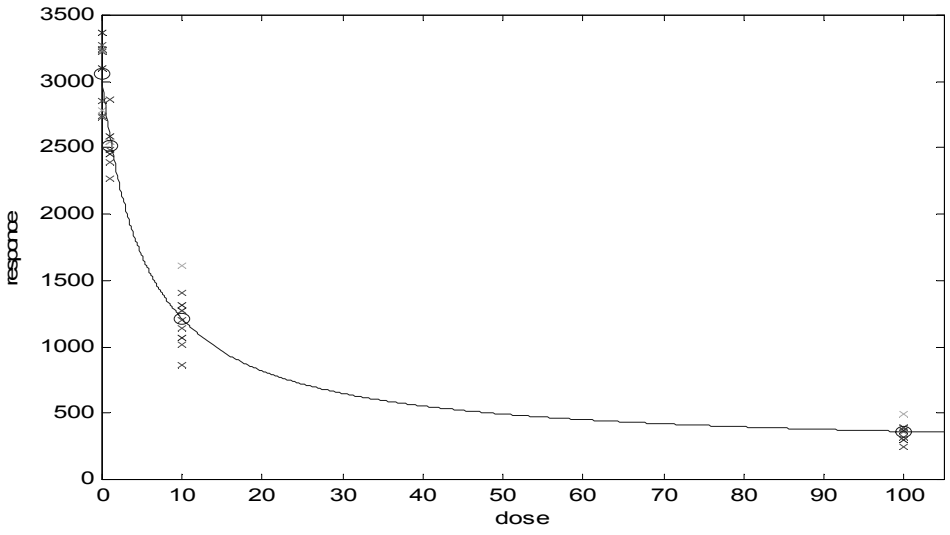

Figure 5.3 Liver retinyl plamitate, fitted by equation (4.2) with variance depending on the dose level.

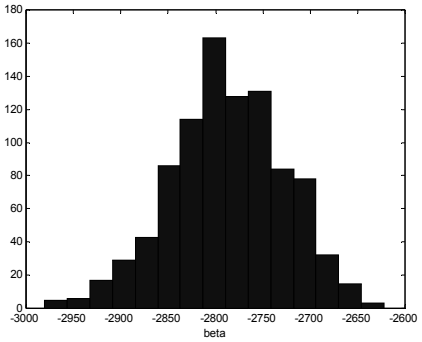

Figure 5.4 Result of bootstrap simulation for parameter $\hat{\beta}$, according to equation (4.2), selected to fit liver retinyl palmitate

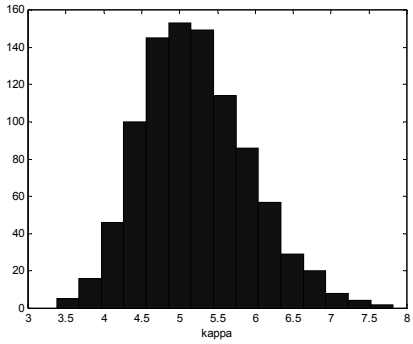

Figure 5.5 result of bootstrap simulation for parameter $\hat{\kappa}$ according to equation (4.2), selected to fit liver retinyl palmitate

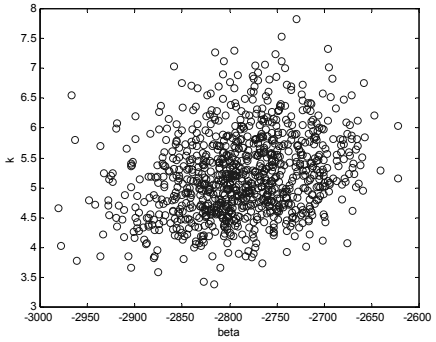

Figure 5.6 plot of $\hat{\beta}$ versus $\hat{\kappa}$, according to equation (4.2), estimated with bootstrap simulation to fit liver retinyl palmitate

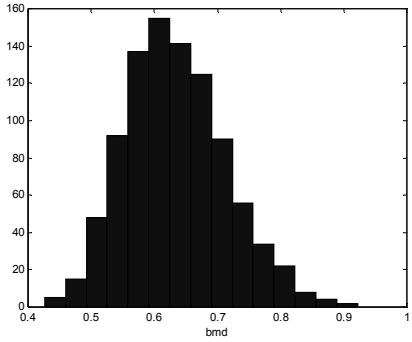

Figure 5.7 BMD, for liver retinyl palmitate, corresponding to equation (4.9), estimated by bootstrap simulation using equation (4.2).

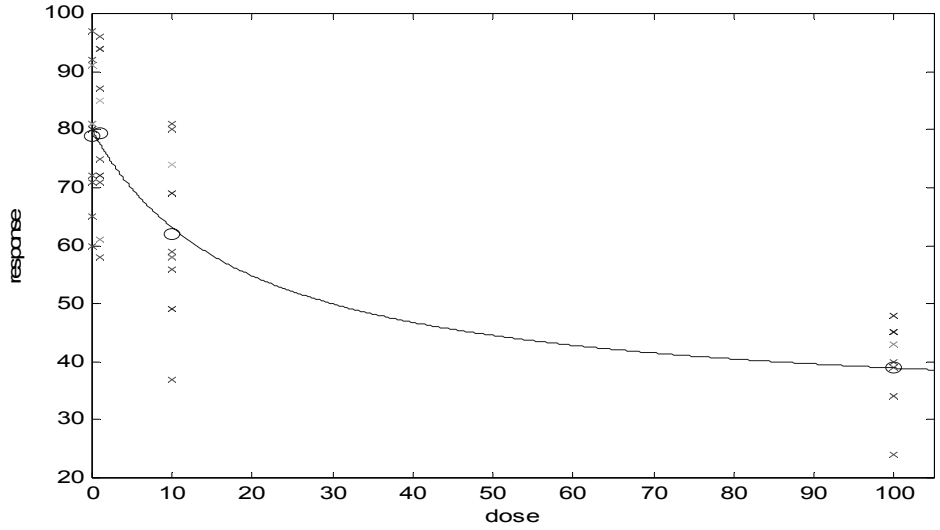

Figure 5.8 Body weight gain, fitted by equation (4.2) with variance depending on the dose level.

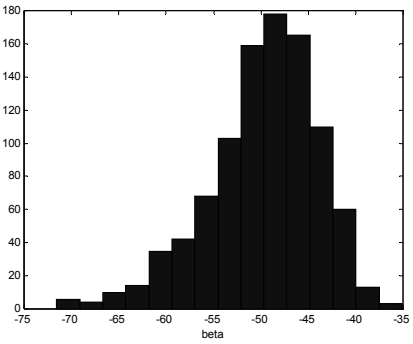

Figure 5.9 Result of bootstrap simulation for parameter $\hat{\beta}$, according to equation (4.2), selected to fit body weight gain

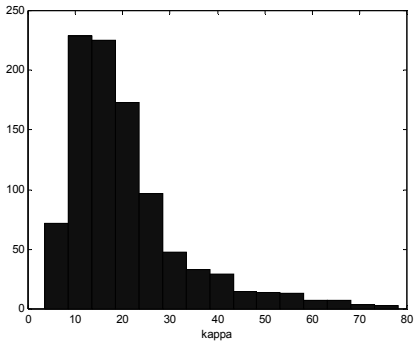

Figure 5.10 Result of bootstrap simulation for parameter $\hat{\kappa}$, according to equation (4.2), selected to fit body weight gain

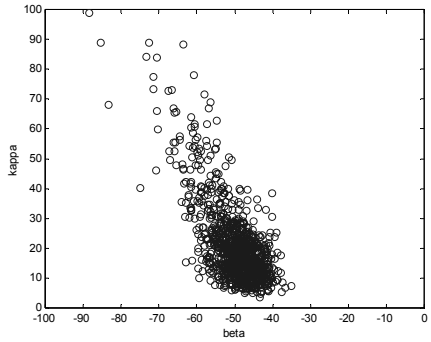

Figure 5.11 plot of $\hat{\beta}$ versus $\hat{\kappa}$, according to equation (4.2), estimated with bootstrap simulation to fit body weight gain

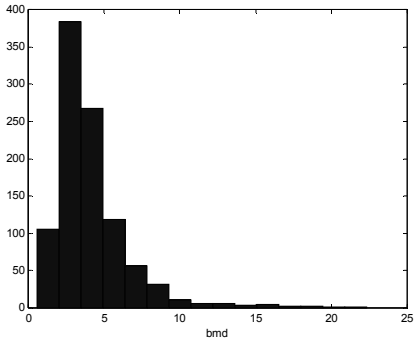

Figure 5.12 BMD, for body weight gain, corresponding to equation (4.9), estimated by bootstrap simulation using equation (4.2).

This problem can be fixed if we calculate a confidence interval for log (BMD) with the delta method and thereafter using Gauss approximations formula to transform the result to normal scale. BMDL can be calculated according to following:

We assume that

$$BMD = \log(BMD)$$

a lower limit for g(BMD) is calculated according to

$$\log(\hat{BMD}) - z_{1-\alpha}\hat{std}(\log(\hat{BMD}))$$

which yields

$$BMDL = \hat{BMD}\exp(-z_{1-\alpha}\hat{std}(\log(BMD))$$

where $\hat{std}(\log(BMD))$ is derived from

$$\text{var}(\log(BMD)) = \left(\frac{1}{BMD}\right)^2 \text{var}(BMD)$$

Tables 5.5, 5.6 and 5.7 show estimated BMDLs, calculated with all the methods, for Body weight gain, Total liver weight, and Liver retinyl palmitate respectively. BMDL calculated with log(BMD) resulted in similar values to bootstrap and profile-likelihood except for the assumption that the variance depends on the response level, which yields lower BMDL values even with log(BMD). For Liver retinyl palmitate,

which has an approximate symmetric distribution function for BMD, BMDL is similar for all three variance assumptions.

Table 5.5 BMDL, for body weight gain, corresponding to equation (4.9) with BMR equal to – 0.1, estimated by delta, delta (log), profile-likelihood and bootstrap, respectively. The selected model is according to equation (4.2).

Variance	BMD	BMDL			
		Delta	Delta(log)	Profile-likelihood	Bootstrap
Constant (λ)	3.42	1.08	1.72	1.51	1.65
$\sigma^2(d) = \lambda\mu(d)^\tau$	3.68	-1.92	0.80	1.69	1.73
$\sigma^2(d) = e^{\lambda+\tau d}$	3.40	0.92	1.64	1.46	1.66

Table 5.6 BMDL, for total liver weight, corresponding to equation (4.9) with BMR equal to – 0.1, estimated by delta, delta (log), profile-likelihood and bootstrap, respectively. The selected model is according to equation (4.2)

Variance	BMD	BMDL			
		Delta	Delta(log)	Profile-likelihood	Bootstrap
Constant (λ)	2.27	-0.57	0.65	0.52	0.72
$\sigma^2(d) = \lambda\mu(d)^\tau$	1.85	-4.37	0.012	0.63	0.73
$\sigma^2(d) = e^{\lambda+\tau d}$	1.96	-0.54	0.55	0.50	0.63

Table 5.7 BMDL, for liver retinyl palmitate concentration, corresponding to equation (4.9) with BMR equal to – 0.1, estimated by delta, delta (log), profile-likelihood and bootstrap, respectively. The selected model is according to equation (4.2)

Variance	BMD	BMDL			
		Delta	Delta(log)	Profile-likelihood	Bootstrap
Constant (λ)	0,63	0.51	0.52	0.49	0.52
$\sigma^2(d) = \lambda\mu(d)^\tau$	0,66	0.62	0.62	0.54	0.56
$\sigma^2(d) = e^{\lambda+\tau d}$	0,64	0.52	0.53	0.50	0.53

The calculations show that the profile-likelihood resulted in lower BMDL value than bootstrap or delta-log, but the differences are not too large. Because of this and the following reasons, the profile-likelihood method is more preferable:

1. The profile-likelihood method's main advantage is that it is very fast. Given a good algorithm, the user will rapidly find a BMDL that fulfills the above condition.
2. The Bootstrap method is most reliable, because the method isn't based on assumptions about theoretical distribution functions and it is easy to compute the parameter distributions and thereby to get an understanding of how different parameters are related to each other. However, the computer time bootstrap needs, is a disadvantage. The method needs thousands of simulation runs to get stable results. Another disadvantage is the choice of the start value for maximizing procedures can be problematic.
3. The calculation of the information matrix, when we use the delta method, may be sensitive for nonlinear models, and may results in incorrect values.

A disadvantage with profile-likelihood is that BMDL can get negative value if the uncertainty in the data is high.

5.2 TOXICOLOGICAL EFFECTS

Organ weight:

The results of the analyzed data in this work show that body weight gain follows a decreasing curve with increasing TCDD dose level. Using BMR definitions, according to equations (4.9) and (4.11) (with BMR equal to 0.05) to define BMD, 95% BMDL equal 0.68 and 0.33 (ng/kg/day), respectively. BMDL calculated with the hybrid method (5 % relative change in probability with chosen c, so that P(0) equal 0.05) becomes 0.671 (ng/kg/day). The estimated models follow increasing curves for total liver weight and total lungs weight, while total thymus weight and total spleen weight follow decreasing curves. However, it's worth noting that the total lungs weight is poorly fitted and the uncertainty in the total spleen samples is too high. Using the same criteria as body weight gain to calculate BMDL and excluding total spleen weight, because of the uncertainty, the total liver weight get the lowest BMDL.

Clinical chemistry:

The responses, analyzed by the benchmark-dose method in this group, are gamma-glutamyl transpeptidase (GGT), triglycerides and relative albumin. GGT and triglycerides follow increasing curves, while relative albumin has a decreasing curve. With the models we used, GGT is poorly fitted, while a comparison between triglycerides and relative albumin show that the effect of TCDD on triglycerides is higher. However, it should be mentioned that the distribution in triglycerides samples make the results uncertain.

Cancer promotion:

The results show increasing curves for both volume fraction of foci and the number of foci. The influence of TCDD on volume fraction of foci was bigger.

Retinoids:

The influence of TCDD on the retinoids is different depending to where in the body the retinoid activities were studied. TCDD exposure results in decreased retinoid activity in the liver, while the activity in the kidneys increase with increased TCDD dose level. For plasma retinoids, which were analyzed with benchmark-dose methods, plasma retinoic acid and plasma retinol follow increasing curves, while plasma 13-cis retinoic acid follows a decreasing curve. Results show that retinoid activity was highly affected by exposure to TCDD. In the liver, liver retinyl palmitates activity was the most affected, while liver retinyl stearate and liver retinol were equally affected. BMDL, (calculated with BMR definition according to equation (4.9) with BMR equal 0.05), becomes 0.3599, 0.5267 and 0.5115 for liver retinyl palmitate, liver stearate and liver retinol, respectively (Data is fitted according to equation (4.2) with variance dependent on the expected means). However, it should be noted that if we accept a p-value equal to 0.05 to fit the data, which means that we can assume that the variance is constant for these responses, BMDL calculated with the hybrid method for liver retinyl stearate and liver retinol show that TCDD has a bigger influence on liver retinyl stearate than on liver retinol. For plasma retinoids, plasma retinoic acid is poorly fitted, while the BMDL for plasma 13-cis retinoic acid, calculated with all definitions, is smaller then plasma retinol.

6. REFERENCES

1. Öberg, M. (2003). Health risk assessment of dioxin-like compounds in complex samples. Institute of Environmental Medicine, Karolinska Institutet, Stockholm.
2. The Swedish national chemicals inspectorate. (2003). Human Health Risk Assessment. Report nr 1/03.
3. U.S. Environmental Protection Agency (USEPA). (1995). The Use of the Benchmark Dose (BMD) Approach in Health Risk Assessment. Final report, EPA/630/R-94/007, Risk Assessment Forum. Washington, DC: U.S. Environmental Protection Agency.
4. Crump, K. (1984). A new method for determining allowable daily intakes. Fundamental and Applied Toxicology, 4, 854-871.
5. Viluksala, M., Barger, Y., Tuomisto, J., Scheu, G., Unkila, M., Pohjanvirta, R., Flodström, S., Kosma, V., Mäki-Paakkanen, J., Vartiainen, T., Klimm, C., Schramm, K., Wärngård, L., Tuomisto, J. (2000) Liver Tumor-promoting Activity of 2,3,7,8-Tetrachloridibenzo-p-dioxin (TCDD) in TCDD-sensitive and TCDD-resistant Rat Strains. Cancer research, 60, 6911-6920.
6. Fletcher, N., Giese, N., Schmidt, C., Stern, N., Lind, M., Viluksela, M., Tuomisto, J., Tuomisto, J., Nau, H. and Håkansson, H. (2004). Altered retinoic acid metabolism in Long-Evans and Han/Wistar rats following long-term 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD)-treatment.
7. Nilsson, C. (1999). Studies on the effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin on vitamin A homeostasis. Institute of Environmental Medicine, Karolinska Institutet, Stockholm.
8. Crump, K. (2002). Critical issues in benchmark calculations from continuous data. Critical Reviews in Toxicology, 32, 133-153.
9. Slob, W. (2002). Dose-response modeling of continuous endpoints. Toxicology Science, 66, 298-312
10. Cox, D., & Hinkley, D. (1974). Theoretical Statistics. London: Chapman & Hall.
11. Ross, G. J. S. (1990). Nonlinear Estimation. Springer-Verlag.
12. Sand, S., von Rosen, D., Falk Filipsson, A. (2003). Benchmark Calculations in Risk Assessment Using Continuous Dose-Response Information: The Influence of Variance and the Determination of a Cut-off Value. Risk Analysis, 23, 1059-1067
13. Blom, G. (1984). Sannolikhetsteori med tillämpningar. Lund: Studentlitteratur.
14. Efron, B., & Tibshirani, R. J. (1993). An Inroduction to the Bootstrap. San Francisco: Chapman & Hall.
15. U.S. Environmental Protection Agency (USEPA). (2004). The Benchmark Dose Software 3.1.1. Available at http://www.epa.gov/ncea.

APPENDICES

n_i = number of observations in sample number i.

$\overline{y}_i$ = the sample mean in dose group number i.

s_i^2 = the unbiased sample variance in dose group number i.

Models:

Hill1: $\mu(d) = \alpha + \beta \frac{d}{\kappa + d}$

Hill2: $\mu(d) = \alpha + (e^{\beta} - \alpha) \frac{d}{\kappa + d}$

Exponential: $\mu(d) = \alpha + \beta(1 - e^{-d/\kappa})$

Variance models:

1: $\sigma^2 = \text{constant} = \lambda$

2: $\sigma^2 = \lambda(\mu(d))^{\tau}$

3: $\sigma^2 = e^{\lambda + \pi d}$

BMD:

1. Relative change to in the mean[1]:

BMD 1:

$BMR = \frac{\mu(BMD) - \mu(0)}{\mu(0)}$ which yields $\mu(BMD) = \mu(0) + BMR\mu(0)$

Hill1: $BMD = \kappa \frac{\alpha BMR}{\beta - \alpha BMR}$

Hill2: $BMD = \kappa \frac{\alpha BMR}{e^{\beta} - \alpha(1 + BMR)}$

Exponential: $BMD = -\kappa \log\left(\frac{\beta - \alpha BMR}{\beta}\right)$

BMD 2:

$BMR = \frac{\mu(BMD) - \mu(0)}{\mu(\infty) - \mu(0)}$ which yields $\mu(bmd) = \mu(0) - BMR(\mu(\infty) - \mu(0))$

Hill1: $BMD = \kappa \frac{BMR}{1 - BMR}$

Hill2: $BMD = \kappa \frac{BMR}{1 - BMR}$

Exponential: $BMD = -\kappa \log(1 - BMR)$

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[1] For relative albumin, $BMD1 \to \infty$ when BMR equal to -0.1.

2. Hybrid method:

The cut-off value is chosen so that *P(0)* = 0.05

Additional risk:

$$BMR = P(BMD) - P(0)$$

which yields

$$\mu(BMD) = \mu(0) + \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)$$

Hill 1: $$BMD = \kappa \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)}{\beta - \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)}$$

Hill 2: $$BMD = \kappa \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)}{e^{\beta} - \alpha - \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)}$$

Exponential: $$BMD = -\kappa \log\left(1 - \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - BMR]\right)}{\beta}\right)$$

Relative risk:

$$BMR = \frac{P(BMD) - P(0)}{1 - P(0)}$$

which yields

$$\mu(BMD) = \mu(0) + \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)$$

Hill 1: $$BMD = \kappa \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)}{\beta - \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)}$$

Hill 2: $$BMD = \kappa \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)}{e^{\beta} - \alpha - \sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)}$$

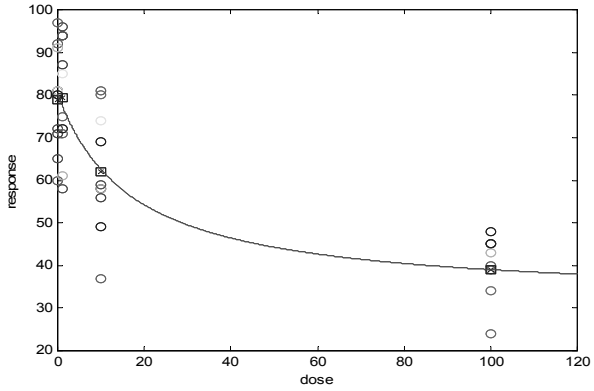
Exponential: $$BMD = -\kappa \log\left(1 - \frac{\sigma\left(\Phi^{-1}[1 - P(0)] - \Phi^{-1}[1 - P(0) - (1 - P(0))BMR]\right)}{\beta}\right)$$

BMDL is calculated with the profile-likelihood method[2]

[2] Because of the uncertainty in total spleen samples, BMDLs get negative values.

Body weight gain (g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	79	146.22
1	10	79.3	192.46
10	10	62.1	194.32
100	9	39.11	55.61



Hill 1:

Parameter estimates:

Variance model	df	α	κ	β	λ	τ	test	p
1	4	80.31	17.16	-48.36	135.59		4.39	0.356
2	3	80.00	18.77	-48.80	0.17	1.60	1.54	0.674
3	3	80.33	17.02	-48.26	5.13	-0.012	0.70	0.873

BMD:

Variance model	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	1.55	0.90	3.42	1.91
2	1.68	0.99	3.68	2.09
3	1.54	0.90	3.40	1.89

BMD(HYBRID)(HYBRID):

Variance model	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.64	1.57	2.95	2.82

BMDL:

Variance model	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.68	0.34	1.51	0.71
2	0.76	0.41	1.69	0.86
3	0.66	0.35	1.46	0.73

BMDL(HYBRID):

Variance model	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.70	0.67	1.26	1.21

Exponential:

Parameter estimates:

Variance model	df	α	κ	β	λ	τ	test	p
1	4	80.18	17.64	-41.24	135.28		4.30	0.368
2	3	79.88	18.83	-41.16	0.17	1.59	1.47	0.689
3	3	80.19	17.58	-41.23	5.13	-0.012	0.63	0.891

BMD:

Variance model	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	1.80	0.90	3.81	1.86
2	1.92	0.97	4.06	1.98
3	1.80	0.90	3.80	1.85

BMD(HYBRID):

Variance model	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.91	1.83	3.32	3.19

BMDL:

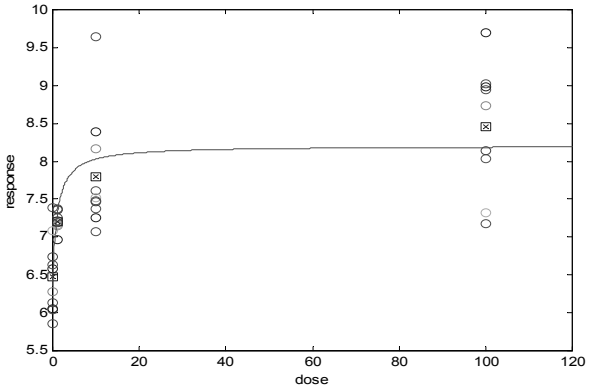
Variance model	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	1.00	0.48	2.12	0.98
2	1.09	0.55	2.30	1.12
3	0.98	0.49	2.06	1.01

BMDL(HYBRID):

Variance model	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
2	1.02	0.98	1.78	1.71

Total liver weight (g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	6.48	0.25
1	10	7.03	0.17
10	10	7.80	0.58
100	9	8.45	0.71



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	6.55	4.44	1.93	0.38		7.08	0.132
2	3	6.52	3.58	1.91	0.0000133	5.07	2.09	0.554
3	3	6.53	3.61	1.86	-1.26	0.0090	4.45	0.216

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.91	0.23	2.27	0.49
2	0.74	0.19	1.85	0.40
3	0.77	0.19	1.96	0.40

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.21	0.055	0.52	0.115
2	0.25	0.06	0.63	0.129
3	0.20	0.044	0.50	0.093

BMD(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.58	0.56	1.07	1.03

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.15	0.14	0.27	0.26

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	6.64	8.89	1.80	0.39		8.03	0.091
2	3	6.61	7.22	1.77	0.000019	4.93	3.69	0.296
3	3	6.64	8.58	1.77	-1.20	0.0081	5.83	0.120

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	1.82	0.46	4.11	0.94
2	1.49	0.37	3.37	0.76
3	1.78	0.44	4.02	0.90

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.34	0.085	0.77	0.17
2	0.31	0.075	0.70	0.15
3	0.22	0.049	0.50	0.10

BMD(HYBRID):

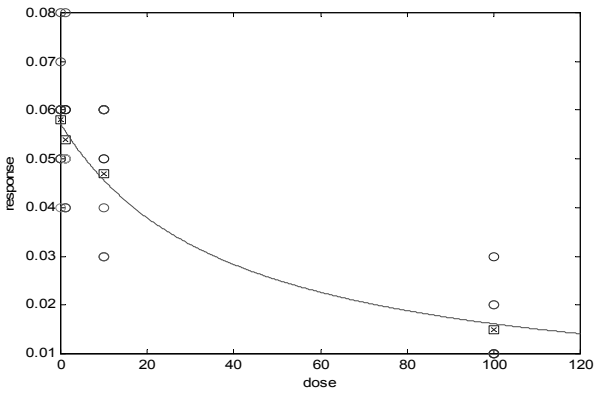
variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.21	1.16	2.12	2.04

BMDL(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.25	0.24	0.44	0.42

Total thymus weight (g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.058	0.00012889
1	10	0.054	0.00013778
10	10	0.047	0.00017889
100	8	0.015	5.7143e-5



Hill 2:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.057	39.37	-32.98	0.00012		3.39	0.495
2	3	0.057	37.82	-111.48	0.0012	0.75	1.37	0.714
3	3	0.057	37.50	-35.03	-8.87	-0.0099	1.12	0.773

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	2.07	2.07	4.37	4.37
2	1.99	1.99	4.20	4.20
3	1.97	1.97	4.17	4.17

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	1.21	1.20	2.55	2.55
2	1.27	1.27	2.68	2.68
3	1.26	1.26	2.66	2.66

BMD(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	2.92	2.79	5.14	4.94

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.62	1.55	2.85	2.73

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.057	41.18	-0.05	0.00012		3.17	0.530
2	3	0.056	44.42	-0.05	0.0012	0.74	0.96	0.811
3	3	0.057	41.35	-0.05	-8.87	-0.01	0.85	0.838

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	2.64	2.11	5.45	4.34
2	2.79	2.28	5.76	4.68
3	2.64	2.12	5.47	4.36

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	1.26	0.89	2.61	1.83
2	1.30	0.95	2.70	1.96
3	1.22	0.89	2.54	1.84

BMD(HYBRID):

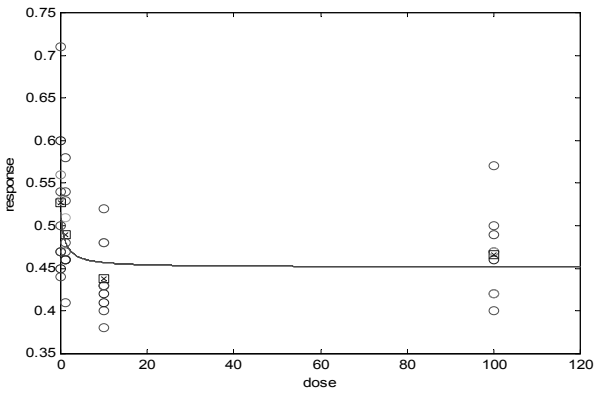
variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	3.71	3.56	6.41	6.17

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.69	1.62	2.93	2.81

Total spleen weight (g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.53	0.0068
1	10	0.49	0.0025
10	10	0.44	0.0018
100	9	0.47	0.0027



Hill 1:

Parameter estimates:

Variance	df	α	κ	β	λ	τ	test	p
1	4	0.53	0.83	-0.08	0.0032		7.18	0.127
2	3	0.53	0.55	-0.08	0.31	6.33	3.64	0.304
3	3	0.53	0.74	-0.08	-5.64	-0.0039	6.74	0.081

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.43	0.044	1.75	0.092
2	0.28	0.029	1.15	0.061
3	0.41	0.039	1.79	0.082

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	X	X	X	X
2	X	X	X	X
3	X	X	X	X

BMD(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.30	0.28	0.66	0.62

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	X	X	X	X

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.53	1.46	-0.08	0.0032		6.60	0.158
2	3	0.53	1.16	-0.08	0.0073	1.18	5.40	0.145
3	3	0.53	1.40	-0.07	-5.66	-0.0038	6.18	0.103

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.63	0.075	1.75	0.15
2	0.46	0.059	1.23	0.12
3	0.62	0.072	1.78	0.15

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	X	X	X	X
2	X	X	X	X
3	X	X	X	X

BMD(HYBRID):

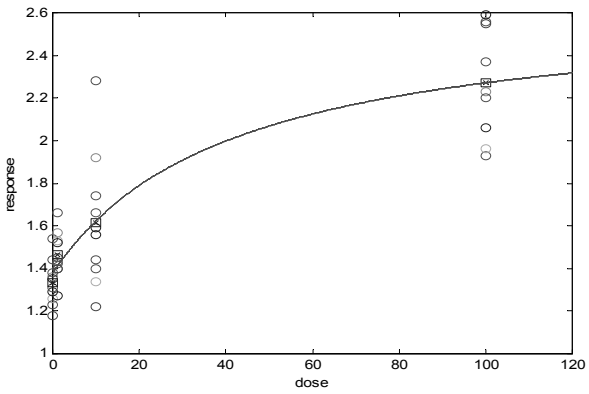
variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.46	0.44	0.89	0.84

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	X	X	X	X

Total lungs weight (g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	1.33	0.011
1	10	1.47	0.012
10	10	1.62	0.096
100	9	2.27	0.067



Hill 1:

Parameter estimates:

Variance	df	α	κ	β	λ	τ	Test	p
1	4	1.38	42.04	1.27	0.043		18.31	0.001
2	3	1.35	12.87	0.87	0.0023	5.73	11.72	0.008
3	3	1.38	41.70	1.26	-3.38	0.0072	16.74	0.001

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	2.43	2.21	5.15	4.67
2	1.09	0.68	2.38	1.43
3	2.41	2.19	5.12	4.63

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	1.02	0.80	2.18	1.69
2	0.33	0.16	0.74	0.33
3	1.07	0.80	2.30	1.69

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.65	2.54	4.63	4.45

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.19	1.14	2.10	2.01

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	1.38	33.83	0.94	0.043		18.36	0.001
2	3	1.36	18.70	0.82	0.0037	4.72	12.20	0.007
3	3	1.38	33.67	0.94	-3.38	0.0072	16.80	0.001

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	2.59	1.74	5.39	3.56
2	1.62	0.96	3.40	1.97
3	2.58	1.73	5.37	3.55

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	1.26	0.86	2.61	1.77
2	0.53	0.24	1.13	0.51
3	1.32	0.86	2.74	1.77

BMD(HYBRID):

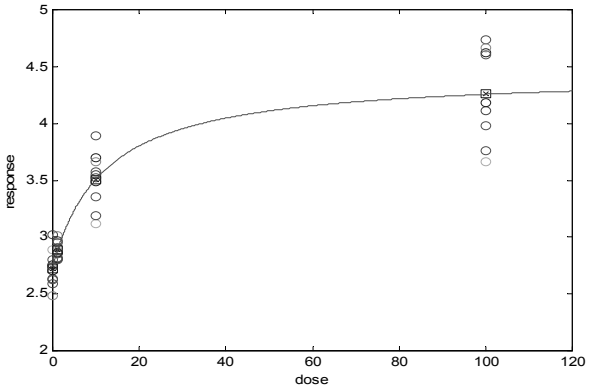
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.82	2.70	4.86	4.67

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.44	1.38	2.49	2.39

Relative liver weight:

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	2.72	0.024
1	10	2.89	0.005
10	10	3.51	0.054
100	9	4.26	0.17



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2.74	12.00	1.70	0.054		25.98	3.2e-5
2	3	2.74	12.07	1.71	6.28e-5	5.31	7.72	0.052
3	3	2.74	11.79	1.69	-3.84	0.02	10.57	0.014

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.05	0.63	2.30	1.33
2	1.05	0.64	2.30	1.34
3	1.04	0.62	2.28	1.31

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.62	0.36	1.35	0.77
2	0.68	0.37	1.49	0.78
3	0.66	0.33	1.48	0.69

BMD(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.63	0.60	1.09	1.05

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.38	0.36	0.66	0.63

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2.75	14.25	1.51	0.055		26.21	2.9e-5
2	3	2.75	14.08	1.51	6.34e-5	5.31	8.18	0.042
3	3	2.75	14.12	1.50	-3.81	0.020	11.15	0.011

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.36	0.73	2.87	1.50
2	1.34	0.72	2.83	1.48
3	1.36	0.72	2.86	1.49

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.94	0.52	1.98	1.06
2	0.96	0.48	2.02	1.00
3	1.03	0.48	2.17	0.98

BMD(HYBRID):

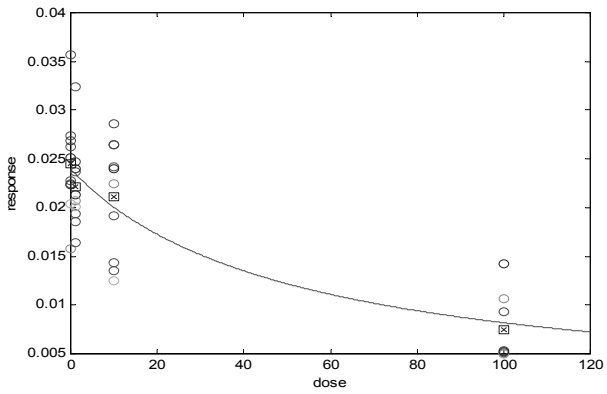
variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.83	0.79	1.41	1.36

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.57	0.55	0.97	0.94

Relative thymus weight:

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.024	2.75e-5
1	10	0.022	1.98e-5
10	10	0.021	3.49e-5
100	8	0.007	1.25e-5



Hill 2:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.024	52.00	-38.20	2.27e-5		4.23	0.376
2	3	0.024	49.38	-42.74	0.0004	0.73	2.79	0.425
3	3	0.024	41.24	-6.89	-10.53	-0.008	2.92	0.404

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	2.74	2.74	5.78	5.78
2	2.60	2.60	5.49	5.49
3	2.27	2.17	4.81	4.58

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	1.57	1.57	3.31	3.31
2	1.63	1.63	3.44	3.44
3	1.20	0.95	2.56	2.00

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	4.06	3.89	7.18	6.89

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.23	2.13	3.91	3.76

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.024	50.78	-0.0235	2.24e-5		3.58	0.465
2	3	0.024	63.40	-0.0234	0.0004	0.73	1.89	0.596
3	3	0.024	51.56	-0.0235	-10.55	-0.008	1.90	0.593

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	2.60	2.60	5.35	5.35
2	3.25	3.25	6.68	6.68
3	2.64	2.64	5.43	5.43

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	1.61	1.07	3.35	2.19
2	1.67	1.14	3.46	2.34
3	1.56	1.06	3.24	2.18

BMD(HYBRID):

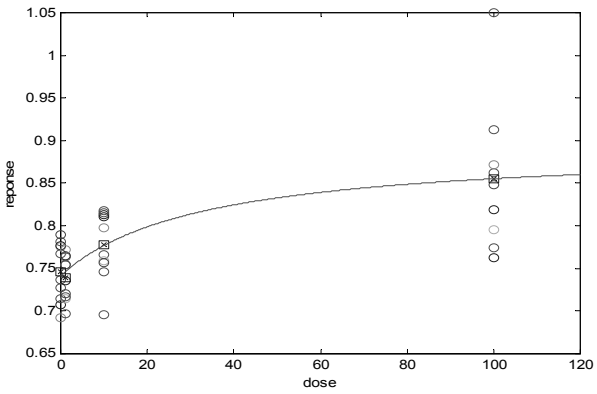
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	3.85	3.70	6.63	6.38

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.30	2.20	3.99	3.84

Relative kidneys weight:

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.75	0.0013
1	10	0.74	0.0007
10	10	0.78	0.0016
100	9	0.86	0.0077



Hill 1:

Parameter estimates:

Variance	df	α	κ	β	λ	τ	test	p
1	4	0.74	32.78	0.15	0.0024		17.12	0.002
2	3	0.74	40.69	0.16	0.057	14.07	2.02	0.569
3	3	0.74	33.60	0.15	-6.92	0.020	2.05	0.562

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	10.64	1.73	31.49	3.64
2	12.00	2.14	34.07	4.52
3	10.75	1.77	31.60	3.73

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	2.81	0.34	8.83	0.73
2	4.64	0.54	13.94	1.13
3	4.09	0.30	13.11	0.64

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	4.38	4.18	8.05	7.70

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.19	1.13	2.23	2.13

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.74	27.57	0.12	0.0024		17.10	0.002
2	3	0.74	31.92	0.12	0.057	14.10	1.97	0.579
3	3	0.74	27.91	0.12	-6.92	0.020	2.00	0.571

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	10.51	1.41	27.70	2.90
2	11.72	1.64	30.44	3.36
3	10.60	1.43	27.89	2.94

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	3.66	0.48	9.21	0.99
2	5.10	0.61	12.90	1.26
3	4.83	0.40	12.44	0.83

BMD(HYBRID):

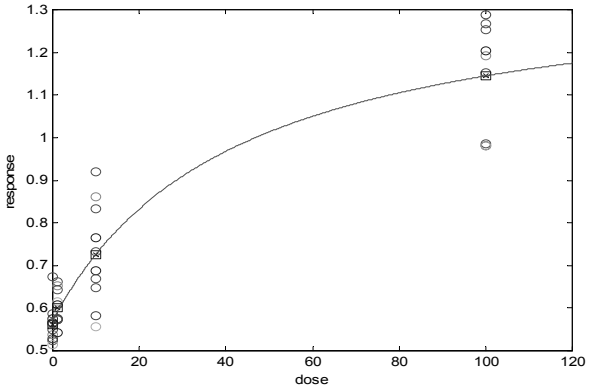
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	4.56	4.36	8.12	7.78

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.66	1.59	2.95	2.83

Relative lungs weight:

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.56	0.0021
1	10	0.60	0.0016
10	10	0.73	0.014
100	9	1.14	0.017



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.57	42.57	0.82	0.0076		19.46	0.001
2	3	0.57	30.90	0.73	0.018	3.51	8.05	0.045
3	3	0.57	42.35	0.82	-5.35	0.013	14.04	0.003

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.54	2.24	3.20	4.73
2	1.25	1.63	2.60	3.43
3	1.54	2.23	3.19	4.71

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.84	1.11	1.75	2.34
2	0.70	0.69	1.48	1.45
3	0.92	1.15	1.91	2.42

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.72	1.65	2.96	2.85

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.99	0.95	1.70	1.64

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.57	33.84	0.60	0.0076		19.49	0.001
2	3	0.57	27.04	0.57	0.018	3.48	8.175	0.043
3	3	0.57	33.73	0.60	-5.35	0.013	14.09	0.003

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.64	1.74	3.37	3.57
2	1.38	1.39	2.83	2.85
3	1.64	1.73	3.36	3.55

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL2**	**BMDL 1**	**BMDL 2**
1	0.97	1.06	2.00	2.17
2	0.88	0.77	1.81	1.58
3	1.05	1.08	2.16	2.23

BMD(HYBRID):

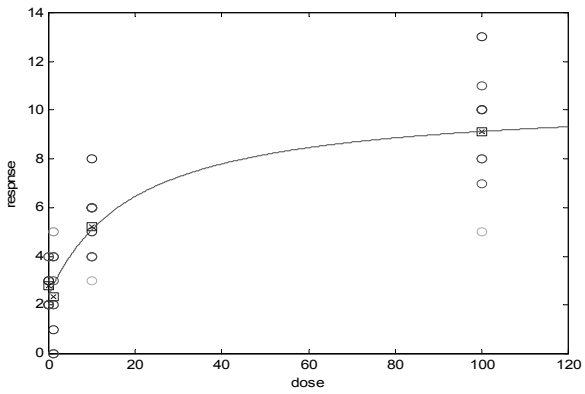
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.83	1.75	3.12	3.00

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.13	1.08	1.92	1.85

Gamma-glutamyl transpeptidase (IU/l):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	9	2.78	0.69
1	9	2.33	3.25
10	10	5.20	2.18
100	9	9.11	5.61



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2.41	19.82	8.05	2.68		10.12	0.038
2	3	2.44	21.18	8.14	0.77	0.80	6.77	0.079
3	3	2.41	20.11	8.10	0.62	0.0099	6.61	0.085

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.30	1.04	0.61	2.20
2	0.32	1.12	0.65	2.35
3	0.30	1.06	0.62	2.23

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.10	0.44	0.21	0.94
2	0.12	0.45	0.25	0.95
3	0.12	0.45	0.24	0.95

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.58	1.51	2.80	2.69

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.75	0.72	1.34	1.28

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2.42	19.33	6.74	2.67		9.97	0.041
2	3	2.45	20.22	6.74	0.78	0.79	6.65	0.084
3	3	2.42	19.44	6.75	0.62	0.0099	6.40	0.094

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.35	0.99	0.71	2.04
2	0.37	1.04	0.75	2.13
3	0.35	1.00	0.71	2.05

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.14	0.56	0.29	1.16
2	0.17	0.56	0.34	1.15
3	0.16	0.56	0.33	1.15

BMD(HYBRID):

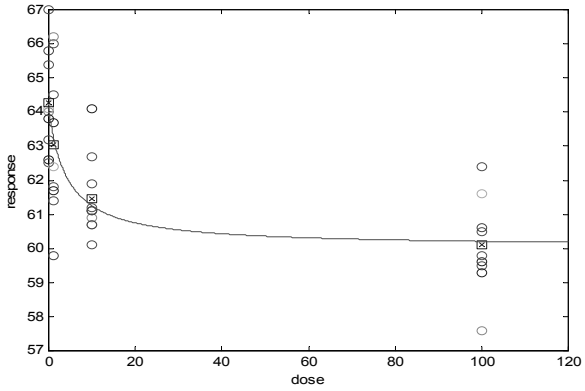
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.78	1.71	3.09	2.97

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.01	0.97	1.74	1.68

Relative albumin (g/l):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	9	64.27	2.31
1	9	63.06	4.78
10	10	61.45	1.37
100	9	60.10	1.94



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	64.11	4.21	-4.06	2.32		4.70	0.319
2	3	64.23	2.94	-4.01	0.0067	1.41	4.54	0.208
3	3	64.10	4.46	-4.10	0.95	-0.0045	4.08	0.253

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	15.75	0.22	X	0.47
2	11.86	0.15	X	0.33
3	15.93	0.23	X	0.50

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	2.45	0.04	X	0.08
2	2.83	0.05	X	0.11
3	2.35	0.04	X	0.09

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.66	0.63	1.25	1.19

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.13	0.12	0.24	0.23

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	63.91	8.74	-3.77	2.37		5.41	0.248
2	3	63.80	9.08	-3.67	3.50e-12	6.59	4.02	0.259
3	3	63.91	8.84	-3.78	0.97	-0.0047	4.71	0.195

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	16.41	0.45	X	0.92
2	18.41	0.47	X	0.96
3	16.45	0.45	X	0.93

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	2.14	0.06	X	0.12
2	4.06	0.10	X	0.21
3	2.34	0.07	X	0.14

BMD(HYBRID):

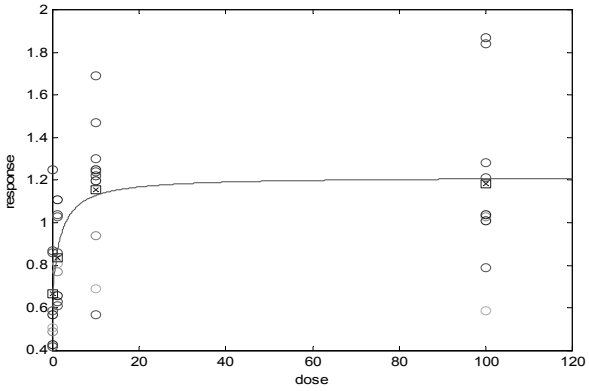
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.40	1.34	2.49	2.39

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.20	0.19	0.35	0.32

Triglycerides (mmol/l):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	9	0.67	0.076
1	9	0.84	0.036
10	10	1.16	0.115
100	9	1.18	0.19



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.66	1.88	0.56	0.093		6.26	0.180
2	3	0.68	3.13	0.57	0.095	1.72	3.17	0.366
3	3	0.66	2.06	0.57	2.74	0.010	2.84	0.416

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.12	0.10	0.25	0.21
2	0.20	0.16	0.43	0.35
3	0.13	0.11	0.27	0.23

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.02	0.01	0.03	0.03
2	0.03	0.03	0.06	0.05
3	0.02	0.02	0.05	0.04

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.47	0.44	0.94	0.89

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.07	0.07	0.15	0.14

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.67	2.54	0.51	0.092		6.11	0.191
2	3	0.69	4.40	0.51	0.095	1.75	3.00	0.393
3	3	0.67	2.48	0.50	-2.75	0.010	2.70	0.441

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.17	0.13	0.36	0.27
2	0.31	0.23	0.64	0.46
3	0.17	0.13	0.35	0.26

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.03	0.03	0.07	0.06
2	0.05	0.05	0.11	0.09
3	0.04	0.04	0.08	0.07

BMD(HYBRID):

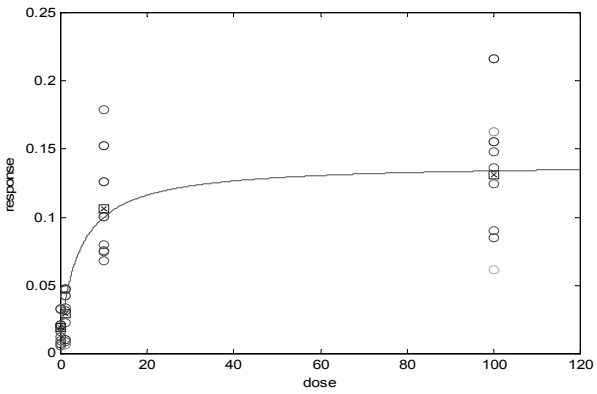
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.62	0.59	1.15	1.10

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.15	0.14	0.27	0.26

Volume fraction of foci (%):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	0.019	0.0001
1	10	0.029	0.0003
10	10	0.106	0.0013
100	9	0.131	0.0022



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.015	4.58	0.13	0.0009		24.38	6.7e-5
2	3	0.018	6.83	0.13	0.040	1.48	2.49	0.477
3	3	0.015	5.42	0.13	-7.70	0.017	15.48	0.001

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.03	0.24	0.05	0.51
2	0.05	0.36	0.10	0.76
3	0.03	0.29	0.06	0.60

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	7.8e-6	0.11	7.9e-5	0.23
2	0.02	0.18	0.04	0.38
3	0.004	0.13	0.007	0.28

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.43	0.41	0.77	0.74

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.20	0.19	0.36	0.34

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	0.017	6.86	0.11	0.0009		23.20	0.0001
2	3	0.018	7.88	0.12	0.036	1.46	0.59	0.898
3	3	0.017	7.11	0.12	-7.74	0.017	13.73	0.003

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.05	0.35	0.10	0.72
2	0.06	0.40	0.12	0.83
3	0.05	0.36	0.10	0.75

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.003	0.168	0.006	0.346
2	0.030	0.220	0.061	0.454
3	0.012	0.168	0.024	0.346

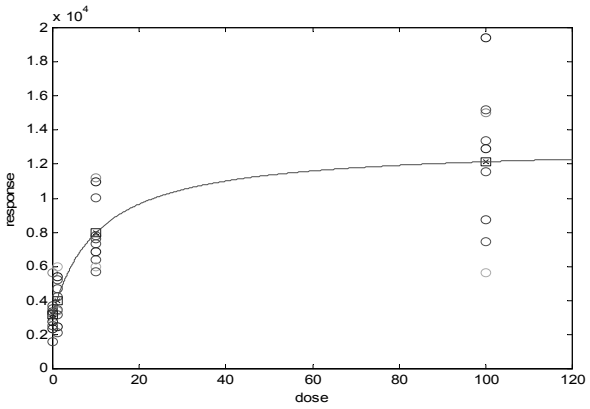
BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.67	0.64	1.15	1.11

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.34	0.32	0.58	0.56

Number of foci/ cm^3:



Hill 1:

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	3163.7	1.15e+6
1	10	4002.8	1.68e+6
10	10	7978.3	4.12e+6
100	9	12139	1.87e+7

Parameter estimates:

variance	**df**	**α**	**κ**	**β**	**λ**	**τ**	**test**	**p**
1	4	3157	10.62	9937.2	5.43e+6		23.18	0.0001
2	3	3207	12.37	10281	0.12	1.96	1.03	0.794
3	3	3157.5	10.65	9947.2	14.39	0.023	2.94	0.402

BMD:

Variance	**BMR = 5 %**		**BMR = 10 %**	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.17	0.56	0.35	1.18
2	0.20	0.65	0.40	1.37
3	0.17	0.56	0.35	1.18

BMDL:

variance	**BMR = 5 %**		**BMR = 10 %**	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.05	0.21	0.10	0.45
2	0.09	0.28	0.18	0.59
3	0.07	0.19	0.14	0.41

BMD(HYBRID):

Variance	**BMR = 5 %**		**BMR = 10 %**	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.99	0.95	1.77	1.70

BMDL(HYBRID):

variance	**BMR = 5 %**		**BMR = 10 %**	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.42	0.40	0.76	0.73

Exponential:

Parameter estimates:

variance	**df**	**α**	**κ**	**β**	**λ**	**τ**	**test**	**p**
1	4	3247.8	13.09	8891.7	5.4e+6		23.21	0.0001
2	3	3265.2	14.20	9086.9	0.14	1.96	1.23	0.745
3	3	3245.6	12.99	8860.7	14.40	0.023	3.03	0.387

BMD:

Variance	**BMR = 5 %**		**BMR = 10 %**	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.24	0.67	0.49	1.38
2	0.26	0.73	0.52	1.50
3	0.24	0.67	0.48	1.37

BMDL:

variance	**BMR = 5 %**		**BMR = 10 %**	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.10	0.37	0.20	0.76
2	0.138	0.38	0.28	0.78
3	0.13	0.28	0.26	0.57

BMD(HYBRID):

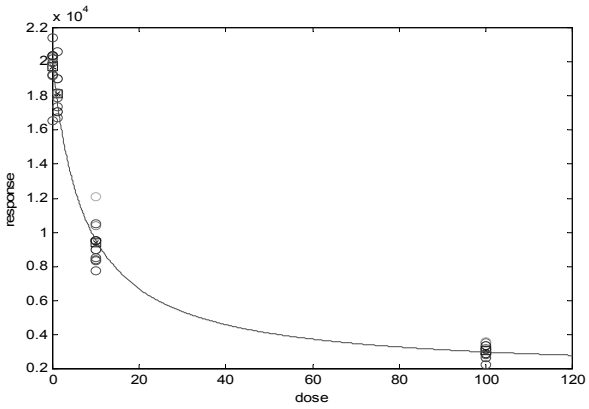
Variance	**BMR = 5 %**		**BMR = 10 %**	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.31	1.26	2.28	2.19

BMDL(HYBRID):

variance	**BMR = 5 %**		**BMR = 10 %**	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.74	0.71	1.29	1.24

Total liver retinyl palmitate (nmol):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	19720	1.64e+6
1	8	18137	1.51e+6
10	10	9390.2	1.72e+6
100	9	3034.1	2.01e+5



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	19918	7.52	-18207	1.19e+6		11.45	0.022
2	3	19928	7.41	-18169	33.86	1.11	4.03	0.258
3	3	19928	7.41	-18152	14.32	-0.022	1.37	0.713

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.43	0.40	0.92	0.84
2	0.43	0.39	0.91	0.82
3	0.43	0.39	0.91	0.82

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.36	0.32	0.76	0.67
2	0.36	0.32	0.76	0.68
3	0.35	0.31	0.75	0.66

BMD(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.17	0.16	0.28	0.27

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.13	0.12	0.21	0.21

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	19700	10.36	-16666	1.15e+6		10.08	0.039
2	3	19680	10.46	-16661	46.64	1.08	3.19	0.363
3	3	19700	10.37	-16667	14.28	-0.022	0.33	0.954

BMD:

variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.63	0.53	1.30	1.09
2	0.64	0.54	1.32	1.10
3	0.63	0.53	1.30	1.09

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.56	0.46	1.15	0.95
2	0.57	0.48	1.18	0.98
3	0.55	0.47	1.15	0.96

BMD(HYBRID):

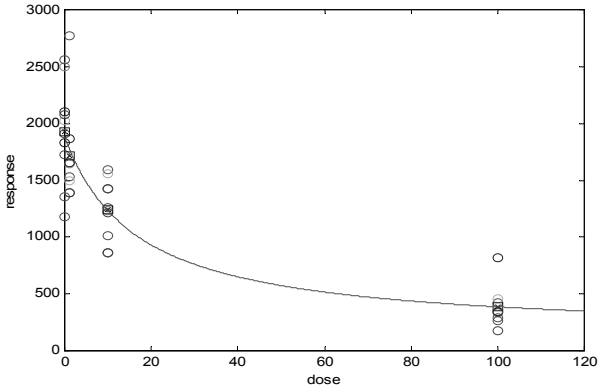
variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.24	0.23	0.41	0.40

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.19	0.18	0.32	0.31

Total liver retinol (nmol):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	1926.6	1.92e+5
1	8	1717.3	2.06e+5
10	10	1243.3	70940
100	9	383.43	33248



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	1883.6	16.74	-1748.2	1.12e+5		9.37	0.053
2	3	1890.3	15.98	-1742.6	31.64	1.13	1.41	0.702
3	3	1882.9	16.91	-1752.4	11.89	-0.016	2.57	0.464

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.95	0.88	2.02	1.86
2	0.92	0.84	1.94	1.78
3	0.96	0.89	2.04	1.88

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.48	0.39	1.03	0.81
2	0.51	0.44	1.09	0.92
3	0.48	0.41	1.03	0.86

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.25	1.20	2.20	2.11

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.61	0.58	1.08	1.03

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	1874.9	17.84	-1495.8	1.12e+5		9.52	0.049
2	3	1881.4	17.21	-1498.3	31.01	1.14	1.52	0.677
3	3	1874.2	17.93	-1496.2	11.89	-0.016	2.68	0.443

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.15	0.92	2.39	1.88
2	1.12	0.88	2.31	1.81
3	1.16	0.92	2.40	1.89

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.72	0.54	1.50	1.12
2	0.74	0.58	1.53	1.20
3	0.71	0.56	1.47	1.15

BMD(HYBRID):

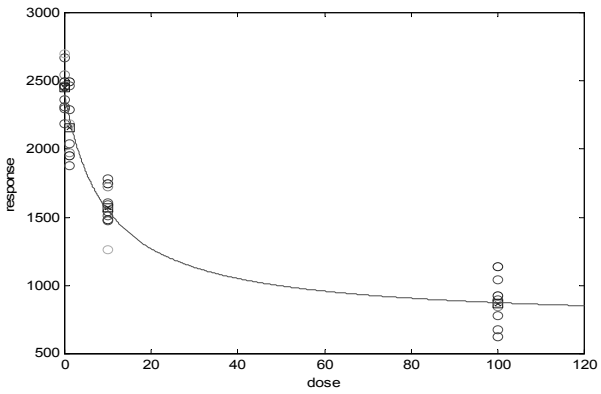
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.51	1.45	2.61	2.51

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.90	0.86	1.54	1.48

Total liver retinyl stearate (nmol):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	2447.5	26823
1	8	2160	56509
10	10	1572.8	24383
100	9	866.82	26581



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2399.4	9.64	-1671.8	31159		4.85	0.303
2	3	2397.5	9.82	-1675.5	616.35	0.53	4.05	0.256
3	3	2398.9	9.71	-1675.2	10.44	-0.0040	4.36	0.225

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.75	0.51	1.62	1.07
2	0.76	0.52	1.64	1.09
3	0.75	0.51	1.62	1.08

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.51	0.33	1.11	0.69
2	0.53	0.34	1.15	0.72
3	0.51	0.33	1.11	0.70

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.38	0.37	0.66	0.64

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.25	0.24	0.43	0.42

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	2378.3	12.70	-1508.8	32605		6.53	0.163
2	3	2377.1	12.77	-1508.4	334.5	0.61	5.41	0.144
3	3	2378	12.73	-1509.7	10.50	-0.0046	5.87	0.118

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.04	0.65	2.18	1.34
2	1.05	0.66	2.19	1.35
3	1.04	0.65	2.18	1.34

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.82	0.50	1.71	1.02
2	0.83	0.51	1.73	1.05
3	0.81	0.50	1.70	1.03

BMD(HYBRID):

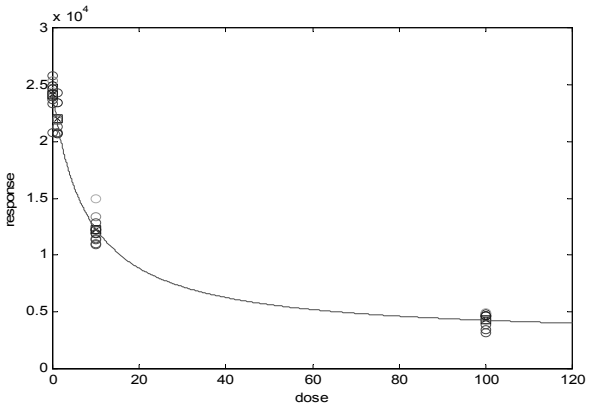
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.56	0.54	0.96	0.92

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.41	0.39	0.70	0.65

Total liver retinoids (nmol):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	24094	1.9647e+6
1	8	22014	1.5411e+6
10	10	12215	1.519e+6
100	9	4284.1	4.0442e+5



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	24200	8.08	-21551	1.24e+6		5.90	0.207
2	3	24206	8.04	-21535	280.74	0.87	1.00	0.801
3	3	24203	8.04	-21528	14.29	-0.015	0.43	0.934

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.48	0.43	1.02	0.90
2	0.48	0.42	1.02	0.89
3	0.48	0.42	1.02	0.89

BMDL:

Variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.41	0.35	0.86	0.74
2	0.41	0.36	0.87	0.75
3	0.40	0.35	0.86	0.74

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.15	0.15	0.26	0.25

BMDL(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.12	0.11	0.20	0.19

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	23950	10.94	-19659	1.24e+6		6.03	0.197
2	3	23937	11.00	-19658	274.84	0.88	1.12	0.772
3	3	23948	10.96	-19664	14.294	-0.015	0.53	0.912

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.69	0.56	1.42	1.15
2	0.69	0.56	1.43	1.16
3	0.69	0.56	1.42	1.15

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.62	0.50	1.27	1.02
2	0.62	0.51	1.29	1.04
3	0.61	0.50	1.27	1.03

BMD(HYBRID):

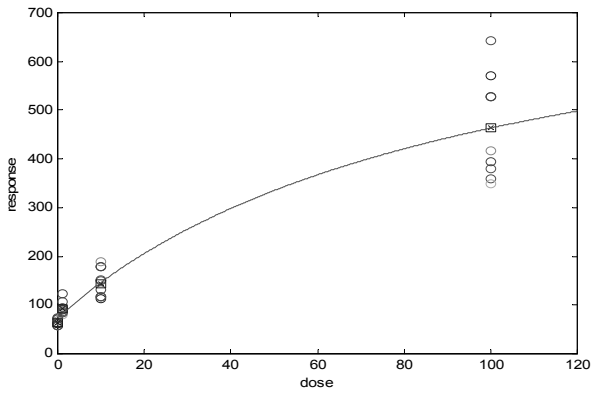
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.23	0.22	0.38	0.37

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.18	0.17	0.30	0.29

Kidney retinol (nmol/g kidneys):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	10	4.16	0.011
1	10	4.53	0.016
10	10	4.95	0.041
100	9	6.12	0.050



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	74.13	96.86	765.74	2613.6		70.17	2.10e-14
2	3	72.24	75.41	678.14	0.011	2.24	18.46	0.0004
3	3	73.88	93.32	749.07	5.75	0.036	23.25	3.58e-5

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.47	5.10	0.95	10.76
2	0.40	3.97	0.81	8.38
3	0.46	4.91	0.93	10.37

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.19	2.01	0.38	4.25
2	0.25	1.80	0.51	3.79
3	0.33	2.61	0.65	5.51

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.41	2.31	4.10	3.95

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.33	1.28	2.28	2.20

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	74.25	63.55	490.55	2614.4		70.18	2.09e-14
2	3	72.54	53.47	458.64	0.012	2.22	18.73	0.0003
3	3	74.02	61.94	484.37	5.75	0.035	23.35	3.41e-5

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.48	3.26	0.97	6.70
2	0.42	2.74	0.85	5.63
3	0.48	3.18	0.95	6.53

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.21	1.61	0.41	3.30
2	0.29	1.58	0.58	3.25
3	0.34	1.96	0.69	4.02

BMD(HYBRID):

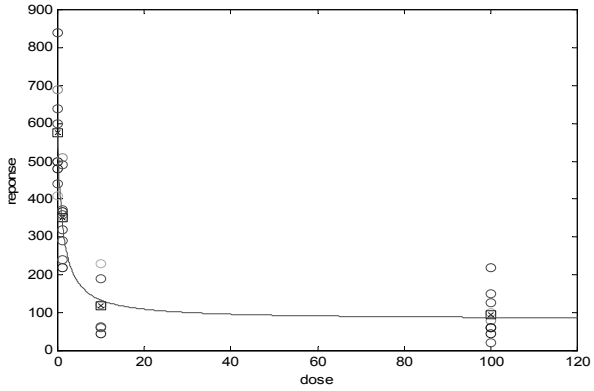
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	2.45	2.35	4.16	4.01

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	1.42	1.36	2.41	2.33

Plasma 13-cis retinoic acid (nmol/g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	8	574.86	21164
1	10	352	8842.5
10	6	118.44	5887
100	9	95.83	3826.7



Hill 1:

Parameter estimates:

Variance	df	α	κ	β	λ	τ	test	p
1	4	575.91	1.17	-493.60	8 785.26		7.06	0.133
2	3	579.66	1.09	-494.14	79.69	0.83	0.87	0.833
3	3	576.21	1.14	-489.04	9.32	-0.012	3.14	0.370

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.07	0.06	0.16	0.13
2	0.07	0.06	0.15	0.12
3	0.07	0.06	0.15	0.13

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.04	0.03	0.09	0.07
2	0.04	0.03	0.08	0.06
3	0.04	0.03	0.08	0.07

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.09	0.08	0.15	0.15

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.05	0.04	0.08	0.08

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	574.71	1.56	-470.24	8 772.57		7.01	0.135
2	3	576.39	1.53	-471.24	93.51	0.80	1.14	0.767
3	3	574.61	1.58	-474.88	9.32	-0.012	3.12	0.373

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	BMD 2	BMD 1	BMD 2
1	0.10	0.08	0.20	0.16
2	0.10	0.08	0.20	0.16
3	0.10	0.08	0.20	0.17

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	BMDL 2	BMDL 1	BMDL 2
1	0.07	0.05	0.14	0.11
2	0.06	0.05	0.13	0.10
3	0.06	0.05	0.13	0.11

BMD(HYBRID):

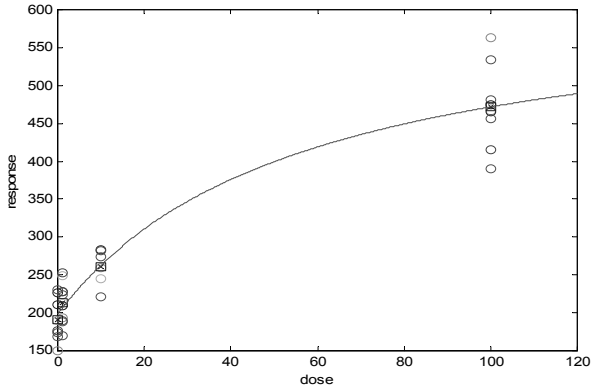
Variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.12	0.11	0.20	0.19

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	Rel.	Add.	Rel.
1	0.07	0.07	0.12	0.12

Plasma retinol (nmol/g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	8	190.96	844.51
1	10	213.18	760.16
10	6	261.17	592.93
100	9	471.75	2803.90



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	Test	p
1	4	198.62	56.44	427.20	1186.20		7.86	0.097
2	3	198.78	57.88	431.23	0.38	1.41	2.70	0.440
3	3	198.55	55.88	425.55	6.53	0.013	2.00	0.573

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.34	2.97	2.75	6.27
2	1.37	3.05	2.80	6.43
3	1.33	2.94	2.73	6.21

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.69	1.31	1.41	2.77
2	0.73	1.35	1.51	2.85
3	0.77	1.44	1.59	3.04

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.70	1.63	2.91	2.80

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.93	0.89	1.59	1.53

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	Test	p
1	4	198.86	41.84	300.38	1187.40		7.90	0.095
2	3	199.02	42.75	302.10	0.39	1.41	2.75	0.431
3	3	198.80	41.57	299.89	6.53	0.013	2.06	0.560

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	1.41	2.15	2.87	4.41
2	1.43	2.19	2.91	4.50
3	1.40	2.13	2.85	4.38

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.78	1.19	1.59	2.45
2	0.83	1.22	1.70	2.50
3	0.87	1.27	1.76	2.61

BMD(HYBRID):

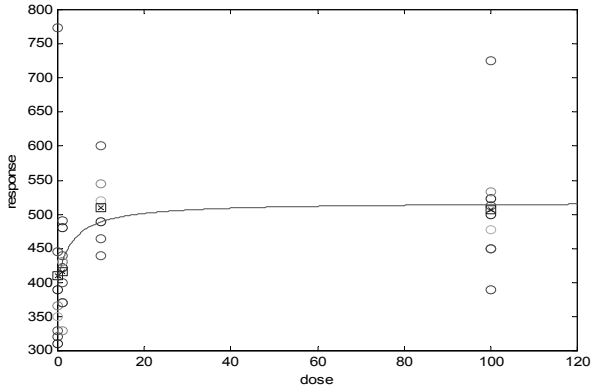
Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.78	1.71	3.03	2.92

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.04	1.00	1.77	1.70

Plasma retinoic acid (nmol/g):

Dose (ng/kg/day)	n_i	$\bar{y}_i$	s_i^2
0	8	410.56	23368
1	10	416.22	2495.8
10	6	509.56	3369.4
100	9	506.70	8695.4



Hill 1:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	402.33	3.41	115.73	8413.7		13.45	0.009
2	3	376.58	0.89	129.69	8.62e+14	-4.17	9.80	0.020
3	3	402.30	3.34	114.72	9.09	-0.0018	13.35	0.004

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.72	0.18	1.82	0.38
2	0.15	0.05	0.37	0.10
3	0.71	0.18	1.81	0.37

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.05	0.010	0.13	0.020
2	0.03	0.024	0.15	0.048
3	0.05	0.009	0.12	0.020

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.38	1.30	3.18	2.96

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.12	0.11	0.28	0.26

Exponential:

Parameter estimates:

variance	df	α	κ	β	λ	τ	test	p
1	4	403.05	4.50	107.92	8314.80		13.06	0.011
2	3	377.93	1.41	125.30	1.23e+15	-4.23	9.36	0.025
3	3	402.99	4.45	107.47	9.07	-0.0017	12.97	0.005

BMD:

Variance	BMR = 5 %		BMR = 10 %	
	BMD 1	**BMD 2**	**BMD 1**	**BMD 2**
1	0.93	0.23	2.10	0.47
2	0.23	0.07	0.51	0.15
3	0.92	0.23	2.09	0.47

BMDL:

variance	BMR = 5 %		BMR = 10 %	
	BMDL 1	**BMDL 2**	**BMDL 1**	**BMDL 2**
1	0.12	0.031	0.26	0.064
2	0.13	0.017	1.40	0.033
3	0.12	0.030	0.25	0.063

BMD(HYBRID):

Variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	1.65	1.57	3.25	3.08

BMDL(HYBRID):

variance	BMR = 5 %		BMR = 10 %	
	Add.	**Rel.**	**Add.**	**Rel.**
1	0.13	0.13	0.46	0.44