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Extending the BMD approach to censored time-to-tumor data for applications in quantitative risk assessment of carcinogens

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Erweiterung des BMD-Verfahrens auf zensierte Tumorinzidenzzeiten für die Risikobeurteilung von Karzinogenen

Zusammenfassung

Das *Benchmark Dose (BMD)*-Verfahren, erstmals 1984 von K. Crump vorgeschlagen [CRUMP (1984)] ist in der Risikobeurteilung von Substanzen heute ein viel genutztes Instrument für die Ableitung von Grenzwerten für gesundheitsgefährdende Substanzen in der Umwelt und in der Nahrung. Das BMD-Verfahren bestimmt dazu einen Referenzpunkt (RfP) auf der statistisch geschätzten Dosis-Wirkungs-Kurve, für den das Risiko ausreichend sicher bestimmt werden kann. Ausgehend von diesem RfP wird dann im Schritt einer Risikocharakterisierung auf der Grundlage toxikologischer Betrachtungen ein Grenzwert ermittelt. Das Verfahren konnte sich insbesondere deshalb profilieren, weil es einige Nachteile des traditionell genutzten *No-Observed-Adverse-Health-Effect-Level (NOAEL)*-Ansatzes vermeidet [EFSA (2009)]. Im Gegensatz zu dem NOAEL-Verfahren, das diejenige Expositionsdosis als Referenzpunkt festlegt, bei der kein statistisch signifikanter Unterschied in der Reaktion (Zielvariable) verglichen mit der Kontrollgruppe nachgewiesen werden kann, basiert das BMD-Verfahren auf der Anpassung eines Dosis-Wirkungs-Modells auf alle vorliegenden Daten auf Grundlage einer Wahrscheinlichkeitsverteilung für den Endpunkt. Letztlich reflektiert die BMD dann die Dosis, bei der eine zuvor spezifizierte Steigerung der gesundheitsschädlichen Reaktion (die sogenannte Benchmarkresponse) zu erwarten ist. Das Verfahren gliedert sich in vier Schritte:

1.) Ein statistisches Verteilungsmodell der Zielvariablen und ein empirisch begründetes Modell der Dosiswirkung werden an experimentelle Daten angepasst.
2.) Eine *Benchmark Response (BMR)* wird festgelegt, die die kleine, aber messbare Änderung in der gesundheitsschädlichen Reaktion widerspiegelt. Bei dichotomen Zielvariablen ist der Standardwert der BMR eine Änderung um 10%, bei kontinuierlichen eine Änderung um 5% verglichen mit der vorhergesagten Reaktion der nicht-exponierten Individuen (d.h. bei Hintergrundbelastung).
3.) Die Dosis, für die diese BMR durch das Modell vorhergesagt wird, wird über die Modellanpassung bestimmt und wird als *Benchmark Dose (BMD)* bezeichnet.
4.) Eine (einseitige) untere 95% Konfidenzschranke wird geschätzt und als sogenannte *BMDL (Benchmark Dose Lower Limit)* als Referenzpunkt bestimmt.

Üblicherweise wird der BMD und die BMDL aus Daten von Tierstudien bestimmt und es erfolgt dann eine Extrapolation vom Tier auf den Menschen und von den noch relativ hohen Expositionsdosen auf so niedrige Dosen, dass die Chance für das Auftreten einer Reaktion beim Menschen noch einmal deutlich niedriger ist als die BMR. Das BMD-Verfahren kann auch auf Beobachtungsdaten beim Menschen direkt angewendet werden, was den Vorteil hat, dass dann die Extrapolation vom Tier auf den Menschen entfällt. Auch wenn dies im Folgenden nicht weiter erörtert wird, besitzen die in dieser Arbeit entwickelten Methoden das Potential, auch in solchen Fällen angewendet zu werden.

Eine Illustration des Verfahrens bietet die folgende Graphik.

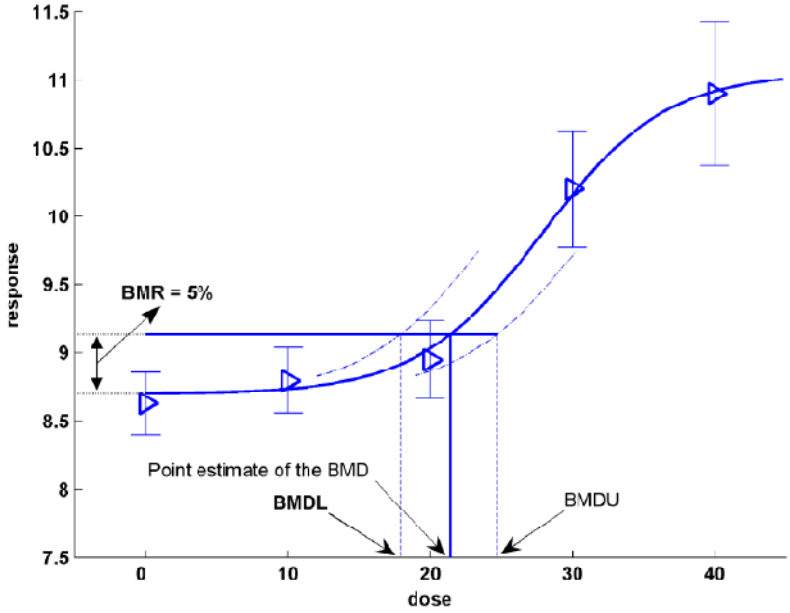


Fig.1: Schlüsselkonzepte des BMD Verfahrens, illustriert mit hypothetischen kontinuierlichen Daten, entnommen aus EFSA (2009) 1150, 10. Die Dreiecke stellten die beobachteten mittleren Reaktionen in den unterschiedlichen Dosisgruppen dar und sind mit ihren 95% Konfidenzintervallen eingezeichnet. Die durchgezogene Linie repräsentiert das angepasste Dosis-Wirkungs-Modell. Die BMD gehört zu einer BMR von 5% Änderung relativ zu der modellierten Hintergrund-Reaktion der nicht-exponierten Individuen. Die untere gestrichelte Kurve gibt die untere Konfidenzgrenze eines einseitigen 95% Konfidenzintervalls an. Manchmal wird zusätzlich auch die einseitige obere 95% Konfidenzgrenze verwendet, die hier durch die obere gestrichelte Kurve repräsentiert wird.

Das BMD-Verfahren ist bisher nur für dichotome und kontinuierliche Zielvariablen beschrieben worden. In der Risikoabschätzung von Karzinogenen sind aber insbesondere sogenannte Ereigniszeiten (*time-to-event* Daten) von großem Interesse, da sie mehr Informationen über die Tumorentstehung enthalten als quantale Inzidenzdaten. In vielen Tierexperimenten wurde die Zeit bis zum Auftreten eines Tumors (*time-to-tumor*) erfasst,

aber dieser Endpunkt nur selten auf die Dosis-Wirkungsbeziehung bei Risikoabschätzungen untersucht. Diese Zielvariable ist um einiges komplizierter, da Zensierungen in Betracht gezogen werden müssen. In der vorliegenden Arbeit soll das *BMD*-Verfahren für solche *TTT* Daten erweitert werden.

In Anlehnung an die oben skizzierten Schritte können Inhalt und Ergebnisse der Arbeit wie folgt zusammengefasst werden:

1.) Modell für die Zielvariable ist die Weibull-Verteilung (mit der Exponentialverteilung als Spezialfall). Sie hat die Überlebensfunktion $S(t) = \exp(-\lambda t^p)$, wobei $t, \lambda, p > 0$. Die Hazardfunktion ergibt sich zu $h(t) = \lambda p t^{p-1}$ und die Dichtefunktion ist durch $f(t) = \lambda p t^{p-1} \exp(-\lambda t^p)$ gegeben. Für $p = 1$ erhält man die Exponentialverteilung.

Reparametrisiert man λ mittels $\exp(\beta_0 + \beta_1 * Dosis)$ oder äquivalent durch $\exp(-p\,\alpha_0 - p\,\alpha_1 * Dosis)$, erhält man ein Regressionsmodell, das zwei im Fachgebiet der Überlebenszeitanalyse wesentliche Annahmen erfüllt, die der *proportional hazards (PH)* und die der *accelerated failure times (AFT)*. PH-Modelle nehmen an, dass der Effekt der Kovariablen (in unserem Fall die Dosis) proportional in Bezug auf das Risiko eines Tumors ist (*hazard*) und AFT-Modelle gehen davon aus, dass der Effekt der Kovariablen proportional in Bezug auf die „Überlebenszeit" (d.h. Zeit bis zu einem Tumor) ist. Anders formuliert, wird in AFT- Modellen angenommen, dass die Überlebenszeit mit zunehmender Dosis „beschleunigt" wird. Die Weibull-Verteilung ist die einzige Verteilung, die sowohl die PH- als auch die AFT-Annahme erfüllt (vorausgesetzt, der Parameter p ist wirklich wie angenommen für alle Dosisgruppen gleich) [KALBFLEISCH (2002), 45f.].

2.) Für die Definition einer BMR sind bei Ereigniszeiten unterschiedliche Möglichkeiten denkbar, die sich allerdings nicht alle als gleich günstig erweisen. Eine Möglichkeit besteht darin, die BMR als Reduktion in der Überlebenswahrscheinlichkeit zu einem festen Zeitpunkt t^* zu definieren. Die Reduktion kann dann entweder absolut oder relativ bezogen auf die (durch das Modell geschätzte) Überlebenswahrscheinlichkeit der Kontrollgruppe angegeben werden. Die BMD ist dann

$$d\colon\ S(t^*, d) = S(t^*, 0) - h \text{ für die Formulierung als absolute Reduktion}$$

bzw.

$$d\colon\ S(t^*, d) = (1 - h)\, S(t^*, 0) \text{ für die Formulierung als relative Reduktion.}$$

Eine andere Möglichkeit besteht darin, die BMR als Reduktion in medianer Überlebenszeit anzugeben. Offensichtlich könnte man auch jedes andere Quantil zugrunde legen, wir beschränken uns an dieser Stelle aber auf den Median als meist genutzten Parameter in der Praxis.

Auch hier kann die Reduktion wieder absolut oder relativ definiert werden. Für die BMD erhält man

$$d\colon\ t_{med}(d) = t_{med}(0) - h \text{ für die Formulierung als absolute Reduktion}$$

bzw.

$$d\colon\ t_{med}(d) = (1-h)\, t_{med}(0) \text{ für die Formulierung als relative Reduktion.}$$

Es spricht nun einiges dafür, die BMR als relative Reduktion in einem Überlebensquantil zu definieren. Denn in diesem Fall ist wegen der AFT-Eigenschaft des Weibull-Modells die errechnete BMD unabhängig von dem Quantil, das man zugrunde gelegt hat. Andere Definitionen einer BMR scheinen nur dann sinnvoll, wenn bestimmte Umstände dafür sprechen, ein gewisses Quantil oder einen bestimmten Zeitpunkt für die Analyse zu betrachten.

3.) Für die Bestimmung der BMD für eine gegebene BMR wird die Maximum-Likelihood-Schätzmethode vorgeschlagen. Die entsprechenden Formeln wurden getrennt für Exponential- und Weibull-Modelle berechnet.

4.) Um auch ein Konfidenzintervall für die BMD angeben zu können, wird eine Re-parametrisierung des Modells vorgeschlagen, so dass die BMD selbst zu einem Modellparameter wird. Dann ist es möglich, ein sogenanntes *Profil-Likelihood-Konfidenzintervall* zu schätzen. Man kann zeigen, dass für $\theta = BMD$ die Menge

$$\left\{\theta\colon \tilde{l}_p(\theta) \geq -\frac{1}{2}\,\chi^2_{1-\alpha}(1)\right\}$$

ein approximatives (zweiseitiges) $(1-\alpha)$ Konfidenzintervall für die BMD bildet. Wichtigster Baustein des Beweises ist die asymptotische Normalverteilung des Maximum-Likelihood-Schätzers. Bezeichnet $\tilde{l}_p(\theta)$ die logarithmierte relative Profil-Likelihood $\log\left(\tilde{L}_p(\theta) := \frac{L_p(\theta)}{L_p(\hat{\theta}_{ML})}\right)$, so ist der Grundgedanke einer Profil-Likelihood-Funktion der, bei einer Likelihood-Funktion, die von mehreren Parametern abhängt, nur einen Parameter zu

untersuchen und für jeden festen Wert dieses interessierenden Parameters die Likelihood-Funktion zu maximieren:

$$L_p(\theta) = \max_{\eta} L(\theta, \eta) = L\big(\theta, \hat{\eta}_{ML}(\theta)\big).$$

In unserem Fall bezeichnet dann θ die BMD und alle anderen Parameter des Dosis-Wirkungs-Modells werden in η, einem weiteren Regressionsparameter(vektor) des reparametrisierten Modells zusammengefasst, der für unsere Analyse nicht von Belang ist (*nuisance parameter).*

Um die untere Grenze des (einseitigen) Konfidenzintervalls $[BMDL, \infty)$ zu finden, muss man schließlich

$$\min\left\{\theta: \tilde{l}_p(\theta) = -\tfrac{1}{2}\,\chi^2_{1-2\alpha}(1)\right\}$$

bestimmen. Man beachte hierbei, dass für das einseitige Konfidenzintervall das $(1-2\alpha)$-Quantil statt des $(1-\alpha)$-Quantils der Chiquadrat-Verteilung verwendet werden muss.

Der Ansatz mit den oben beschriebenen Methoden wurde auf die Daten einer am Deutschen Krebsforschungszentrum vorgenommenen Studie von Schmähl, Port und Wahrendorf aus dem Jahr 1977, die die Kanzerogenität von Ethylcarbamat (Urethan) im Tierexperiment an Mäusen und Ratten untersucht, angewendet, für das die originalen Tumordaten, genauer gesagt die Zeiten bis zum Tod mit Tumor(en), vorlagen. Das Experiment untersuchte zwei Spezies (Mäuse und Ratten) und zwei Geschlechter (männlich und weiblich), weshalb es in vier Teilexperimente untergliedert wurde: Männliche Mäuse, weibliche Mäuse, männliche Ratten und weibliche Ratten. In jedem Teilexperiment wurden fünf Dosisgruppen unterschieden: 0 (Kontrolle), 100, 500, 2500, 12500 µg Urethan/kg BW/day. Die BMD_{10}-Werte für das Weibull-Modell lagen im Bereich von 4030 bis 8523 µg/kg BW/day bei einer BMR von 10% (relativem) Verlust in jedem Überlebensquantil. Die entsprechenden $BMDL_{10}$-Werte deckten einen Bereich zwischen 2590 und 3666 µg/kg BW/day ab. Das Exponential-Modell führte (vermutlich aufgrund des schlechteren Modellfits) zu kleineren Schätzern. Die BMD_{10}-Werte lagen hier zwischen 786.6 und 2198 µg/kg BW/day, die $BMDL_{10}$-Werte zwischen 541.7 und 902.8 µg/kg BW/day. Alle errechneten BMDs und BMDLs wurden mit den Ergebnissen für dichotome Modelle (Loglogistic und Weibull) sowie mit Werten aus der Literatur auf Konsistenz verglichen. Es zeichnete sich der Trend ab, dass die dichotomen Modelle, die weniger der vorliegenden Information ausnutzen, zu niedrigeren BMD- und BMDL-Werten führen. Zu beachten ist bei einem solchen Vergleich allerdings, dass die für

TTT- und dichotome Modelle jeweils zugrundegelegten BMRs nicht unbedingt äquivalent sind.

Abschließend wurde in einer Simulationsstudie der Einfluss des Zensierungsprozentsatzes auf die Überdeckungswahrscheinlichkeit des approximativen Konfidenzintervalls für Exponentialmodelle bei gleichen Dosisgruppen und Tierzahlen wie im Experiment von Schmähl und Kollegen untersucht. Es stellte sich heraus, dass die Überdeckungswahrscheinlichkeit für alle betrachteten Zensierungsprozentsätze sehr nahe am approximativen Wert lagen, die Variabilität des BMDLs aber wie erwartet insbesondere für hohe Zensierungssätze zunahm, wobei dann auch der Median der geschätzten BMDLs kleiner wurde. Das kann als vorsichtiger Hinweis interpretiert werden, dass hohe Zensierungssätze im Mittel zu konservativeren Schätzern führen könnten.

Die Arbeit schließt mit Überlegungen dazu, wie dieser Ansatz weiter entwickelt werden kann, z.B. für andere Modelle von Tumor-Daten, und welche Maßnahmen getroffen werden können, so dass ein solcher Ansatz von praktisch arbeitenden Forscher in der Risikobeurteilung angenommen wird.

Extending the BMD approach to censored time-to-tumor data for applications in quantitative risk assessment of carcinogens

Summary

The *Benchmark Dose (BMD)* approach, which was suggested firstly in 1984 by K. Crump [CRUMP (1984)], is a widely used instrument in risk assessment of substances in the environment and in food. In this context, the BMD approach determines a reference point (RfP) on the statistically estimated dose-response curve, for which the risk can be determined with adequate certainty and confidence. In the next step of risk characterization a threshold is calculated, based on this RfP and toxicological considerations. The 'success' of the BMD approach can be led back to the fact that it avoids some disadvantages of the traditionally used *No-Observed-Adverse-Health-Effect-Level (NOAEL)* approach. The NOAEL approach determines the exposition dose for which no statistically significant difference in the response (endpoint) compared to the control group can be detected as reference point. In contrast, the BMD approach bases upon the fit of a dose-response model on the data. For this fit a stochastic distribution of the response endpoint is taken as a basis. Ultimately, the BMD reflects the dose for which a pre-specified increase in an adverse health effect (the benchmark response) can be expected.

The BMD approach consists of four steps:

1.) A statistical distribution model for the endpoint variable and an empirically justified model of the dose-response interrelationship have to be fitted on the data.
2.) A *Benchmark Response (BMR)* is determined, which reflects a small but measurable increase in the adverse health effect. For dichotomous endpoints the default value for the BMR is an increase of 10%, for continuous endpoints of 5% compared to the predicted response of the non-exposed individuals (background response).
3.) The dose, for which this BMR is predicted by the fitted model, is determined and is called B*enchmark Dose (BMD).*
4.) An (one-sided) lower 95% confidence bound is estimated and as so called *BMDL (Benchmark Dose Lower Limit)* determined to build the reference point for the further risk assessment.

Typically, the BMD and BMDL are determined on the basis of data from animal studies and afterwards an extrapolation from animal to human, such as from relatively high exposition doses to doses for which the risk of an adverse effect in humans is much less than the BMR, has to be performed. It is also possible to apply the BMD approach to observational data for humans, which has the advantage that no extrapolation from animal to human is needed. Although not subject of this thesis, the methods developed here have the potential to be applied therefore as well.

The following figure provides an illustration of the approach:

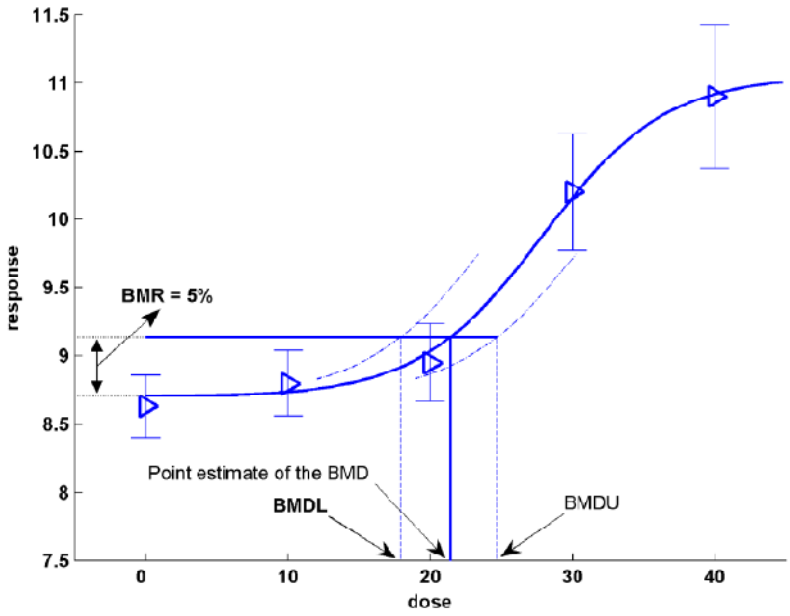


Fig. 1: Key concepts of the BMD approach, illustrated by using hypothetical continuous data, taken from EFSA (2009) 1150, 10. The triangles denote the observed mean responses in the different dose groups, and are plotted together with their confidence intervals. The solid curve represents a fitted dose-response model. The BMD corresponds to a 5% change in response relative to the modeled background response (BMR=5%). The lower dashed curve is the lower one sided 95% confidence bound for the effect size as a function of dose. Analogously, the upper dashed curve represents the upper one sided 95% confidence bound for the effect size.

Until now, the BMD approach has been specified only for quantal and continuous endpoints. But in risk assessment of carcinogens especially so called *time-to-event* data are of high interest since they contain more information on the tumor development than quantal incidence data. Many animal experiments have recorded the time until a tumor occurs (*time-to-tumor*) but that endpoint has been rarely investigated for dose-response in risk assessment. This endpoint is much more complicated because censoring has to be taken into account. The goal of this diploma thesis is to extend the BMD approach to such *time-to-tumor (TTT)* data.

Following the steps outlined before, the contents and results of the thesis can be summarized as follows:

1.) We have chosen the Weibull distribution (including the Exponential distribution) to model the endpoint *time-to-tumor*. The Weibull distribution has the survival function $S(t) = \exp(-\lambda t^p)$, where $t, \lambda, p > 0$. The Hazard function is given by $h(t) = \lambda p t^{p-1}$ and the density function by $f(t) = \lambda p t^{p-1} \exp(-\lambda t^p)$. If $p = 1$, this characterizes the Exponential distribution.

In the next step, λ was re-parameterized through $\exp(\beta_0 + \beta_1 * Dosis)$ or equivalently through $\exp(-p\,\alpha_0 - p\,\alpha_1 * Dosis)$, which led to a regression model that fulfills two essential assumptions often considered in survival analysis: *proportional hazards (PH)* and *accelerated failure times (AFT)*. PH models suppose that the effect of covariates (in our case the dose) is proportional with respect to the risk of a tumor (*hazard*). On the other hand, AFT models assume the effect of covariates to be proportional with respect to the endpoint (time to tumor). In other words, AFT suppose the time to tumor to be "accelerated" with increasing dose. A unique property of the Weibull distribution is the equivalence of PH and AFT assumption (pretended that the parameter p does not vary over different levels of covariates) [KALBFLEISCH (2002), 45f.].

2.) In order to define a BMR for time-to-event data different possibilities are thinkable, but not all of them are equally appropriate. One possibility would be to define the BMR as reduction of the survival probability at a fixed time point t^*. This reduction of survival probability can be stated either in absolute or in relative terms with respect to the survival probability of the non-exposed individuals predicted by the model. Then, the BMD equals

d: $S(t^*, d) = S(t^*, 0) - h$ for the definition in terms of an absolute reduction

resp.

d: $S(t^*, d) = (1 - h)\, S(t^*, 0)$ for the definition in terms of a relative reduction.

Another possibility is to define the BMR as reduction in median survival time. An extension to any other quantile would be obvious, however this thesis focuses on the median as the most used parameter in praxis.

Again, the reduction can be defined either in absolute or in relative terms. The BMD is

d: $t_{med}(d) = t_{med}(0) - h$ for the definition in terms of an absolute reduction

resp.

d: $t_{med}(d) = (1-h)\, t_{med}(0)$ for the definition in terms of an relative reduction.

Defining the BMR as relative reduction has the advantage that the BMD calculated on the basis of this definition does not depend on the choice of the quantile when the Weibull model is assumed due to its AFT property. Other possibilities of defining the BMR seem to be only reasonable if additional information is on hand, e.g. suggesting the evaluating of a specific quantile or a key time point during tumor development.

3.) We suggest determining the BMD for a pre-specified BMR with Maximum Likelihood estimates. The formulas have been calculated individually for the Exponential and Weibull models.

4.) In order to estimate a confidence interval for the BMD, a re-parameterization of the model is suggested, which includes the BMD as a parameter itself. Then a so called *profile likelihood confidence interval* can be estimated. It can be shown that for $\theta = BMD$ the set

$$\left\{\theta: \tilde{l}_p(\theta) \geq -\frac{1}{2}\, \chi^2_{1-\alpha}(1)\right\}$$

builds an approximate (two-sided) confidence interval for the BMD. An important result for the proof is the asymptotic normality of Maximum Likelihood estimates. Here, $\tilde{l}_p(\theta)$ denotes the logarithm of the profile likelihood, i.e. $\log\left(\tilde{L}_p(\theta) := \frac{L_p(\theta)}{L_p(\hat{\theta}_{ML})}\right)$. The main idea of a profile likelihood function is to 'concentrate' on only one parameter of a likelihood function that depends on several parameters. The likelihood function is maximized for every fixed value of the parameter of interest:

$$L_p(\theta) = \max_{\eta} L(\theta, \eta) = L\big(\theta, \hat{\eta}_{ML}(\theta)\big).$$

In our case, θ denotes the BMD and all other parameters of the dose-response model are summarized in the nuisance paramter η, which denotes a parameter(vector) of the re-parameterized regression model that is not of interest for our analysis.

To gain the lower limit of a (one-sided) confidence interval $[BMDL, \infty)$, finally

$$\min\left\{\theta: \tilde{l}_p(\theta) = -\frac{1}{2}\,\chi^2_{1-2\alpha}(1)\right\}$$

has to be determined. Note, that for a one-sided confidence interval the $(1-2\alpha)$-quantile instead of the $(1-\alpha)$-quantile of the chi-squared distribution has to be used.

The approach and methods outlined above were then applied to data of an animal experiment conducted at the German Cancer Research Center by Schmähl, Port and Wahrendorf in 1977, which investigated the carcinogenicity of ethyl carbamate (urethane) in mice and rats and for which the original time-to-tumor data were available, more precisely tumor specific survival time, namely time-to-death with tumor. The experiment investigated two species (mouse and rat) and two sexes (male and female) and was therefore divided into four sub-experiments: Mouse male, mouse female, rat male and rat female. In each sub-experiment there were five dose groups analyzed: 0 (control), 100, 500, 2500, 12500 µg urethane/kg BW/day. For the Weibull model, the estimated values of BMD_{10} lay in the range of 4030 to 8523 µg/kg BW/day for a relative BMR of 10% loss in each quantile of survival. The values for $BMDL_{10}$ lay between 2590 and 3666 µg/kg BW/day. The exponential model led to smaller estimates (probably due to the poorer fit). Here, the values for the BMD_{10} lay between 786.6 and 2198 µg/kg BW/day and for the $BMDL_{10}$ in the range of 541.7 to 902.8 µg/kg BW/day. All calculated estimates for BMDs and BMDLs were compared with the results for dichotomous models (loglogistic and Weibull) and values published in the literature. We could observe the trend that the dichotomous models, which use only a smaller amount of information, led to lower estimates for the BMD and BMDL. But one has to keep in mind that the underlying BMRs for dichotomous and TTT models might not be equivalent.

Finally, a simulation study was performed to evaluate the influence of the censoring percentages on the coverage probability of the approximate confidence interval. We used the same dose groups and numbers of animals as in the experiment of Schmähl and colleagues. The coverage probability was found to be very close to the approximate value for all censoring percentages. As expected, the variability of the BMDLs increased especially for higher censoring percentages, whereby the median of the estimated BMDLs decreased. This can be interpreted as a hint that high censoring percentages could lead to more conservative estimates.

The thesis closes with some considerations on how the approach could be further extended, e.g. for other models for time-to-tumor data and which steps could be done so that this approach becomes accepted by practical working researchers in risk assessment.

Contents

1. Introduction

„Alle ding sind gifft u. nichts ohn gifft / Allein die dosis macht das ein ding kein gifft ist.“[1]
Paracelsus (7 Defensiones: 3. Defension II, 1537/38)

1.1. Risk assessment and the need for quantitative methods

Almost 500 years ago the famous scientist Paracelsus pointed out that every substance must essentially be regarded as poison and only the dose dictates whether it is harmful or not. Of course, the field of toxicology has made huge progress since then; however, this medieval insight has remained an indisputable concept among toxicologists – at least for non-carcinogenic substances. Therefore the search for a threshold dose that can be defended to be not harmful to humans is the essential goal of modern risk assessment. In general, risk assessment is an important prerequisite step for the risk management process and means the determination of a qualitative or quantitative value of the risk attributed to a substance considered to be hazardous. If carcinogenic substances are analyzed, the results of modern cancer research suggest that no threshold dose can be found below which no adverse health effect could occur [e.g. OEHLMANN, MARKERT (1997)]. This property has been postulated, in particular, for carcinogenic substances which are genotoxic, i.e. having mutational activity and react directly with the DNA. Therefore, it is an important task in toxicology and in risk assessments to find doses which cause only a small increase of cancer risk.

In a nut shell, risk assessment aims to find the maximum dose of a substance which is not harmful to humans. As clear as this goal is, as complicated it is to achieve. For ethical reasons these doses cannot be determined through straight experiments on humans. Hence, they must be estimated either from available epidemiological data or data from animal studies. Both data sources have constraints. Epidemiological data are in most cases obtained through observational studies which are accompanied with a series of difficulties such as uncertainties in estimating the quantity of human exposure or distorting effects due to confounding factors. On the other hand, when data from animal studies are used, the conclusions have to be extrapolated to environmentally relevant exposure levels for humans. Note that risk assessment often faces the problem of extrapolating from high exposure doses to lower doses since one is interested in a human exposure dose which does not constitute a risk to human

[1] Translation: “All things are poison and nothing is without poison. Only the dose makes that a thing is no poison.”

health or which is such low, that it is negligible. When using animal data that extrapolation is usually in the order of several magnitudes (e.g. extrapolating by a factor between 100 and up to 10 000) whereas when using human data that factor is usually smaller and could be not larger than 10 [EDLER (1998), BOKKERS (2009)].

In risk assessment of substances in the environment and in food the *Benchmark Dose (BMD)* approach has become a widely used instrument. In this context, the BMD approach determines a reference point (RfP) on the statistically estimated dose-response curve, for which the risk can be determined with adequate certainty and confidence. In the next step of risk characterization a threshold is calculated, based on this RfP and toxicological considerations.

This thesis will concentrate on the analysis of data generated in animal studies, i.e. we will consider experimental data to determine dose levels which can be used as reference points in the further proceeding of risk assessment. The data type studied here is called *time-to-tumor (TTT)* data: Each individual (animal) is observed from the start of the experiment until a tumor occurred and is recorded. The time span from start to that event is denoted as time-to-tumor. This endpoint is an important example of time-to-event (TTE) data studied in the field of survival analysis and the analysis for failure time data, see e.g. KALBFLEISCH AND PRENTICE (2002). Note that in practice the determination and interpretation of such a time-to-tumor could be rather complicated, see e.g. GART ET AL. (1986), due to restricted observability and events occurring to the animal in a manner competing with the tumor event. For this thesis and the development of the BMD approach for TTT data we assume that the tumor event can be observed and recorded exactly as time, however, we allow (independent) censoring, e.g., when the animal dies and had no tumor developed by that time. Our goal is to calculate a so called *Benchmark Dose*, i.e. a dose producing a predetermined level of change in adverse response compared to the response in untreated animals, and its lower confidence bound. In the further proceeding of risk assessment, the lower confidence bound of this dose can be used as RfP for deriving health-based guidance values for humans, for example an Acceptable Daily Intake (ADI) or margins of exposure (MOE) [EFSA (2005), CONSTABLE, BARLOW (2009)].

We will consider in the following classes of parametric survival distributions focusing at the Weibull distribution.

The approach we will present is an extension of the Benchmark Dose approach described in a scientific opinion of 2009 requested by the European Food Safety Authority [EFSA (2009)], which was basically influenced by the work of the "pioneer" of this approach K. Crump in the 1980s [CRUMP (1984)] and many other biostatisticians doing research in that field.

Traditionally, the No-Observed-Adverse-Effect-Level (NOAEL) has been used as a reference point in risk assessment, which reflects a dose level where no statistically significant differences in response (compared with the observed background response) are found. But this approach has several disadvantages. Most prominent is that its outcome is strongly influenced by the study design.[2] The 'success' of the BMD approach can be led back to the fact that it avoids some disadvantages of the NOAEL approach.

The general proceeding of the Benchmark Dose (BMD) approach can be summarized as follows:

- Firstly, a model of the outcome variable (response) has to be fitted, including a reasonable model of the dose effect (dose-response).
- Secondly, a so called *Benchmark Response* (BMR) has to be defined, which is a small but measurable change in adverse response in contrast to the non-exposed individuals. For quantal data a default value of 10% and for continuous data a default value of 5% is commonly used. In order to avoid misunderstandings, we should already here mention as a side note that the reference is the background response predicted by the fitted model and not the response observed in the control group.
- Thirdly, the dose has to be found for which this BMR is predicted. This dose is called *Benchmark Dose* (BMD).
- Because risk assessment always has to be performed very cautiously, finally, a 95% confidence interval of the BMD has to be calculated and especially the (one sided) 95% lower limit, denoted by the abbreviation BMDL, is of interest. To include the chosen value of the BMR, the notation BMD_{BMR}/ $BMDL_{BMR}$ is common, i.e. BMD_{10}/ $BMDL_{10}$ for a BMR of 10% change in response.

[2] For more details see e.g. EFSA (2009) 1150.

The following figure provides an illustration of the BMD approach:

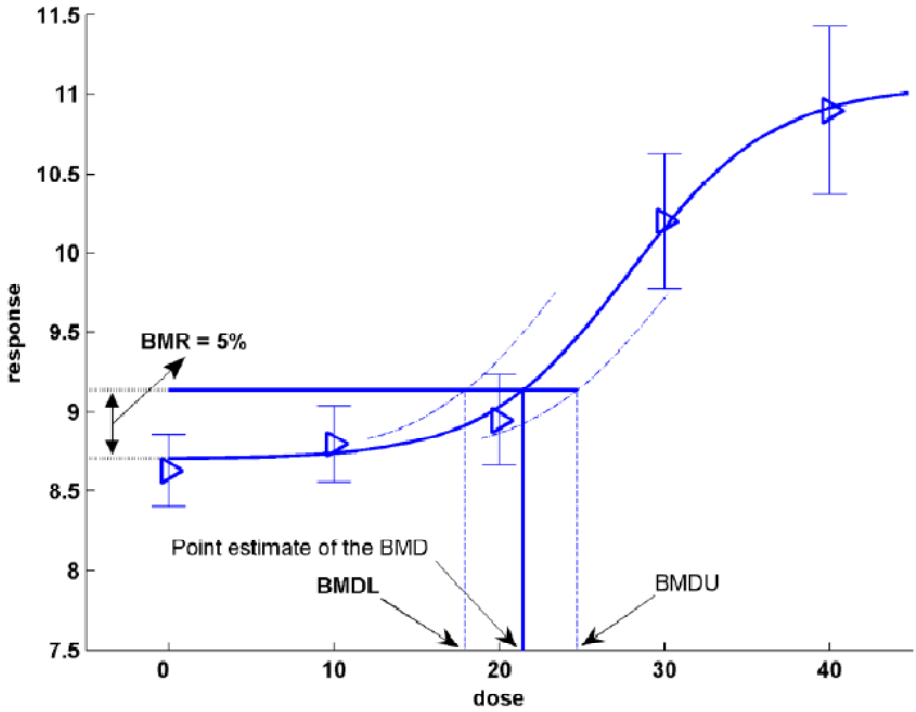


Fig.1: Key concepts of the BMD approach, illustrated by using hypothetical continuous data, taken from EFSA (2009) 1150, 10. The triangles denote the observed mean responses in the different dose groups, and are plotted together with their confidence intervals. The solid curve represents a fitted dose-response model. The BMD corresponds to a 5% change in response relative to the modeled background response (BMR=5%). The lower dashed curve is the lower one sided 95% confidence bound for the effect size as a function of dose. Analogously, the upper dashed curve represents the upper one sided 95% confidence bound for the effect size.

The BMD approach can be used for all experimental data that show at least a significant dose-related trend in the considered endpoint which hints at a graded monotonic (non-decreasing) response with dose. It is applicable to all toxicological effects (non-carcinogenic as well as carcinogenic ones) and to studies in which all dose groups show statistically significant changes in response compared to the control group, a case where there would no NOAEL be defined.

Naturally, the question which doses of critical substances can be tolerated is a question of risk management and therefore also a societal and political one because certain guidelines have to be adopted. In the European Union the European Food Safety Authority (EFSA) performs the task of scientifically informing about how to deal with health risks in connection with food, providing advice for the European Commission and Parliament, which have to decide how the risk management should be performed on the European scale. Therefore the EFSA as well as its counterparts in the USA, the Food and Drug Administration (FDA) and the Environmental

Protection Agency (EPA) have spent considerable effort in developing sustainable statistical methods of determining these doses as RfPs. Nevertheless, this work has so far concentrated on quantal tumor incidence dose-response data or continuous data such as body weight or liver size – but almost never on time-to-event data and on the special case of survival data. The work of this thesis was motivated by a presentation given by Prof. Wout Slob (RIVM, The Netherlands) at an ILSI Workshop and the EUROTOX Meeting in 2008 on Rhodes Island where he addressed the importance to extend the BMD approach to time-to-tumor data. Given the experience with those data at the Department of Biostatistics and the interest in further developing the statistical methods for the BMD approach the theme of this thesis was developed in cooperation between the Mathematical Institute of the University of Frankfurt (JProf G. Schneider) and the Department of Biostatistics of the German Cancer Research Center (Dr. L. Edler) with the aim to find a statistical solution for calculating Benchmark Doses for this type of data which is much more difficult to handle because both censoring and (dependent on the study design) also competing risks have to be taken into account.

1.2. Research Questions

The research questions underlying the task of extending the BMD approach to TTE data to be solved in this thesis are as follows:

1) How can a reasonable model be formulated for the data, if the outcome variable is a TTE?
2) Which meaningful risk functionals can be defined in order to state a *Benchmark Response* (BMR)? Which criteria are useful to evaluate their appropriateness?
3) Which statistical method is most appropriate to estimate the BMD and its lower confidence limit (BMDL)?

We will suggest estimating the BMD and BMDL using the concept of Maximum Likelihood Estimation. This will lead to the following mathematical tasks:

a) The models chosen for fitting the dose-response data have to be re-parameterized in a way that makes it possible to determine a profile likelihood confidence interval for the BMD.
b) The coverage of the estimated profile likelihood confidence interval and its dependence on the percentage of censoring in the sample has to be investigated.

Firstly, we try to answer the questions posed above rather theoretically and describe the statistical and numerical methods. Then we will apply the approach and methods outlined before to real data, taking an experimental study as a basis which had been conducted at the German Cancer Research Center (DKFZ) by Schmähl, Port and Wahrendorf [SCHMÄHL (1977)] and for which the original time-to-tumor data were available, more precisely time-to-death with tumor. These researchers tried to investigate the carcinogenicity of ethyl carbamate (urethane) in female and male mice as well as in female and male rats. Afterwards we will attempt to examine the validity of the approach using simulated data, focusing on the influence of the censoring percentages on the coverage probability of the approximate profile likelihood confidence intervals. Finally, we will discuss the results as well as the methods used in this work and make some remarks on how these results obtained for TTE data can be transferred to the risk analysis of humans. The thesis closes with some considerations on how the approach could be further extended, e.g. for other models for time-to-tumor data and which steps could be done so that this approach becomes accepted by practical working researchers in risk assessment.

Part I: Statistical and numerical Methods

The risk analysis methods presented in this thesis have the overall goal to determine the carcinogenic potential of substances studied in animal experiments for human risk assessment. In contrast to most carcinogenic risk analyses which merely consider tumor incidence data we are interested in two endpoints at the same time related to the two questions "How many animals in different dose groups develop a tumor or not during the experimental observation time?" and "If they have developed a tumor, when did it occur?". That means, we do not have to deal with quantal data (tumor: yes or no) but with survival data, which contain information about the dose of the analyzed substance an individual was exposed to during the study and either the time until a tumor occurred or – if no tumor was observed – the time until the animal left the study. This type of data is in the following called "time-to-tumor" (TTT) data.

Before the research questions formulated in the introduction will be approached some background information is provided next, including a short description of survival data and the (two parameter) Weibull distribution. In this context, the so-called Kaplan-Meier curves are introduced, which are an important instrument of non-parametric survival analysis. We will finish this section making some remarks on the numerical methods used in this thesis. The subsequent section will contain a stepwise description of our proposed procedure for applying the BMD approach to time-to-tumor data. To become not too theoretical, we will illustrate the proceeding using some small example data sets.

2. Basic concepts

2. 1. Introduction to survival data

Survival data occur in very different fields such as economics, sociology, biology or medicine. They are always dealing with the same outcome variable: the time until an event (sometimes also called failure) occurs. In our case, the event is specified by occurrence of a malignant tumor. Therefore we speak of time-to-tumor (TTT) data. This survival time (or time to event) is denoted by a capital T, indicating that it is a random variable, with possible values the nonnegative real numbers, $\mathbb{R}_{\geq 0}$.

A typical situation in survival analysis is that not all individuals face the event during the study period. The explanation can be e.g. death from other reasons or end of the experiment and the individual is still alive. Those individuals are called (*right*) *censored* because we only know that they did not face the event until a specific time point - but not the primary endpoint "tumor". Therefore, in a typical survival analysis, there are two specifications given for each individual: The time after which the individual has left the study and the reason, why it has left it. In principle, each individual i is characterized by two random variables: the time of observation, denoted T_i^*, and the type of the event which terminates the observation, the status, denoted δ_i. For n individuals we denote this information using two (vector) variables $t^* = (t_1^*, \dots, t_n^*)$ and $\delta = (\delta_i, \dots, \delta_n)$, where t^* gives the observed times and δ the status, i.e. $\delta_i = 1$ if the individual i has faced the event at time point t_i^* or $\delta_i = 0$ if it has been censored at time point t_i^*.

The observed time T_i^* can be specified as minimum of two random variables: The time to event T_i and the censoring time C_i: $T_i^* = \min(T_i, C_i)$. Following this definition, the status variable is a censoring indicator: $\delta_i = \mathbb{I}\{T_i \leq C_i\}$. The focus of survival analysis is modeling the real time to event T_i appropriately.

Next we introduce two essential functions used in survival analysis – the survival function and the hazard function of T:

Definition: The survival function $S(t)$ gives the probability that an individual survives a time point t (without facing the event), i.e. the probability $P(T > t)$.

A survival function is non-increasing and, by definition of T on $\mathbb{R}_{\geq 0}$ we know that $S(0) = 1$, since at the start of the study no one has faced the event yet. When time increases the survival function should approach the value zero: $\lim_{t\to\infty} S(t) = 0$.

Definition: The hazard function $h(t)$ is defined to be the following limit:

$$h(t) = \lim_{\Delta t \to 0} \frac{P(t \leq T < t + \Delta t \mid T \geq t)}{\Delta t}$$

So the hazard is the conditional probability that an individual's survival time lies in the time interval between t and $t + \Delta t$, given that the survival time is greater than or equal to t, divided by the length of the interval Δt. Therefore the hazard function is sometimes called a

conditional failure rate and it can take values between zero and infinity. In practical terms, this rate can be interpreted as the instantaneous potential per unit time for the event to occur, given that the individual has survived up to time t. In contrast to a survival function, the graph of $h(t)$ can start at any nonnegative value and can go up and down in any direction over time with no upper bound, pretended that it does not become smaller than zero [KLEINBAUM (2005), 8-14].

In general, these two functions are connected as follows:

$$S(t) = \exp\left(-\int_0^t h(u)\,du\right) \quad \text{or equivalently} \quad h(t) = -\frac{dS(t)/dt}{S(t)}.$$

The density function $f(t)$ is then given by $f(t) = S(t)\,h(t)$.

Note, that we have excluded the possibility of competing risks in these definitions, i.e. we have assumed that only one event is possible for each individual.

2. 2. The Weibull distribution

2. 2. 1. Definition and basic properties

Sometimes it is possible to reasonably assume the variable T to follow some family of distributions. The most widely used parametric family for times to event is the Weibull distribution, also called Weibull model below, with survival function $S(t) = \exp(-\lambda t^p)$, hazard function $h(t) = \lambda p t^{p-1}$ and density function $f(t) = \lambda p t^{p-1}\exp(-\lambda t^p)$, $t > 0,\ \lambda > 0,\ p > 0$. The parameter p is often called shape parameter and we can differentiate between three cases:

- If $p > 1$, the hazard increases over time.
- If $p < 1$, the hazard decreases over time.
- If $p = 1$, the hazard is constant over time.

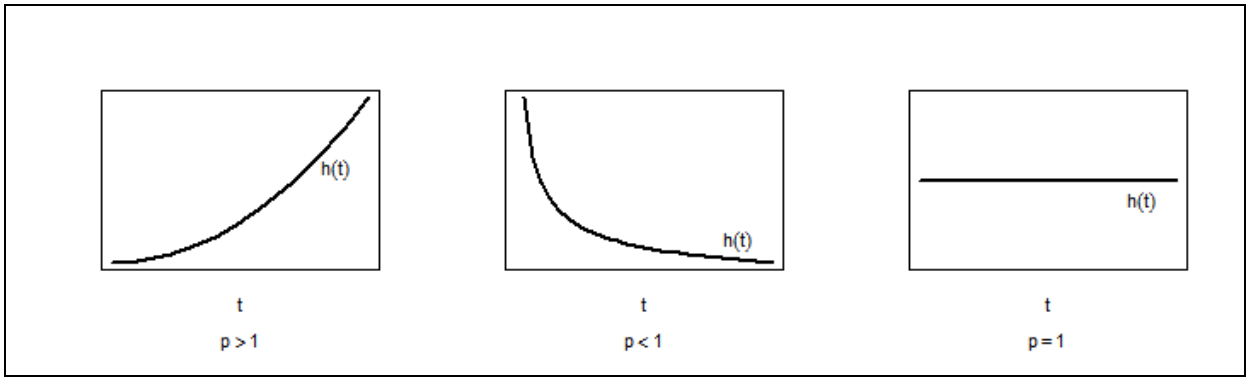


Fig.2.: These graphs show the three possible appearances of the Weibull hazard function dependent on the shape parameter p.

Note, that in all three cases the hazard function is monotonic. Thus, when using the Weibull distribution a hazard cannot change directions during time.

The third case, i.e. $p = 1$, leads to survival function $S(t) = \exp(-\lambda t)$, hazard function $h(t) = \lambda$ and density function $f(t) = S(t)h(t) = \lambda \exp(-\lambda t)$, which characterizes the Exponential distribution. This shows that the Exponential distribution is a special case of the Weibull distribution. The Exponential distribution has a constant hazard function: $\lambda(t) = \lambda > 0$, over the range of T. As the instantaneous failure rate is independent of t, the conditional potential of an event per unit time is the same regardless of how long the individual has been on study. This characteristic is referred to as the *memoryless property* of the exponential distribution.

2. 2. 2. Goodness of fit

When a dataset is on hand, we probably want to know at first how appropriate a Weibull or Exponential model would be for the given data. Fortunately, in case of the Weibull or Exponential distribution there is a very straight graphical test for assessing its adequacy. That is the so called log-log plot which makes use of the fact that $\log(-\log)$ of $S(t)$ is linear with the log of time:

$$\text{Weibull: } \log(-\log(\exp(-\lambda t^p))) = \log(\lambda) + p \log(t)$$

$$\text{Exponential: } \log(-\log(\exp(-\lambda t))) = \log(\lambda) + \log(t)$$

Kaplan-Meier Curves. In order to make use of this property, we have to introduce the so called *Kaplan-Meier estimates* for survival times. In order to determine these estimates we proceed as follows: At first, only the non-censored individuals are taken into account because

they give the times to event (survival times/ failure times) observed in the study of full information. We order these times from smallest to largest. With $t_{(1)} < t_{(2)} < \ldots < t_{(k)}$, $k \leq n$, we denote the distinct times to event. Then we count the number of failures m_j at each distinct failure time, $m_j \geq 1$, and afterwards we count the number q_j , $q_j \geq 0$, of individuals censored in the time interval $[t_{(j)}, t_{(j+1)})$ starting with failure time $t_{(j)}$ up to but not including the next failure time $t_{(j+1)}$. At last we note the size of risk set n_j, which is given by the number of individuals who have survived at least to time $t_{(j)}$. Now we are able to estimate the probabilities $P\big(T > t_{(j)} \big| T \geq t_{(j)}\big)$ by the relative frequencies of survivors of time $t_{(j)}$, pretended availability (i.e. being in the risk set) at time $t_{(j)}$.

Definition: The Kaplan-Meier estimate or Product-limit estimate is defined to be

$$\hat{S}\big(t_{(j)}\big) = \prod_{i=0}^{j} \hat{P}\big(T > t_{(i)} \big| T \geq t_{(i)}\big) = \prod_{i=0}^{j} \frac{n_i - m_i}{n_i}.$$

With the law of total probability it follows that $\hat{S}\big(t_{(j)}\big) = \hat{S}\big(t_{(j-1)}\big) * \hat{P}\big(T > t_{(j)} \big| T \geq t_{(j)}\big)$, $j \geq 1,\ S(t_0 = 0) = 1$.

Example (Kaplan-Meier): The proceeding is illustrated using a small simulated data set (Exponential distribution with rate $\lambda = 0.1$).

Time t^* [days]	Status δ
12	1
7	1
1	1
6	0
13	1

Tab.1: Simulated example data set with underlying distribution $Exp(0.1)$, $n = 5$.

The calculation of the Kaplan-Meier estimates can be performed as follows:

j	$t_{(j)}$	n_j	m_j	q_j	$\hat{S}\big(t_{(j)}\big)$
0	0	5	0	0	1
1	1	$5 - 0 - 0 = 5$	1	1	$1 * \frac{5-1}{5} = 0.8$
2	7	$5 - 1 - 1 = 3$	1	0	$0.8 * \frac{3-1}{3} \approx 0.53$
3	12	$3 - 1 - 0 = 2$	1	0	$0.53 * \frac{2-1}{2} \approx 0.27$
4	13	$2 - 1 - 0 = 1$	1	0	$0.27 * \frac{1-1}{1} = 0$

Tab.2: Calculation of Kaplan-Meier estimates $\hat{S}\big(t_{(j)}\big)$ for the example data set of Tab.1.

A plot of the Kaplan Meier survival probabilities estimates leads to a *Kaplan Meier curve*, which provides an empirical approximation of the survival function.

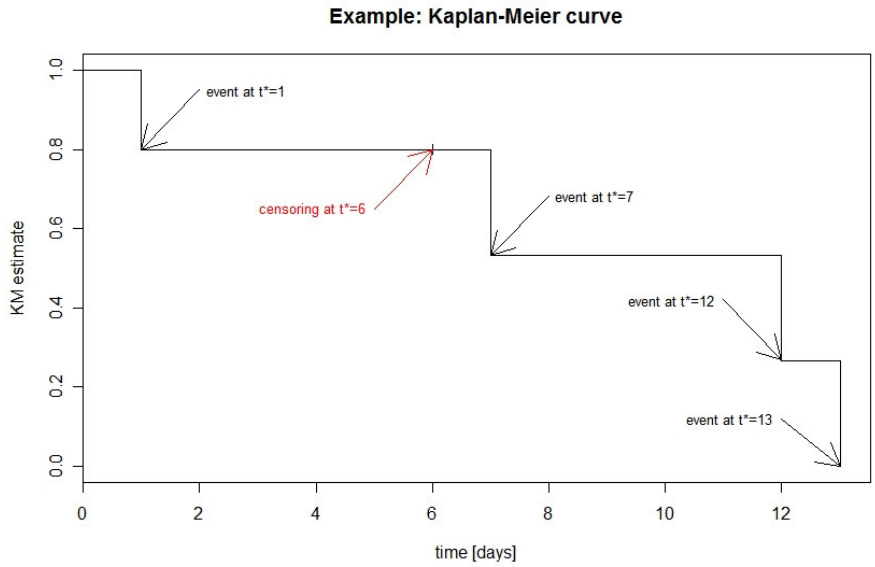


Fig.3: Kaplan-Meier curve for the example data set of Tab.1. A censoring is marked with a small tick.

Now we return to our test of the appropriateness of a Weibull model: We only have to plot the log negative log Kaplan-Meier survival estimates against the log of time ("log-log survival plot"). Approximately straight lines would indicate that the Weibull assumption is reasonable and a slope p of 1 hints at an exponential distribution of the survival times. Further, the slope and y intercept provide a rough estimate of p and $\log \lambda$, respectively.

Note, that this test constitutes only an approximate method because it yields a qualitative assessment of the goodness of fit. Quantitative methods can be found in EVANS (1989).

Because Exponential and Weibull models are 'nested', it is possible to evaluate whether the Weibull distribution is more appropriate than the Exponential with the so called *Likelihood ratio statistic*. The term 'nested' means, that a simpler model can be extended to a more complex one (with more parameters) such that the more complex one includes the simpler one. Usually, one starts with a simple model and then checks whether including more parameters leads to significant improvement of the fit [EFSA (2009), 27]. We will describe this statistical test in section 3.3.2.

Example: As an illustration of this graphical goodness of fit test with a log-log-plot we again use the example data set of Tab.1. The values for the plot are summarized in the following table:

$t_{(j)}$	$\hat{S}(t_{(j)})$	$\log(t_{(j)})$	$\log(-\log(\hat{S}(t_{(j)})))$
0	1	Not defined	Not Defined
1	***0.8***	***0***	$\mathbf{-1.50}$
7	$\mathbf{0.53}$	$\mathbf{1.95}$	$\mathbf{-0.45}$
12	**0.27**	**2.48**	$\mathbf{0.27}$
13	0	2.56	Not Defined

Tab.3: Calculations for a Log-log-plot of the example data set of Tab.1.: Only the event times and associated Kaplan-Meier estimates printed in bold can be used for the graphical assessment of the goodness of fit.

This leads to the following plot, which provides rough estimates for the parameters λ and p of the Weibull model due to the small number of events:

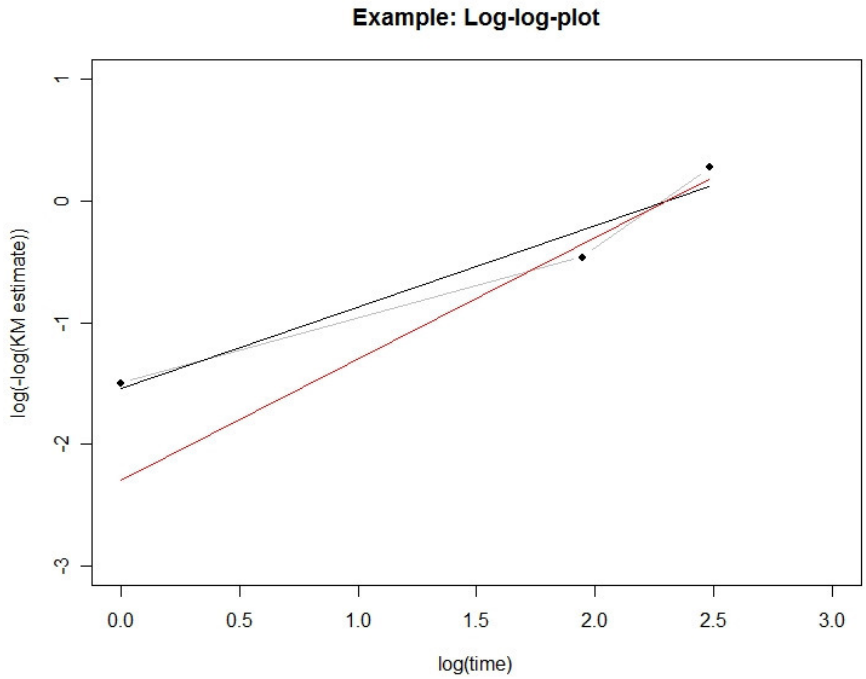


Fig.4: Log-log-plot of the example data set of Tab.1.: The red line reflects the straight line $y = x - 0.1$ expected from the underlying distribution Exp(0.1) out of which the sampling was performed. The lines printed in grey are a frequency polygon of the" observed" values (black points) and the black line displays a linear regression line of these values. The equation of the regression line is $y \approx 0.67 * x - 1.54$, which leads to (the rough) estimates $\hat{p} \approx 0.67$ of p and $\hat{\lambda} \approx \exp(-1.54) \approx 0.21$ of λ.

3.3. Numerical Methods

In our further calculations we will need to determine a maximum of a nonlinear function numerically and will use the procedure `nlm` implemented in the software package R. This procedure can only determine a minimum, i.e. we have to consider the negative function. The algorithm in `nlm` is a 'generalization' of Newton's method [DENNIS/SCHNABEL (1983)]. In its default mode `nlm` uses finite differences to calculate the derivatives but it is also possible to supply them.

The general form of an *unconstrained minimization problem* is:

Given $f: \mathbb{R}^n \longrightarrow \mathbb{R}$
find $x^* \in \mathbb{R}^n$ *for which* $f(x^*) \leq f(x) \quad \forall\, x \in \mathbb{R}^n$, *abbreviated by* $\min_{x \in \mathbb{R}^n} f: \mathbb{R}^n \longrightarrow \mathbb{R}$.

The usual scenario in the numerical solution of a nonlinear minimization problem is that the user is asked to provide a subroutine to evaluate the considered function, and a starting point x_0 that should be a rough approximation to the solution x^*. A widely used method for minimization purposes is to re-formulate it as a 'zeros-problem', i.e. finding the root of the equation $\nabla(f) = 0$, where $\nabla(f)$ denotes the gradient of f, and use Newton's method to solve it. Afterwards it has to be checked, whether the Hessian matrix is positive definite for the possible solution.

One has to keep in mind that numerical procedures for finding numerical solutions of minimization problems usually are not able to find the "global minimum", i.e. the absolute lowest point of the function f in the case when there are several distinct local minimizers. The `nlm` procedure can also provide only *local* minimizers, but it is "*global*" in another sense: The numerical method "line searches" used in `nlm` converges to a local minimizer from almost any starting point and thus is called a *globally convergent algorithm.*

Newton's Method can be shown to have the very convenient characteristic of being locally q-quadratically convergent (inside its convergence region!) but has the disadvantage that it is highly dependent on the starting point. Therefore, a good approximation of the solution by the starting point could be essential. The basic idea of improved nonlinear minimization algorithms is to combine the fast local Newton Method with a globally convergent strategy (in our case the "line searches method"). This approach can be very briefly summarized by the three steps [DENNIS/SCHNABEL (1983)]:

- Try Newton's method (or some modification of it) first at each iteration.
- Decide, whether it seems to be taking a reasonable step (in a minimization process: if value of f decreases).
- If it seems to be appropriate, use it. If not, fall back on a step dictated by a global method, e.g. linear searches.
- Terminate when $\|\nabla f(x_k)\| <$ tol.

The advantage of such a quasi-Newton algorithm is that it combines the advantages of both global methods and Newton's method as it will always end up using Newton's method close to the solution and thus holds on to its fast convergence rate, but being also globally convergent.

The basic idea of a global method for unconstrained minimization problems is intuitive and geometrically obvious: it takes steps that lead "downhill" for the function f. That means, one has to take a direction p from the current point x_c in which f decreases initially, and a new point x_+ in this direction from x_c such that $f(x_+) < f(x_c)$. Such a direction is called a *descendent direction.* More precisely, p is a descendent direction from x_c if the directional derivative of f at x_c in the direction of p is negative:

$$\nabla f(x_c)^T\, p < 0.$$

Under this circumstances it is guaranteed that $f(x_c + \delta p) < f(x_c)$ for sufficiently small $\delta > 0$.

A very natural application of this concept is the method of line searches. The idea behind this algorithm is simple: After the calculation of a descendent direction p_k, it is set $x_{k+1} \coloneqq x_k + \lambda_k\, p_k$ for some $\lambda_k > 0$. That makes x_{k+1} an acceptable next iterate.

The direction p_k is set to $-H_k^{-1}\, \nabla f(x_k)$, where $H_k = \nabla^2 f(x_k) + \mu_k I$ is positive definite with $\mu_k = 0$ if $\nabla^2 f(x_k)$ is safely positive definite and $\nabla^2 f$ the Hessian matrix of f. For more details and numerical background see [DENNIS/SCHNABEL (1983)].

3. Description of a BMD approach for TTT data

Now we describe the BMD approach for TTT data. The study design we have in mind is an animal experiment with k different dose groups $d_1, \dots, d_k$. Following the notation introduced before, the observed times are given by $t_i^* = (t_{i1}^*, \dots, t_{in_i}^*)$ for dose group i and the related status by $\delta_i = (\delta_{i1}, \dots, \delta_{in})$, where $i,\ i = 1, \dots, k$, denotes the dose group. Note, we do not assume here that $d_1 = 0$ and that necessarily the experiment must have a control of dose equal to 0. However, in most applications the data are based on a design with such a control group. Actually, it is a prerequisite of a good experiment that a control with dose $d_1 = 0$ is present.

Dose group	Observed Times	Status
1	t_{11}^* ⋮ $t_{1n_1}^*$	δ_{11} ⋮ δ_{1n_1}
⋮	⋮	⋮
k	t_{k1}^* ⋮ $t_{kn_k}^*$	δ_{k1} ⋮ δ_{kn_k}

Tab. 4: Design of dose- response data with k dose groups.

3.1. Question 1: A reasonable model for TTT data, assuming a Weibull distribution

The underlying assumption of our approach is that the outcome variable time-to-tumor T follows a Weibull distribution. The next step in finding an adequate model for the dose-response data above (Tab.4) is to include appropriately the predictor variables, i.e. generalizing it to obtain a regression model. We assume the hazard of an event to be dependent on the dose. The typical strategy for Weibull models is to re-parameterize λ while the shape parameter p is usually held fixed over the doses. A common re-parameterization for λ, is $\exp(\beta_0 + \beta_1 * \text{pred})$ where pred denotes the predictor variable – in our case the dose. This form of a re-parameterization can be seen as natural since it takes only positive values (λ has to be positive, see section 2.2.).

The Weibull regression procedure in the R-package use a slightly different but – as we will see later – equivalent re-parameterization, namely $\lambda = \exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{pred})$, where p denotes the shape parameter of the Weibull distribution.

Thus we will suggest modeling the survival function of time-to-tumor T with

$$S(t,d) = \exp(-\exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{dose}) * t^P).$$

This model is convenient because it fulfills two assumptions, which are often considered in survival models: proportional hazards and accelerated failure times. Both properties will be briefly presented next.

The underlying assumption of the proportional hazard (PH) models is that the effect of explanatory variables (covariates) is proportional (i.e. multiplicative) with respect to the hazard, where the proportionality constant is independent of time. That means the hazard ratio comparing any two individuals is constant over time. PH regression models treat the hazard function as a product of a baseline hazard $h_0(t)$ and an exponential expression with a linear combination of covariates in its argument:

$$h(t,\boldsymbol{x}) = h_0(t) * \exp\left(\sum_{j=1}^{n} \beta_j\, x_j\right),$$ where $\boldsymbol{x}$ denotes a vector of covariates.

We will consider only one covariate, namely the $dose$, which then leads to the form

$$h(t,\boldsymbol{x}) = h_0(t) * \exp(\beta_1 * dose).$$

The class of PH regression models is applied frequently in survival analysis because it includes the Exponential, Weibull and Gompertz parametric models. It is widely used as Cox semi-parametric model, where the baseline hazard has not to be specified [KLEINBAUM (2005)].

Example: Let HR $= \frac{1}{4}$ be the hazard ratio of two dose groups d_1 and d_2. Then, at any time point, the hazard of an event in group d_2 is 4 times higher than in group d_1:

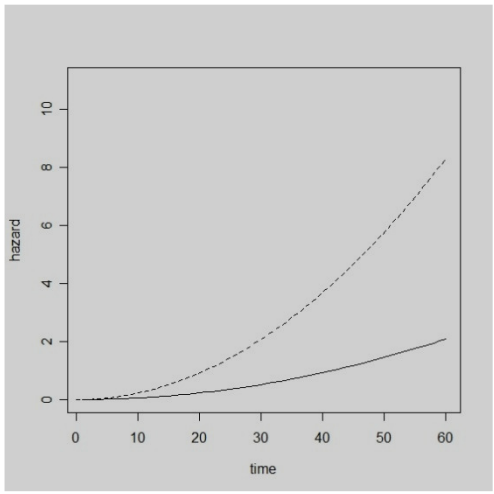


Fig. 5: Proportional Hazard (PH) characteristic: The graph constructed with R reflects the hazard functions for two different dose groups. The Hazard Ratio is constant over time, namely $\frac{\exp(1.35)}{\exp(2.73)} \approx 0.25\ (t > 0)$.

The PH assumption can also be evaluated by plotting $\log(-\log \hat{S}(t))$ against $\log(t)$. Parallel curves support the PH assumption. For more details see [KLEINBAUM (2005)].

The accelerated failure time (AFT) assumption takes a slightly different perspective: Here, the effect of predictor variables is assumed to be multiplicative with respect to survival time, i.e. AFT models describe the contraction or "stretching out" of survival time as a function of covariates [KLEINBAUM (2005), KALBFLEISCH (2002), NIKOLIN (2005)]:

Definition: Let $T \geq 0$ be a survival time and $\boldsymbol{x} = (x_1, \dots, x_n)^T$ a p-dimensional vector of explanatory variables. An AFT model is defined through the following connection:

$$S(t|\boldsymbol{x}) = S_0(\exp((\boldsymbol{\alpha}^*)^{\boldsymbol{T}}\boldsymbol{x})\, t), \quad t \geq 0, \tag{2.1.}$$

where $\boldsymbol{\alpha}^* = (\alpha_1^*, \dots, \alpha_p^*)^T \in \mathbb{R}^p$ denotes a vector of regression coefficients and S_0 the baseline survival function, i.e. the survival function of an individual with vector of covariates $\boldsymbol{x} = \boldsymbol{0}$. $\gamma = \exp((\boldsymbol{\alpha}^*)^{\boldsymbol{T}}\boldsymbol{x})$ is called *accelerating factor*.

The AFT assumption can also be expressed in terms of random variables. If T_0 is a random variable (following some distribution) which represents the survival time for individuals when the vector of covariates $\boldsymbol{x} = \boldsymbol{0}$, the AFT assumption can be expressed as:

$$T = \gamma\, T_0,$$

where γ is the accelerating factor. Alternatively, AFT models can be described as linear models for the logarithm of the survival time $Y = \log(T)$:

Theorem: Let $T \geq 0$ be a survival time, $Y = \log(T)$ and $\boldsymbol{x} = (x_1, \dots, x_n)^T$ a p-dimensional vector of explanatory variables. An AFT model describes the influence of the covariates on the survival time in the following manner:

$$Y = \log(T) = \alpha_0 + \boldsymbol{\alpha}^T\boldsymbol{x} + bZ, \qquad (2.2)$$

where $\boldsymbol{\alpha} = (\alpha_1, \dots, \alpha_p)^T$ denotes a vector of regression parameters and Z denotes a random error, following some distribution.

Proof: If S_0 denotes the survival function of $T = \exp(Y)$ with $\boldsymbol{x} = \boldsymbol{0}$, the following equation holds for the survival probability of an individual with arbitrary vector $\boldsymbol{x}$:

$$\begin{aligned} S(t|\boldsymbol{x}) &= P(\exp(\alpha_0 + \boldsymbol{\alpha}^T\boldsymbol{x} + bZ) > t) = P(\exp(\alpha_0 + bZ) > t\ \exp(-\boldsymbol{\alpha}^T\boldsymbol{x})) \\ &= S_0(t\ \exp(-\boldsymbol{\alpha}^T\boldsymbol{x})). \end{aligned}$$

Therefore, the linear model (2.2.) is equivalent to (2.1.) with $\boldsymbol{\alpha}^* = -\boldsymbol{\alpha}$.

■

Thus, AFT models are additive in terms of $\log(T)$ and multiplicative with respect to T.

AFT regression models are even easier to interpret than PH models because the accelerating factor describes the direct effect of an exposure on every quantile of survival time. A commonly used distribution for AFT models is the log-logistic distribution because it can model non-monotonic hazard functions. Other suitable distributions for T besides the Weibull are the log-normal, Gamma and inverse Gaussian distributions. The Weibull distribution is the only family that can be parameterized as either a PH or as AFT model. We will see later that if T follows a Weibull distribution, $\log(T)$ follows an extreme value distribution $EV(u, b)$ with density function $f(y) = \frac{1}{b}\exp\left(\frac{y-u}{b} - \exp\left(\frac{y-u}{b}\right)\right), y \in (-\infty, \infty)$ [KALBFLEISCH (2002), LAWLESS (2002)].

The accelerating factor γ is illustrated in Fig. 6: Let γ be the accelerating factor, e.g. $\gamma = 2$. That means that for all fixed values of $S(t)$ the ratio of the survival time in group 2 divided by the survival time in group 1 equals 2.

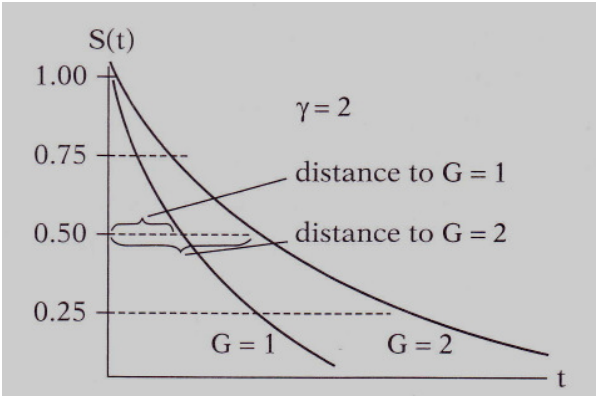


Fig.6: Accelerated Failure Time (AFT) characteristic: Survival curves for Group 1 $(G = 1)$ *and Group 2* $(G = 2)$*. The horizontal lines are twice as long to* $G = 2$ *compared to* $G = 1$ *because* $\gamma = 2$. [KLEINBAUM (2005), 268].

A graphical test of the appropriateness of an AFT model would be a so called "QQ-Plot", where estimated survival quantiles of two different dose groups are plotted against each other. If the AFT assumption holds, the points (referring to the observed survival times) of this plot should approximately lie on a straight line [e.g. PATEL (2006)].

Now we return to the Weibull model. As already mentioned, a unique property of the Weibull model is the equivalence of AFT and PH assumption pretended that p does not vary over different levels of covariates [KALBFLEISCH (2002), 45f.]. That means, if p is held fixed, a Weibull model fulfills the PH assumption as well as the AFT assumption and can therefore be interpreted in either framework. If in the log-log survival plot parallel and straight lines occur, we can conclude, that the Weibull assumptions as well as the PH (and thus also the AFT) assumption hold. If parallel but not straight lines arise, the Weibull assumption is violated. If non parallel but straight lines occur, we can conclude that the Weibull assumption holds but not the PH (and therefore also not the AFT) assumption and thus that p is not constant across levels of covariates. A Weibull PH model with '$dose$' as explanatory variable can be formulated

$$h(t) = \exp(\beta_0)\, p\, t^{p-1} \exp(\beta_1\, dose) = \exp(\beta_0 + \beta_1\, dose) p\, t^{p-1},$$

where the baseline hazard is specified parametrically as $h_0(t) := \exp(\beta_0)\, p\, t^{p-1}$.

This is equivalent to a re-parameterization of λ by $\exp(\beta_0 + \beta_1 * \text{dose})$. Thus, we gain $S(t) = \exp(-(\beta_0 + \beta_1 * \text{dose}) * t^p)$.

How do then two individuals exposed to different doses differ in their hazard? The hazard ratio for two arbitrary individuals in dose groups d_i and d_j equals a constant:

$$\text{HR} = \frac{\exp(\beta_0 + \beta_1\, d_i)\; p\; t^{p-1}}{\exp\big(\beta_0 + \beta_1\, d_j\big)\; p\; t^{p-1}} = \frac{\exp(\beta_1\, d_i)}{\exp(\beta_1\, d_j)}.$$

Because of the equivalence of PH and AFT for Weibull regression models with fixed shape parameter p, we can formulate this model also in the AFT framework.

We first solve for t in terms of a fixed value of $S(t)$:

$$S(t) = \exp(-\lambda\, t^p) \qquad => t = (-\log(S(t)))^{\frac{1}{p}}\, \lambda^{-\frac{1}{p}}$$

Using the parameterization $\lambda = \exp(\beta_0 + \beta_1 * \text{dose})$ of the PH model, we get

$$\lambda^{-\frac{1}{p}} = \exp(\beta_0 + \beta_1 * \text{dose})^{-\frac{1}{p}} = \exp\left(-\frac{1}{p}\beta_0 - \frac{1}{p}\beta_1 * \text{dose}\right) := \exp\,(\alpha_0 + \alpha_1 * \text{dose})$$

and thus

$$S(t) = \exp(-\exp(-p\;\alpha_0 - p\;\alpha_1 * \text{dose}) * t^p).$$

Then for any fixed probability $S(t) = q$ we obtain for doses d_i and d_j:

$$\frac{(-\log(q))^{\frac{1}{p}} \exp\,(\alpha_0\; + \alpha_1\, d_i)}{(-\log(q))^{\frac{1}{p}} \exp\,(\alpha_0\; + \alpha_1\, d_j)} = \frac{\exp(\alpha_1\, d_i)}{\exp(\alpha_1\, d_j)},$$

which is a constant, that does not depend on q.

The survival time of an individual in dose group d_j in comparison to that of an unexposed individual is accelerated with factor $\gamma = \frac{\exp(\alpha_1 * 0)}{\exp(\alpha_1\, d_j)} = \exp\big(-\alpha_1\, d_j\big)$:

$$S(t|\, d_j) = S_0\big(\exp(-\alpha_1\, d_j)\, t\big) = \exp\big(-\exp(-p\,\alpha_0)\,\big(\exp(-\alpha_1\, d_j)\, t\big)^p\big)$$
$$= \exp\,(-\exp\big(-p\,\alpha_0 - p\,\alpha_1\, d_j\big)\, t^p) \quad t \geq 0.$$

If, for example, $\alpha_1 = -1.5$, an individual in dose group $d_j = 1$ can be viewed, on average, as accelerating through life $\gamma = \exp(-(-1.5)) \approx 4.5$ times faster than an unexposed one.

In terms of $\log(T)$ we gain the following model:

$$\log(T) = \alpha_0 + \alpha_1 * dose + \frac{1}{p}\, Z, \qquad (2.3.)$$

Z following a standard extreme value distribution.

Proof: We use the density transformation theorem [HELD (2008), 264]. Let $Y = \log(T)$, then we gain with substitutions $p = \frac{1}{b}$ and $\lambda = \exp\left(-\frac{u}{b}\right)$:

$$f_Y(y) = f_T(\log^{-1}(y)) \left|\frac{d\log^{-1}(y)}{dy}\right| = f_T(\exp(y))\,|\exp(y)|$$

$$= \lambda\, p\, (\exp(y))^{\mathrm{p}-1} \exp(-\lambda\, (\exp(y))^{\mathrm{p}})\ \exp(y)$$

$$= \exp\left(-\frac{u}{b}\right)\frac{1}{b}\exp\left(\frac{y}{b} - y\right)\exp\left(-\exp\left(-\frac{u}{b}\right)\exp\left(\frac{y}{b}\right)\right)\exp(y)$$

$$= \frac{1}{b}\,\exp\left(\frac{y-u}{b} - \exp\left(\frac{y-u}{b}\right)\right)$$

Thus, if T follows a Weibull distribution $Wei(\lambda, p)$, $\log(T)$ follows an extreme value distribution $EV(u = -b\log(\lambda), b = p^{-1})$.

The re-parameterization $\lambda = \exp\,(-p\,\alpha_0 - p\,\alpha_1\, d)$ leads to $u = \alpha_0 + \alpha_1\, d$ and thus to the linear model

$$\log(T) = \alpha_0 + \alpha_1\, d + \tfrac{1}{p}\, Z, \qquad Z \text{ following a standard extreme value distribution.}$$

■

Note, that the corresponding coefficients of the PH and AFT model formulations are related in the following manner: $\beta_j = -\,p\,\alpha_j$, j=1,2.

In the case of an Exponential distribution, the relationship of the coefficients occurring in the PH and AFT model is simply given by $\beta_j = -\,\alpha_j$, $j = 1,2$.

Conclusion: A reasonable model for the outcome variable TTT in our case should be of the form $S(t,d) = \exp(-\exp(\beta_0 + \beta_1 * \mathrm{d}) * t^P)$, where d denotes the dosage.

The hazard function of our model is given by $h(t,d) = \exp(\beta_0 + \beta_1 * \mathrm{d})$ and the density function by $f(t,d) = \exp(-\exp(\beta_0 + \beta_1 * \mathrm{d}) * t^P) * \exp(\beta_0 + \beta_1 * \mathrm{d})$.

This PH model is equivalent to the AFT model with survival function given by $S(t,d) = \exp(-\exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{d}) * t^P)$.

3.2. Question 2: Risk functionals for the *Benchmark Response* (BMR)

3.2.1. Definitions of BMRs for TTT data in general

The Benchmark Response (BMR) is a small but measurable change in adverse response in contrast to non-exposed individuals. Ideally, the BMR would reflect an effect size that can be tolerated because it is negligible or even non-adverse. However, there is a practical constraint when animal data are used: The increase in effect should not be too small because otherwise the estimate of the BMD will lie outside the range of observation and its lower confidence limit will heavily depend on the chosen model [CRUMP (1984), EFSA (2009)].

In principle, there are two different approaches possible in specifying it, referring to TTT:

The first point of view is to consider the survival curves of different dose groups at a fixed time point t^* and define the BMR in terms of reduction in survival probability compared to the predicted one in the control group ($d = 0$) at that time t^*:

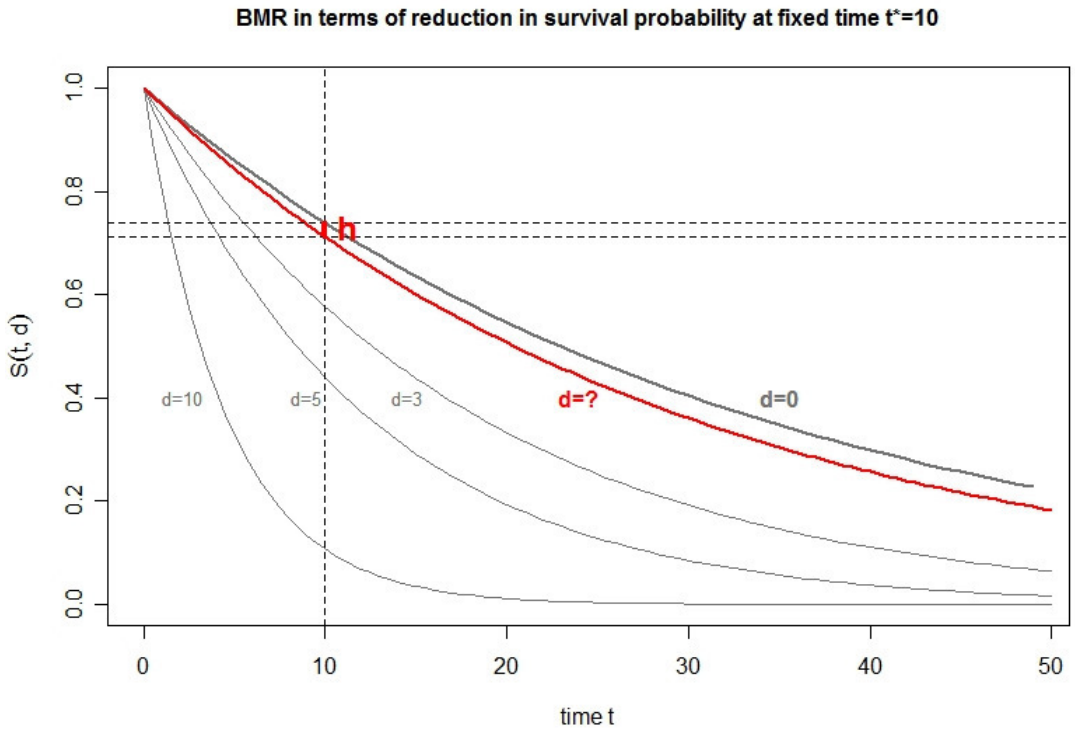


Fig.7. BMR in terms of reduction in survival probability at fixed time: The BMR is defined in terms of a reduction h in survival probability at fixed time $t^* = 10$ (in contrast to the unexposed individuals). The goal is then to determine the dose d, for which this reduction is expected.

This reduction can be declared either in absolute or in relative terms.

In the first case, we state an absolute reduction h that can be just tolerated and the dose d which leads to the survival function with value $S(t^*, 0) - h$ at point t^* is the dose we define as Benchmark Dose (BMD):

$$d\text{:}\;\; S(t^*, d) = S(t^*, 0) - h.$$

In the second case, we state a relative reduction $h = \frac{S(t^*,0) - S(t^*,d)}{S(t^*,0)}$ and the BMD is given by the dose d for which holds

$$d\text{:}\;\; S(t^*, d) = (1 - h)\, S(t^*, 0).$$

The second point of view is to consider the BMR as reduction in time at a fixed quantile of the survival distribution, e.g. the median survival time. Note that again we refer to the background values predicted by the model, not the observed ones:

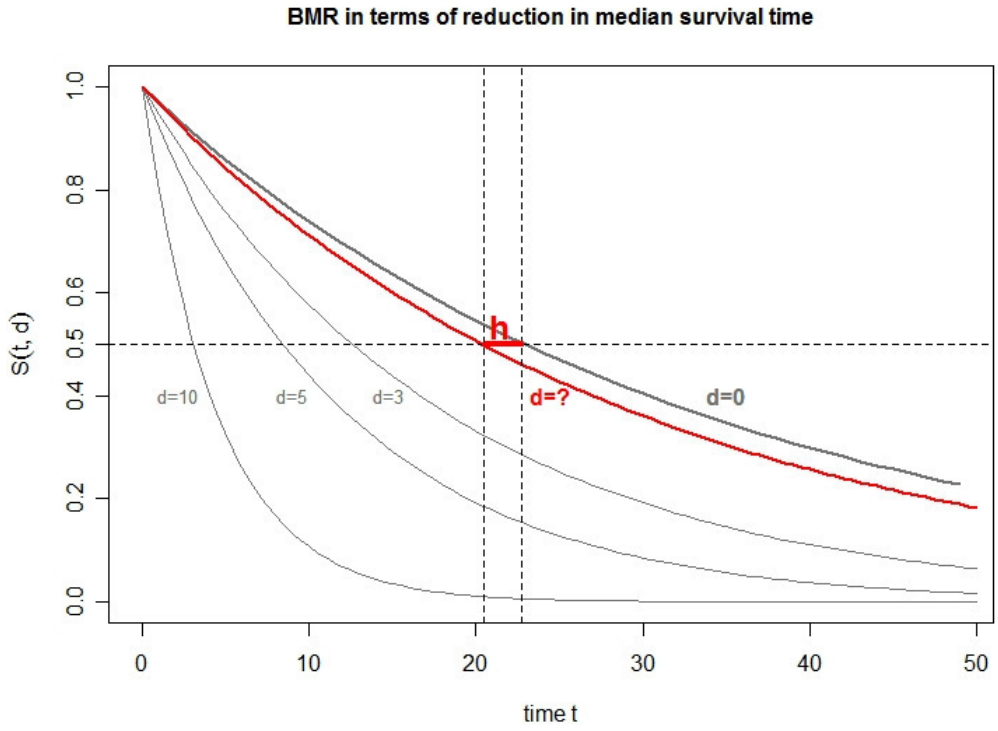


Fig. 8. BMR in terms of reduction in median survival time: The BMR is defined in terms of a reduction in median survival in contrast to the unexposed individuals. The dose d for which this reduction is expected, has to be determined.

Once more, we can define the BMR in absolute and relative terms:

In the first case, we consider the BMR as a reduction of h units (in animal experiments most conveniently in weeks) in median survival. Then we are looking for the dose d for which holds

$$d\colon\ t_{med}(d) = t_{med}(0) - h.$$

In the second case, we define the relative BMR as $h = \frac{t_{med}(0) - t_{med}(d)}{t_{med}(0)}$ and the BMD is the dose d with

$$d\colon\ t_{med}(d) = (1-h)\, t_{med}(0).$$

3.2.2. Definitions of BMRs for TTT data under assumption of a Weibull model

For our case of Weibull models not all of the presented ways of defining a BMR are similarly suitable. Especially the definitions in terms of a reduction of survival probability at a fixed time point t^* are accompanied with difficulties: The main problem is that the calculated BMD depends highly on the choice of t^* - which becomes crucial when the value of t^* is chosen rather arbitrarily. For a definition of the BMR in absolute terms, the estimates for the BMD do not even decrease monotonically for increasing values of t^* (Fig.9a).

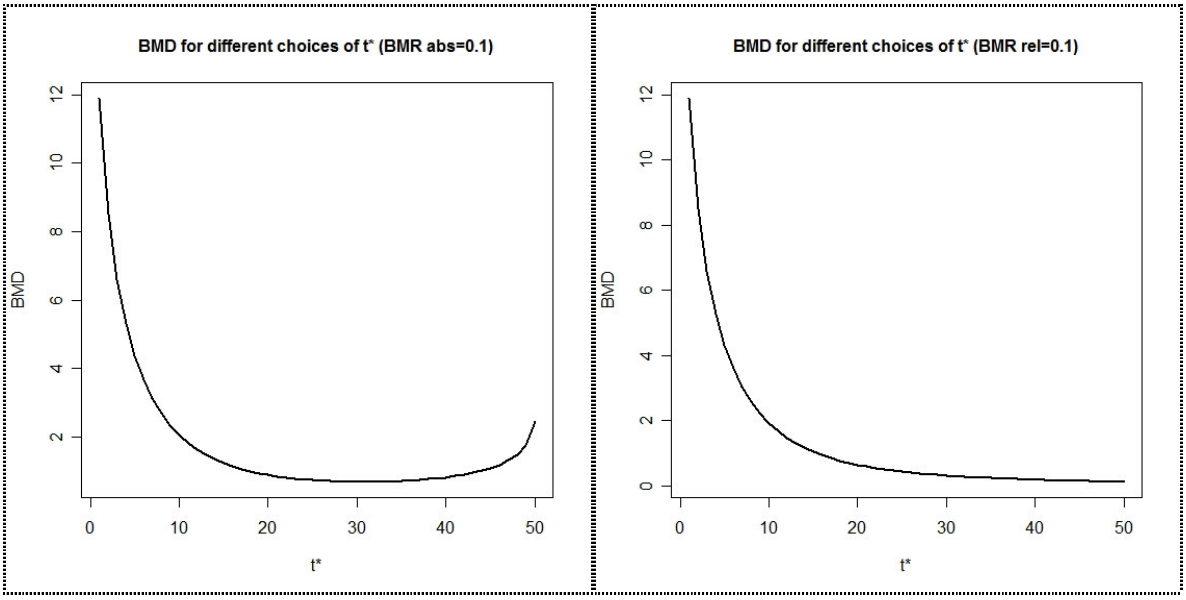


Fig. 9: Dependence of the resulting value for the BMD for different choices of t^*, illustrated using a Weibull model of the form $S(t,d) = \exp(-\exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{d}) * t^P)$ with parameters $\alpha_0 = 3.5$, $\alpha_1 = -0.2$, $p = 2$. Note, that the underlying formulas used for the calculation of the BMDs will be derived in section 3.3.

On the other hand, defining the BMR in terms of a reduction in a specified quantile of survival seems to be more appropriate. If the BMR is stated in absolute terms, it is also highly dependent on the choice of the quantile – nevertheless, the choice of the median can be defended to be quite "natural" and therefore the whole proceeding does not seem as arbitrary as in the case discussed above.

If the BMR is formulated in relative terms, the situation becomes very comfortable because this way of definition makes use of the characteristic that Weibull models are accelerated failure time models. Therefore, following simply the definition of AFT, the value of the BMD is independent of the choice of the quantile used for the definition of the relative BMR.

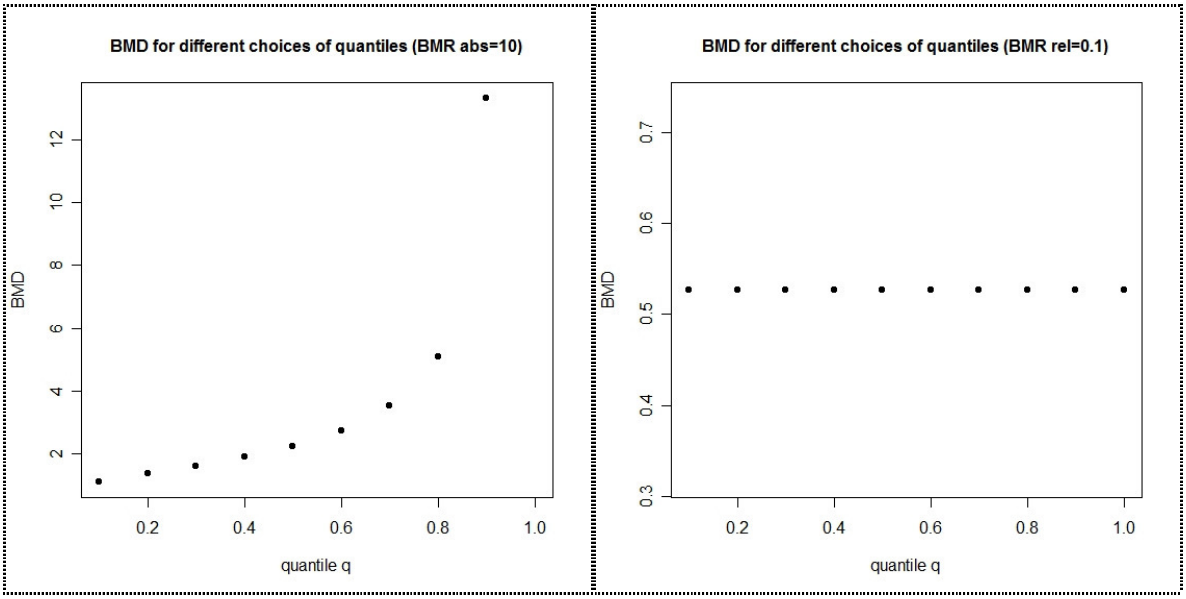


Fig.10: Dependence of the resulting value for the BMD for different choices of the quantile, illustrated using a Weibull model of the form $S(t,d) = \exp(-\exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{d}) * t^P)$ *with parameters* $\alpha_0 = 3.5$, $\alpha_1 = -0.2$, $p = 2$. *If the BMR is stated in terms of a relative reduction, the value of the BMD is independent of the specific choice of a quantile because of the AFT property of Weibull models.*

Beside of this, the definitions of a BMR which base on an absolute reduction either in survival probability at a fixed time point or in a specified quantile of survival generally lead to the question of which value should be chosen. It seems that considerations of relative reductions can be handled easier because – as already mentioned – here some "default" values like 5% or 10% already exist from non-cancer contexts.

Conclusion: If no specific circumstances are fulfilled which would suggest another proceeding, the most appropriate way of defining a BMR for a Weibull model seems to be stating the BMR in terms of a relative reduction in the median and thereby quasi automatically in all quantiles of the TTT distribution. Nevertheless, we will provide the further calculations for all ways of defining the BMR presented above because in some cases one could be interested in analyzing the absolute or relative reduction at a certain time point (e.g. the 1 year or 5 year survival probability, which are common measures in clinical applications) or an absolute reduction in median survival time

3.3. Question 3: Estimating the BMD and its lower confidence limit (BMDL)

3.3.1. Maximum Likelihood Estimation of the BMD

In order to find the BMD we have to estimate the unknown parameters of our model at first. Therefore we have to calculate the Maximum Likelihood Estimates (MLEs) of the parameters, which can be summarized in a vector θ.
We consider the AFT form of the models. Thus, in the Exponential case we have $\theta = (\alpha_0,\ \alpha_1)$ and in the general Weibull case we have $\theta = (\alpha_0,\ \alpha_1,\ p)$.

The likelihood for partly censored data is given by

$$L(\theta) = \prod_{i=1}^{k}\prod_{j=1}^{n_j} f\left(x_j, d_i; \theta\right)^{\delta_j} * S\left(x_j, d_i; \theta\right)^{1-\delta_j}.$$

Recall that under presumption of a statistical model, parameterized by a yet unknown parameter vector θ, the probability of the observed data as a function of θ is called *likelihood function* or just *likelihood*. In our case, the individual observations are assumed independent and therefore we can write the likelihood as product of the individual contributions to it. The contribution of an individual who faced the event is given by $f\left(x_j, d_i; \theta\right)$.[1] This notation indicates that one has to plug in the observed time of event x_j and the dose group d_i the individual belongs to and to interpret the result as function of θ. In contrast, the contribution of an individual who was censored is given by $\mathrm{S}\left(x_j, d_i; \theta\right)$ because we only know that the (event-free) survival time of the individual exceeds x_j.

The maximum likelihood estimate $\hat{\theta}_{ML}$ of θ is obtained through maximization of the likelihood function:

$$\hat{\theta}_{ML} = \arg\max_{\theta\in\Theta} L(\theta)$$

or – because of the monotony of the logarithm – through maximization of the log likelihood function

$$l(\theta) = \log\left(L(\theta)\right) = \sum_{i=1}^{k}\sum_{j=1}^{n_j} f\left(x_j, d_i; \theta\right)^{\delta_j} * S\left(x_j, d_i; \theta\right)^{1-\delta_j}: \qquad (2.1)$$

[1] For discrete models it would be obvious that this is the individual contribution. For continuous models it can also be defended that the individual contribution is equal to the value of the density function with plugged-in time of event x_j and dosage d_i. The argumentation can be found e.g. in Held, 15f., cited above.

$$\hat{\theta}_{ML} = \arg\max_{\theta \in \Theta} l(\theta).$$

Because the log likelihood can often be handled easier, we will continue with (2.1). In order to find the maximum of $l(\theta)$, where θ consists of the k unknown parameters, we need the k first partial derivatives of the log likelihood function. The MLEs are determined through the so called system of *score equations*

$$s(\theta) = \left(\frac{\partial}{\partial \theta_1} l(\theta), \dots, \frac{\partial}{\partial \theta_k} l(\theta) \right)^T = 0.$$

The negative Hessian matrix of $l(\theta)$, which is a symmetric $k \times k$ matrix whose (i, j) elements are $-\frac{\partial^2 l(\theta)}{\partial \theta_i \partial \theta_j}$, is called *Fisher information matrix* $I(\theta)$. Because we are searching for a maximum of $l(\theta)$, the Fisher information matrix of $\hat{\theta}_{ML}$, i.e. the *observed Fisher information matrix* $I(\hat{\theta}_{ML})$, has to be positive definite for a solution. This is the case when all eigenvalues are positive respectively all main minors of $I(\hat{\theta}_{ML})$.

Now we want to present the calculation of the MLEs for the Weibull AFT model introduced in section 3.1 with survival function

$$S(t, d) = \exp(-\exp(-p\,\alpha_0 - p\,\alpha_1 * \mathrm{d}) * t^P).$$

Before considering the general Weibull model we look at the easier Exponential case.

i. ML estimation in Exponential AFT models

For $p = 1$ the likelihood function is given by

$$L(\alpha_0, \alpha_1) = \prod_{i=1}^{k} \prod_{j=1}^{n_i} \{\exp(-\alpha_0 - \alpha_1 d_i)\ \exp\left(-\exp(-\alpha_0 - \alpha_1 d_i)\, x_{ij}\right)\}^{\delta_{ij}}\ \{\exp\left(-\exp(-\alpha_0 - \alpha_1 d_i)\, x_{ij}\right)\}^{1-\delta_{ij}}$$

$$= \prod_{i=1}^{k} \{\exp(-\alpha_0 - \alpha_1 d_i)\}^{\sum_{j=1}^{n_i} \delta_{ij}}\ \exp(-\exp(-\alpha_0 - \alpha_1 d_i))^{\sum_{j=1}^{n_i} x_{ij}}$$

$$= \{\exp(-\alpha_0)\}^{\sum_{i=1}^{k} \sum_{j=1}^{n_i} \delta_{ij}} * \{\exp(-\alpha_1)\}^{\sum_{i=1}^{k} \left(d_i \sum_{j=1}^{n_i} \delta_{ij}\right)} * \prod_{i=1}^{k} \exp(-\exp(-\alpha_0 - \alpha_1 d_i))^{\sum_{j=1}^{n_i} x_{ij}}$$

With $\delta_i := \sum_{j=1}^{n_i} \delta_{ij}$, the number of events in dose group i, and $x_i := \sum_{j=1}^{n_i} x_{ij}$ we obtain:

$$L(\alpha_0, \alpha_1) = \{\exp(-\alpha_0)\}^{\sum_{i=1}^{k} \delta_i} * \{\exp(-\alpha_1)\}^{\sum_{i=1}^{k} (d_i\, \delta_i)} * \prod_{i=1}^{k} \exp(-\exp(-\alpha_0 - \alpha_1 d_i))^{x_i}$$

This leads to the log likelihood

$$l(\alpha_0, \alpha_1) = -\left(\sum_{i=1}^{k} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{k} d_i \delta_i\right)\alpha_1 - \sum_{i=1}^{k} x_i \exp(-\alpha_0 - \alpha_1 d_i)$$

and thus to the non-linear system of score equations

$$\frac{\partial \log(\mathrm{L})}{\partial \alpha_0} = -\left(\sum_{i=1}^{k} \delta_i\right) + \sum_{i=1}^{k} x_i \exp(-\alpha_0 - \alpha_1 d_i) = 0$$

$$\frac{\partial \log(\mathrm{L})}{\partial \alpha_1} = -\left(\sum_{i=1}^{k} d_i \delta_i\right) + \sum_{i=1}^{k} d_i\, x_i \exp(-\alpha_0 - \alpha_1 d_i) = 0,$$

which has to been solved numerically.

Example: As an illustration an Exponential AFT model is fitted to an example data set.

Dosage $\boldsymbol{d}$ [mg/kg BW]	**Time** $\boldsymbol{x}$ [weeks]	**Status** $\boldsymbol{\delta}$
0	84	1
0	2	1
0	8	0
0	1	1
1	21	1
1	40	1
1	2	0
1	6	1
10	1	1
10	6	1
10	2	1
10	8	1

Tab. 5. Example data set for three dose groups.

Fitting an exponential model with R is shown next when using the procedure `survreg` from the R-Package. The R-code lines are indicated by >. After the call of `survreg` the output is displayed.

```
> rm(list=ls())
> library(splines)
> library(survival)
```

```
>
> bsp<-read.table("bsp.txt",header=T)
> attach(bsp)
> out<-survreg(Surv(weeks,status)~dose,dist='weib',scale=1)
> out
Call:
survreg(formula = Surv(weeks, status) ~ dose, dist = "weib",
    scale = 1)

Coefficients:
(Intercept)        dose
  3.3983780  -0.1956325

Scale fixed at 1

Loglik(model)= -35.6   Loglik(intercept only)= -39
        Chisq= 6.78 on 1 degrees of freedom, p= 0.0092
n= 12
```

Thus the estimates for the parameters α_0 and α_1 are: $\alpha_0 = 3.3984$ and $\alpha_1 = -0.1956$.

Alternatively, we can minimize the negative log-Likelihood function, which leads to the same results:

The log-Likelihood is given by

$$l(\alpha_0, \alpha_1) = -\left(\sum_{i=1}^{k} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{k} d_i \delta_i\right)\alpha_1 - \sum_{i=1}^{k} x_i \exp(-\alpha_0 - \alpha_1 d_i)$$

$$= -10\alpha_0 - 43\alpha_1 - 95\exp(-\alpha_0) - 69\exp(-\alpha_0 - \alpha_1) - 17\exp(-\alpha_0 - 10\alpha_1)$$

Using R we obtain (the function g is the negative log-Likelihood):

```
> f=function(x){10*x[1]+43*x[2]+95*exp(-x[1])+69*exp(-x[1]-x[2])+17*exp(-
x[1]-10*x[2])}
> nlm(f,c(5,3))
$minimum
[1] 35.57158

$estimate
[1]  3.3983779 -0.1956325
```

$\Rightarrow \alpha_0 = 3.3984$, $\alpha_1 = -0.1956$ (R)

Now we are able to compare the observed and expected survival probabilities. For each dose group the Kaplan Meier curve is plotted and the survival curve predicted by the Exponential model

$$S(t) = \exp(-\exp(-3.3984 + 0.1956 * \mathrm{d}) * t).$$

```
rm(list=ls())
library(splines)
library(survival)
```

```
bsp<-read.table("bsp.txt",header=T)
attach(bsp)

#Kaplan Meier estimates
kmbsp<-survfit(Surv(weeks, status==1)~ dose, data=bspw)

#KM-plot
plot (kmbsp, col=c("red", "blue", "green"),main="Example: 'observed' (KM)
and expected Survival probabilities",xlab="time [weeks]", ylab="survival
probability",legend.text=c("dose 0","dose 1", "dose 10"))

#survival curves predicted by the Exponential model
x<-(0:84)
y0<-y1<-exp(-exp(-3.3983)*x)
y1<-exp(-exp(-3.3983+0.1956)*x)
y10<-exp(-exp(-3.3983+0.1956*10)*x)

#expected for dose=0
for (i in 1:85)
      {
       lines(c(x[i-1],x[i]),c(y0[i-1],y0[i]),col="red")
      }

#expected for dose=1
for (i in 1:85)
      {
       lines(c(x[i-1],x[i]),c(y1[i-1],y1[i]),col="blue")
      }

#expected for dose=10
for (i in 1:85)
      {
       lines(c(x[i-1],x[i]),c(y10[i-1],y10[i]),col="green")

      }
```

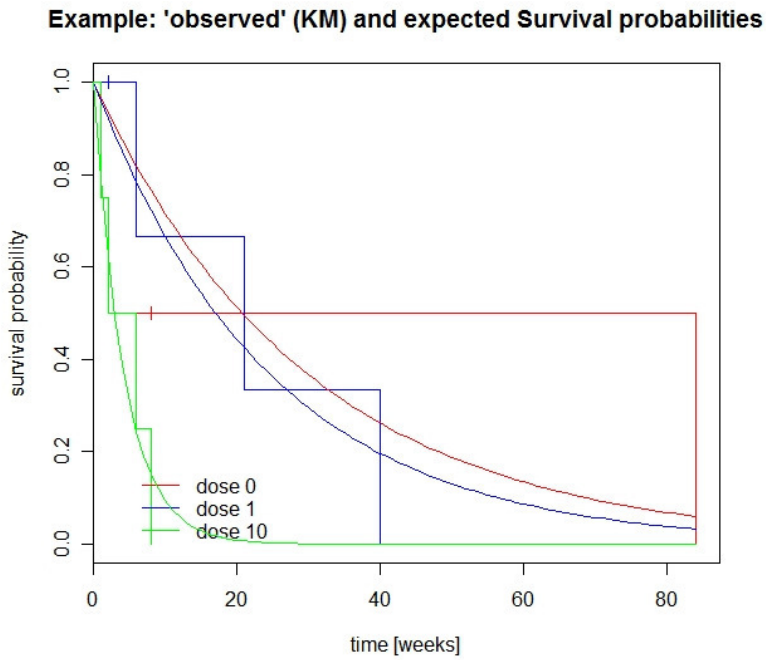


Fig.11. 'Observed' (i.e. Kaplan-Meier estimates obtained from the observed times) and expected survival probability of the example dataset when an Exponential model is fitted.

ii. ML estimation in Weibull AFT models

It is technically more comfortable to use for the following calculation the PH form of the Likelihood, which is given by

$$L(p,\beta_0,\beta_1) = \prod_{i=1}^{k}\prod_{j=1}^{n_i}\{\exp(\beta_0+\beta_1 d_i) * p * (x_{ij})^{p-1}\ \exp(-\exp(\beta_0+\beta_1\, d_i)(x_{ij})^p)\}^{\delta_{ij}}$$

$$* \left\{\exp\left(-\exp(\beta_0+\beta_1\, d_i)\,(x_{ij})^p\right)\right\}^{1-\delta_{ij}}$$

$$= \prod_{i=1}^{k}\{\exp(\beta_0+\beta_1\, d_i)\}^{\sum_{j=1}^{n_i}\delta_{ij}}\ \exp(-\exp(\beta_0+\beta_1\, d_i))^{\sum_{j=1}^{n_i}(x_{ij})^p}$$

$$* \prod_{i=1}^{k}\prod_{j=1}^{n_i} p^{\delta_{ij}} * (x_{ij})^{(p-1)\delta_{ij}}$$

$$= \{\exp(\beta_0)\}^{\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}} * \{\exp(\beta_1)\}^{\sum_{i=1}^{k}\left(d_i\sum_{j=1}^{n_i}\delta_{ij}\right)}$$

$$* \prod_{i=1}^{k}\exp(-\exp(\beta_0+\beta_1\, d_i))^{\sum_{j=1}^{n_i}(x_{ij})^p} * \prod_{i=1}^{k}\prod_{j=1}^{n_i} p^{\delta_{ij}} * (x_{ij})^{(p-1)\delta_{ij}}$$

With $\delta_i := \sum_{j=1}^{n_i}\delta_{ij}$, the number of events in dose group i we obtain:

$$L(p,\beta_0,\beta_1) = \{\exp(\beta_0)\}^{\sum_{i=1}^{k}\delta_i} * \{\exp(\beta_1)\}^{\sum_{i=1}^{k}(d_i\,\delta_i)} * \prod_{i=1}^{k}\exp(-\exp(\beta_0+\beta_1\, d_i))^{\sum_{j=1}^{n_i}(x_{ij})^p}$$

$$* \prod_{i=1}^{k}\prod_{j=1}^{n_i} p^{\delta_{ij}} * (x_{ij})^{(p-1)\delta_{ij}}$$

This leads to the log likelihood

$$l(p,\beta_0,\beta_1) = \left(\sum_{i=1}^{k}\delta_i\right)\beta_0 + \left(\sum_{i=1}^{k} d_i\delta_i\right)\beta_1 - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp(\beta_0+\beta_1\, d_i)$$

$$+ \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$

Thus, the AFT form is given by

$$l(p,\alpha_0,\alpha_1) = \left(\sum_{i=1}^{k}\delta_i\right)(-p\,\alpha_0) + \left(\sum_{i=1}^{k} d_i\delta_i\right)(-p\,\alpha_1) - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp(-p\,\alpha_0 - p\,\alpha_1\,d_i) + \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$

Similar to the Exponential model the proceeding is illustrated using the same dataset as before (Tab.5). Firstly, the estimates for α_0, α_1 and p are calculated using the `survreg` procedure implemented in R. Note, that the output of the `survreg` procedure includes the estimates for α_0 (intercept), α_1 and $\frac{1}{p}$ (scale), following the AFT model form (2.3.):

```
> rm(list=ls())
> library(splines)
> library(survival)
>
> bspw<-read.table("bsp.txt",header=T)
> attach(bspw)
>
> out<-survreg(Surv(weeks,status)~dose,dist='weib')
> out
Call:
survreg(formula = Surv(weeks, status) ~ dose, dist = "weib")

Coefficients:
(Intercept)        dose
  3.3803461  -0.1948039

Scale= 1.044804

Loglik(model)= -35.6   Loglik(intercept only)= -37.9
        Chisq= 4.75 on 1 degrees of freedom, p= 0.029
n= 12
```

$=> \alpha_0 = 3.3803\ ,\ \alpha_1 = -0.1948,\ \frac{1}{p} = 1.0448 => p = 0.9571$

Secondly, the estimates are obtained via maximizing the log likelihood with R. We use the procedure `nlm` to minimize the negative log likelihood.

```
> #Weibull example: MLE for alpha0, alpha1, p
>
> #Tools for loglikelihood
> delta<-sum(status)
> delta
```

```
[1] 10
>
> deltad<-sum(status*dose)
> deltad
[1] 43
>
> #x=(alpha0,alpha1,p)
>
f=function(x){delta*x[3]*x[1]+deltad*x[3]*x[2]+(sum(weeks[dose==0]^x[3]))*e
xp(-x[3]*x[1]-x[3]*x[2]*0)+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-
x[3]*x[2]*1)+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-x[3]*x[2]*10)-
delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}
>
> nlm(f,c(4,1,1))
$minimum
[1] 35.55586
```

$=>\ \alpha_0 = 3.3803,\ \alpha_1 = -0.1948,\ p = 0.9571$

These estimates are almost the same as for the Exponential model.

After having determined the MLEs for the parameters included in our model we can compute the BMD for a pre-specified BMR. Again, the calculation will be presented for the Exponential model at first.

iii. Calculation of BMDs for Exponential AFT models

The calculation of a BMD depends on the way the BMR is defined. As we have seen in section B.2. there are in total four different ways of specifying the BMR with respect to TTT.

a) Absolute value for the BMR at a fixed time point t^*

Let the BMR be the reduction of h in survival probability at time t^*:

$$S(t^*, d_{BMR}) = S(t^*, 0) - h.$$

The survival function $S(t^*, d_{BMR}) = \exp(-\exp(-\alpha_0 - \alpha_1 d_{BMR})\ t^*)$ leads to:

$\exp(-\exp(-\alpha_0 - \alpha_1 d_{BMR})\ t^*) = \exp(-\exp(-\alpha_0)\ t^*) - h \quad |\log$

$\Leftrightarrow\ -\exp(-\alpha_0 - \alpha_1 d_{BMR})\ t^* = \log(\exp(-\exp(-\alpha_0)\ t^*) - h) \quad |*(-1)$

$\Leftrightarrow\ \exp(-\alpha_0 - \alpha_1 d_{BMR})\ t^* = -\log(\exp(-\exp(-\alpha_0)\ t^*) - h) \quad |\log$

$$\Leftrightarrow\ (-\alpha_0 - \alpha_1 d_{BMR}) + \log(t^*) = \log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h))$$

$$\Leftrightarrow\ d_{BMR} = \frac{\log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h)) - \log(t^*) + \alpha_0}{-\alpha_1}$$

This dosage d_{BMR} is just the BMD we are searching for.

b) Relative value for the BMR at a fixed time point t^*

Let the BMR be a $h * 100\%$ reduction of survival probability (of the control group) at time t^*,

$$S(t^*, d_{BMR}) = (1-h)\, S(t^*, 0).$$

With $S(t^*, d_{BMR}) = \exp(-\exp(-\alpha_0 - \alpha_1 d)\, t^*)$ we obtain:

$$\exp(-\exp(-\alpha_0 - \alpha_1 d_{BMR})\, t^*) = (1-h)\ \exp(-\exp(-\alpha_0)\, t^*) \quad |\log$$

$$\Leftrightarrow -\exp(-\alpha_0 - \alpha_1 d_{BMR})\, t^* = \log(1-h) - \exp(-\alpha_0)\, t^* \quad |*(-1)$$

$$\Leftrightarrow \exp(-\alpha_0 - \alpha_1 d_{BMR})\, t^* = -\log(1-h) + \exp(-\alpha_0)\, t^* \quad |\log$$

$$\Leftrightarrow -\alpha_0 - \alpha_1 d_{BMR} + \log(t^*) = \log(-\log(1-h) + \exp(-\alpha_0)\, t^*)$$

$$\Leftrightarrow d_{BMR} = \frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-\alpha_1}$$

c) Absolute value for the BMR as reduction in a specified quantile of survival

Now we state the BMR as reduction of h units in median survival (for any other quantile of survival the proceeding is analogous):

$$t_{med}(d_{BMR}) = t_{med}(0) - h.$$

In our model, $t_{med}(d_{BMR})$ equals $(-\log 0.5)\exp(\alpha_0 + \alpha_1 d_{BMR})$ because by solving the parameterized Exponential survival function $S(t,d) = \exp(-\exp(-\alpha_0 - \alpha_1 d)\, t)$ for t, we obtain

$$t = -\frac{\log\big(S(t)\big)}{\exp(-\alpha_0 - \alpha_1 d)} = -\log\big(S(t)\big) * \exp(\alpha_0 + \alpha_1 d)\,.$$

With $t_{med}(d_{BMR}) = (-\log 0.5)\exp(\alpha_0 + \alpha_1 d_{BMR})$ we gain:

$(-\log 0.5)\exp(\alpha_0 + \alpha_1 d_{BMR}) = (-\log 0.5)\exp(\alpha_0) - h$

$\Leftrightarrow (-\log 0.5)\exp(\alpha_0)\exp(\alpha_1 d_{BMR}) = (-\log 0.5)\exp(\alpha_0) - h$

$\Leftrightarrow \exp(\alpha_1 d_{BMR}) = \dfrac{(-\log 0.5)\exp(\alpha_0) - h}{(-\log 0.5)\exp(\alpha_0)} \quad |\log$

$\Leftrightarrow \alpha_1 d_{BMR} = \log\left(\dfrac{(-\log 0.5)\exp(\alpha_0) - h}{(-\log 0.5)\exp(\alpha_0)}\right)$

$\Leftrightarrow \alpha_1 d_{BMR} = \log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log((-\log 0.5)\exp(\alpha_0))$

$\Leftrightarrow d_{BMR} = \dfrac{\log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log(-\log 0.5) - \alpha_0}{\alpha_1}$

d) Relative value for the BMR as reduction in a specified quantile of survival

Let the BMR be a $h * 100\%$ reduction of a quantile q of survival (in relation to control group),

$$t_q(d_{BMR}) = (1 - h)\, t_q(0).$$

With $t_q(d_{BMR}) = (-\log q)\exp(\alpha_0 + \alpha_1 d_{BMR})$ we gain:

$(-\log q)\exp(\alpha_0 + \alpha_1 d_{BMR}) = (1 - h)(-\log q)\exp(\alpha_0)$

$\Leftrightarrow (-\log q)\exp(\alpha_0)\exp(\alpha_1 d_{BMR}) = (1 - h)(-\log q)\exp(\alpha_0)$

$\Leftrightarrow \exp(\alpha_1 d_{BMR}) = (1 - h) \quad |\log$

$\Leftrightarrow \alpha_1 d_{BMR} = \log\big((1 - h)\big)$

$\Leftrightarrow d_{BMR} = \dfrac{\log(1 - h)}{\alpha_1}$

As already mentioned, the dose we determine through plugging in the value h for the BMR and the estimated values for α_0 and α_1 is the BMD.

Illustration: For our example data set (Tab.5.) we obtained the ML estimates $\alpha_0 = 3.3984$ and $\alpha_1 = -0.1956$. Thus we can easily calculate the estimated BMD for a pre-specified BMR.

For example: Let the BMR be a reduction of 5 weeks in median survival. Then the BMD is given by $d_{BMR} = \frac{\log((-\log 0.5)\exp(3.3984)-5)-\log(-\log 0.5)-3.3984}{-0.1956} \approx 1.41$.

iv. Calculation of BMDs for Weibull AFT models

Next, we present the formulas of the BMD for the different ways of defining the BMR, assuming a general Weibull model with three parameters α_0, α_1 and p. The calculation can be done analogously to the Exponential case.

a) Absolute value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = S(t^*, 0) - h$

The survival function $S(t^*, d_{BMR}) = \exp(-\exp(-p\,\alpha_0 - \alpha_1\, p\, d_{BMR})\,(t^*)^p)$ leads to

$$d_{BMR} = \frac{\log(-\log(\exp(-\exp(-p\,\alpha_0)\,(t^*)^p) - h)) + p\,\alpha_0 - p\log(t^*)}{-p\,\alpha_1}.$$

b) Relative value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = (1-h)\,S(t^*, 0)$

In this case, the BMD is given by

$$d_{BMR} = \frac{\log(-\log(1-h) + \exp(-p\,\alpha_0)\,(t^*)^p) - p\log(t^*) + p\,\alpha_0}{-p\,\alpha_1}.$$

c) Absolute value for the BMR as reduction in a specified quantile of survival, e.g. in median survival time: $t_{med}(d_{BMR}) = t_{med}(0) - h$

Using $t_{med}(d_{BMR}) = (-\log 0.5)^{1/p}\exp(\alpha_0 + \alpha_1 d_{BMR})$, which holds because

$S(t, d_{BMR}) = \exp(-\exp(-p\ \alpha_0 - \alpha_1\ p\ d_{BMR})\ t^p)$

$\Longrightarrow t = (-\log S(t))^{1/p} * \frac{1}{\lambda^{1/p}}$, $\frac{1}{\lambda^{1/p}} = \exp(\alpha_0 + \alpha_1 d_{BMR})$,

we gain

$$d_{BMR} = \frac{\log\left((-\log 0.5)^{1/p} \exp(\alpha_0) - h\right) - \frac{1}{p}\log(-\log 0.5) - \alpha_0}{\alpha_1}.$$

d) Relative value for the BMR as reduction in a specified quantile of survival: $t_q(d_{BMR}) = (1-h)\ t_q(0)$

With $t_q(d_{BMR}) = (-\log q)^{1/p} \exp(\alpha_0 + \alpha_1 d_{BMR})$ we obtain

$$d_{BMR} = \frac{\log(1-h)}{\alpha_1}.$$

Example: For our example dataset (Tab.5) we obtain for a BMR of 5 weeks loss in median survival the BMD

$$d_{BMR} = \frac{\log\left((-\log 0.5)^{\frac{1}{0.9571}} \exp(3.3803) - 5\right) - \frac{1}{0.9571}\log(-\log 0.5) - 3.3803}{-0.1948}$$

$$\approx 1.474.$$

This estimate of the BMD is slightly higher than the estimate gained via the Exponential model. Because of the additional parameter, the Weibull model is more flexible and leads to a more appropriate estimate. As risk assessment has to be done always very cautiously it is an appeasing result that the less adequate model leads to a smaller estimate of the BMD. The

very small difference can be explained by the estimated value of the shape parameter p of the Weibull model, which is almost equal to 1 ($p \approx 0.96$).

3.3.2. Estimation of the BMDL

Whereas the determination of the MLEs for the unknown parameters of the models and thereupon the calculation of the BMD is rather straightforward, the estimation of a lower confidence limit is more complicated. In general, three different methods to calculate confidence intervals for the benchmark dose in risk analysis are often discussed in the literature: Beneath the method we will suggest (profile likelihood ratio method), the delta and bootstrap method occur. Moerbeek and collaborators compared these methods in fitting non-linear dose response models for continuous, ordinal and quantal data. They recommend the likelihood ratio method because it is less time consuming than the bootstrap method and leads to similar results. The delta method appeared to be unreliable for nonlinear dose response models because it led to different and usually narrower intervals compared to the other methods [MOERBEEK (2004)].

We suggest parameterizing the model in a way that the dose itself becomes a parameter and determine a so called *profile likelihood confidence interval* for it. Therefore we have to re-parameterize our model in order to include the BMD as a parameter itself. The idea is – after having defined a BMR – solving the formula of the dose found in the last section for a parameter of the original parameterized model (e.g. α_1) and plug it in. The following explanation is oriented on [HELD (2008)], [SERFLING (1980)], [PAWITAN (2001)] and [BARNDORFF-NIELSON (1994)].

After having introduced the concept of profile likelihood confidence intervals, we will again illustrate the approach for the Exponential case and have a look at the Weibull case afterwards, using the example from above.

i. Introduction to profile likelihood confidence intervals

In order to find a confidence interval for a parameter in a single parameter model the so called *likelihood ratio statistic* is often used. Its definition is based on the relative likelihood:

Definition: Let $L(\theta, x) = \prod_{i=1}^{n} f(x_i, \theta)$, $\theta \in \Theta \subset \mathbb{R}$ be the likelihood function of a sample. The *relative likelihood* is defined to be

$$\tilde{L}(\theta) = \frac{L(\theta)}{L(\hat{\theta}_{ML})}$$

and the relative log likelihood is $\tilde{l}(\theta) = l(\theta) - l(\hat{\theta}_{ML})$.

The construction of a confidence interval using the *likelihood ratio statistic* $W = -2\,\tilde{l}(\theta)$ is based on the following insight, which holds under regularity conditions [SCHIPP (2008)]:

$(R1)$ The parameter space Θ is an open subset of $\mathbb{R}$,
$(R2)$ the support of the distribution $\mathcal{X} := \{x: f(x, \theta) > 0\}$ does not depend on θ,
$(R3)$ $\frac{d \log f(x,\theta)}{d\theta}$ exists and is finite for $\theta \in \Theta$ and $x \in \mathcal{X}$,
$(R4)$ differentiation and integration are commutable for a function $h(x)$, i.e.

$$\frac{d}{d\theta} \left(\int \dots \int h(x) f(x, \theta)\, dx_1 \dots dx_n \right) = \int \dots \int h(x) \left[\frac{d}{d\theta} f(x, \theta) \right] dx_1 \dots dx_n < \infty.$$

(These conditions guarantee the existence of the *expected Fisher information matrix* $J(\theta) = E(I(\theta))$.)

In order to avoid "pathological cases" and prove the asymptotical properties of ML estimates we need some additional conditions:

$(R5)$ $L(., x)\colon \Theta \to [0, \infty)$ is continuous for all $\theta \in \Theta$,
$(R6)$ for all $\theta_1 \neq \theta_2 \in \Theta$ it holds $f(x, \theta_1) \neq f(x, \theta_2)$,
$(R7)$ $E(\log f(X, \theta))$ exists,
$(R8)$ $\frac{1}{n} \log L(\theta, x) \xrightarrow{a.s.} E(\log f(X, \theta)) \;\; \forall\, \theta \in \Theta$,
$(R9)$ $\log L(\theta, x)$ is twice differentiable in an open interval around θ.

A very important result is the asymptotic normality of the ML estimate $\hat{\theta}_{ML}$

$$\hat{\theta}_{ML} \overset{a}{\sim} \mathcal{N}\left(\theta, \left[I\left(\hat{\theta}_{ML}\right)\right]^{-1}\right).$$

A proof can be found e.g. in [SERFLING (1980), p.144-148] or in [HELD (2008), p.79-81].

Theorem: Under assumption of the regularity conditions listed above

$$-2\,\tilde{l}(\theta) \xrightarrow{d} \chi^2(1).$$

Proof (outline): A Taylor expansion of second order of $l(\theta)$ at $\hat{\theta}_{ML}$ leads to

$$l(\theta) \approx l\left(\hat{\theta}_{ML}\right) + \underbrace{\frac{d\,l\left(\hat{\theta}_{ML}\right)}{d\theta}}_{=0}\left(\theta - \hat{\theta}_{ML}\right) + \frac{1}{2}\underbrace{\frac{d^2\,l\left(\hat{\theta}_{ML}\right)}{d\theta^2}}_{=-I(\hat{\theta}_{ML})}\left(\theta - \hat{\theta}_{ML}\right)^2$$

$$\Rightarrow\ \tilde{l}(\theta) = l(\theta) - l\left(\hat{\theta}_{ML}\right) \approx -\frac{1}{2}\,I(\hat{\theta}_{ML})\,\left(\theta - \hat{\theta}_{ML}\right)^2$$

$$\Rightarrow\ -2\tilde{l}(\theta) = 2\left(l\left(\hat{\theta}_{ML}\right) - l(\theta)\right) \approx\ I(\hat{\theta}_{ML})\,\left(\theta - \hat{\theta}_{ML}\right)^2$$

Because $\hat{\theta}_{ML} \overset{a}{\sim} \mathcal{N}(\theta, [I(\hat{\theta}_{ML})]^{-1})$ it follows $I\left(\hat{\theta}_{ML}\right)(\theta - \hat{\theta}_{ML})^2 \overset{a}{\sim} \chi^2(1)$. ■

This theorem can be generalized for the multiparameter case and it can be shown that under generalized regularity conditions the likelihood ratio statistic is asymptotically $\chi^2(k)$ distributed, where *k* denotes the number of parameters that are to estimate. The proof is given e.g. in [SERFLING (1980),154-155].

Likelihood ratio test. The likelihood ratio test can be used to find the optimal model from a family of nested models. If the inclusion of more parameters does not lead to a significantly better fit, the simpler model with fewer parameters is chosen. The likelihood ratio test is based on the insight that minus twice the difference of the log likelihood values associated with two nested models ('generalized likelihood ratio statistic') follows a Chi-square distribution with *k* parameters, where *k* is equal to the difference in number of parameters between the two models [EFSA (2009), 30-31, HELD (2008), 125-126]. In our case, this leads to

$$W = -2\,\frac{\max L_{exp}(\alpha_0, \alpha_1)}{\max L_{weib}(\alpha_0, \alpha_1, p)} = -2\left(l_{exp}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML}) - l_{weib}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML}, \hat{p}_{ML})\right)$$

$$W \overset{a}{\sim} \chi^2(3-2) = \chi^2(1).$$

Now we return to the introduction of profile likelihood confidence intervals. When we use a multi parameter model, it is not unusual, that we are really interested only in a subset of

parameters or even in only one. But even if we are interested in several parameters, it is always easier to describe one parameter at a time. Therefore we need a method to 'concentrate' the likelihood on a single parameter by eliminating the other parameter(s), the so called nuisance parameter(s).

The approach to eliminate the nuisance parameter that we will use is the following: Replace the nuisance parameter by its ML estimate at each fixed value of the parameter of interest. The resulting likelihood is called the *profile likelihood* [PAWITAN (2001), p.61-67,256-259] / [HELD (2008),p.112-138]:

Definition: Let (θ, η) be the full parameter and θ the parameter of interest. Given the joint likelihood $L(\theta, \eta)$ the *profile likelihood* of θ is

$$L_p(\theta) = \max_{\eta} L(\theta, \eta) = L\big(\theta, \hat{\eta}_{ML}(\theta)\big),$$

Where the maximization is performed at fixed value of θ.

The easiest way to obtain the profile likelihood is to solve the score equation $\frac{\partial L(\theta,\eta)}{\partial \eta} = 0$ separately for the nuisance parameter in order to gain $\hat{\eta}(\theta)$ and then plug it in in the joint likelihood $L(\theta, \eta)$. But unfortunately that is not always possible and therefore the profile likelihood can often be only determined numerically.

Our goal was to find a confidence interval for a parameter of our model. When the parameter of interest θ is scalar, we can use the so called *relative profile likelihood*, which is defined to be $\tilde{L}_p(\theta) := \frac{L_p(\theta)}{L_p(\hat{\theta}_{ML})}$.

Thus, the *relative profile log likelihood* has the form the $\tilde{l}_p(\theta) := l_p(\theta) - l_p(\hat{\theta}_{ML})$.

Under assumption of generalized regularity conditions it can be shown that also the distribution of $-2\,\tilde{l}_p$ converges for $n \to \infty$ to a χ^2 distribution with k degrees of freedom, where k denotes the number of parameters that are of interest, i.e. the number of all parameters minus the number of nuisance parameters. The proof can be found in [SERFLING (1980), p.156-160]. Because we are only interested in one parameter (the BMD), we have $k = 1$.

Therefore, the set

$$\left\{\theta: \tilde{l}_p(\theta) \geq -\frac{1}{2}\,\chi^2_{1-\alpha}(1)\right\}$$

builds an approximate $(1-\alpha)$ confidence interval for θ.

Note, that this confidence interval is a two-sided one. In order to gain the lower bound of a one-sided confidence interval of the form $[c_L, \infty)$ we use the $\chi^2_{1-2\alpha}(1)$ quantile because we have to bring the probability mass of the critical area on one side only. A one-sided lower confidence limit c_L equals

$$\min\left\{\theta: \tilde{l}_p(\theta) = -\frac{1}{2}\,\chi^2_{1-2\alpha}(1) =: c\right\} \qquad (2.4.)$$

The (rounded) values for c, given a level α, are summarized in the following table. The calculations were done with R.

α	c
0.1	-0.82
0.05	-1.35
0.01	-2.71

Tab.6. Profile Likelihood confidence intervals: values of c *in* (2.4.) *for different levels of* α.

ii. BMDL for Exponential AFT models

Now we solve the formula of the dose found in the last section for α_1 and plug it in in our model. Again, we carry out these calculations for the four possibilities of defining the BMR:

a) Absolute value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = S(t^*, 0) - h$

$$d_{BMR} = \frac{\log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h)) - \log(t^*) + \alpha_0}{-\alpha_1}$$

$$\Leftrightarrow \alpha_1 = \frac{\log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h)) - \log(t^*) + \alpha_0}{-d_{BMR}}$$

When plugging in this formula for α_1 in the survival function we obtain:

$$S(t, d) = \exp\left(-\exp\left(-\alpha_0 - \frac{\log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log(-\log 0.5) - \alpha_0}{-d_{BMR}} d\right) t\right)$$

The accompanying log likelihood is given by

$$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i \delta_i\right)\frac{\log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h)) - \log(t^*) + \alpha_0}{-d_{BMR}}$$
$$-\sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(-\log(\exp(-\exp(-\alpha_0)\, t^*) - h)) - \log(t^*) + \alpha_0}{-d_{BMR}} d_i\right)$$

Using this parameterization we can obtain both the ML estimate for the BMD and additionally the profile likelihood BMDL.

The value for the BMD gained through ML estimation is of course the same as if it is determined the way presented in the last section. The reason is the invariance property of Maximum Likelihood Estimates [PAWITAN (2001), p.45].

b) Relative value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = (1-h)\, S(t^*, 0)$

Here, we have

$$\alpha_1 = \frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}}$$

and the log likelihood has the form

$$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i \delta_i\right)\frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}}$$
$$-\sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}} d_i\right).$$

c) Absolute value for the BMR as reduction in a specified quantile of survival, e.g. in median survival time: $t_{med}(d_{BMR}) = t_{med}(0) - h$

For this manner of specifying the BMR, we have

$$\alpha_1 = \frac{\log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log(-\log 0.5) - \alpha_0}{d_{BMR}}$$

and thus the log likelihood

$$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i \delta_i\right)\frac{\log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log(-\log 0.5) - \alpha_0}{d_{BMR}}$$
$$-\sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log\big((-\log 0.5)\exp(\alpha_0) - h\big) - \log(-\log 0.5) - \alpha_0}{d_{BMR}} d_i\right).$$

d) Relative value for the BMR as reduction in a specified quantile of survival: $t_q(d_{BMR}) = (1-h)\, t_q(0)$

Finally, in this case

$$\alpha_1 = \frac{\log(1-h)}{d_{BMR}}$$

leads to the log likelihood

$$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i\delta_i\right)\frac{\log(1-h)}{d_{BMR}} - \sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(1-h)}{d_{BMR}} d_i\right).$$

Example: We again use the example data set of Tab.5 and define the BMR as reduction of 5 weeks in median survival. The ML estimates for the re-parameterized function is calculated with the R procedure `nlm`.

```
> rm(list=ls())
>     f=function(x){10*x[1]+43*(log((-log(0.5))*exp(x[1])-5)-log(-log(0.5))-
x[1])/x[2]+95*exp(-x[1])+69*exp(-x[1]-(log((-log(0.5))*exp(x[1])-5)-log(-
log(0.5))-x[1])/x[2])+17*exp(-x[1]-(log((-log(0.5))*exp(x[1])-5)-log(-
log(0.5))-x[1])/x[2]*10)}
> nlm(f,c(3,3))
$minimum
[1] 35.57158

$estimate
[1] 3.398365 1.410442
```

$=>\ \alpha_0 = 3.3984,\ \ BMD = 1.4104.$

Both approaches lead to the same estimates for α_0 and BMD. In order to ensure that these estimates maximize the log likelihood we provide the following contour plot.

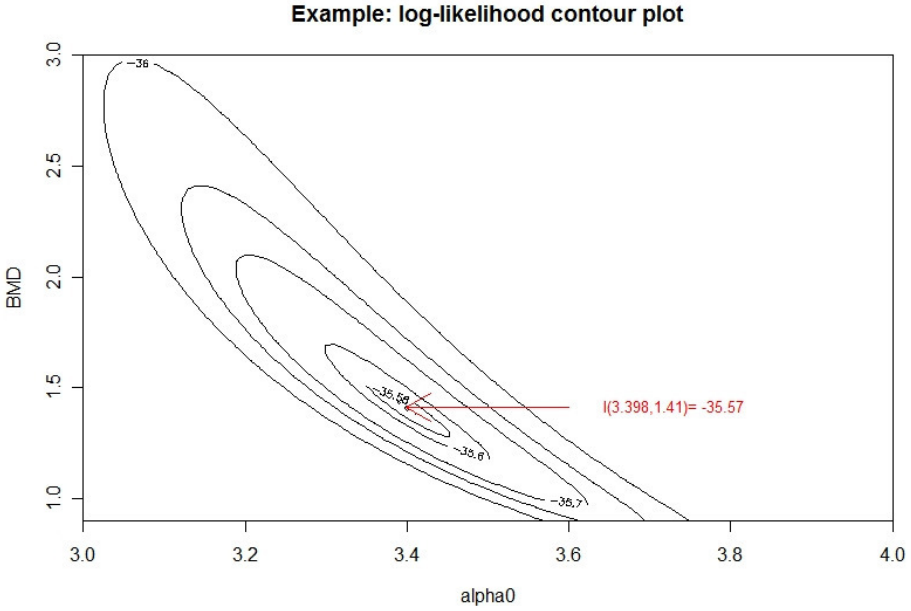


Fig.12. Contour plot of the log-likelihood function of the Exponential model, BMR(abs.) h=5: The log-likelihood function has a maximum of -35.57 *for the estimates* $\alpha_0 = 3.3984$, $BMD = 1.4104$ *for a BMR defined as reduction of 5 weeks in median survival. The R code can be found in the appendix.*

With this re-parameterized log likelihood function, which includes the BMD as a parameter itself, we can also determine the 95% lower bound of the profile likelihood confidence interval for the BMD:

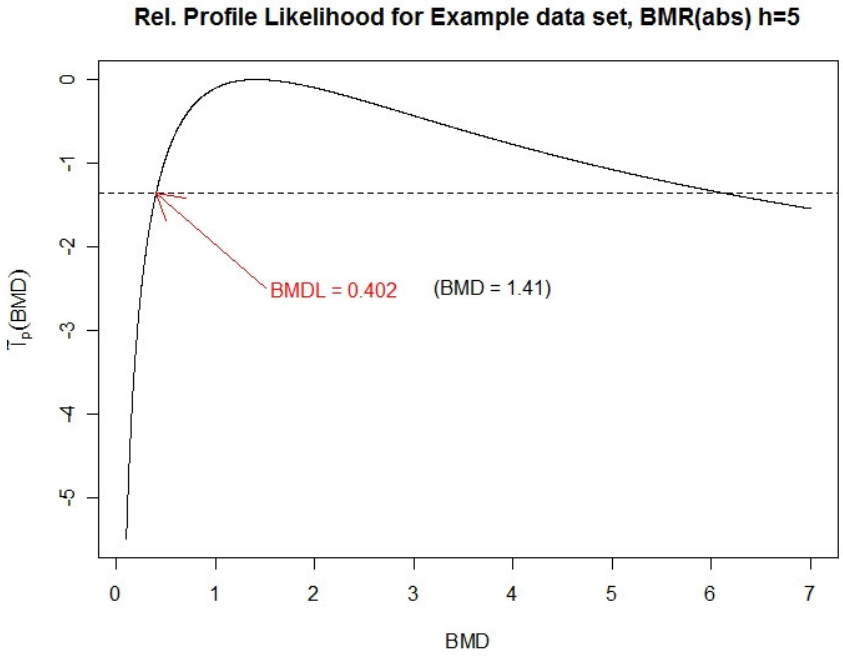


Fig.13. Relative Profile Likelihood for Example data set (Exponential), BMR(abs.) h=5: The BMDL –estimated via the profile likelihood method outlined before – equals $BMDL = 0.402$. *The dashed horizontal lines in the plots of the profile likelihood functions indicate the value of* $-\frac{1}{2}\chi^2_{0.9}(1) \approx$ -1.35. *The BMR was defined as a reduction of 5 weeks in median survival. The R code can be found in the appendix.*

Next, a second illustration is provided, using another specification of the BMR. For a BMR defined in relative terms (10% loss in every quantile of survival) we get the estimates $\alpha_0 = 3.3984,\ \ BMD = 0.539$. The calculation of these estimates was done analogously to the example above, using the appropriate re-parameterized log likelihood function for this case. A log likelihood contour plot is given below.

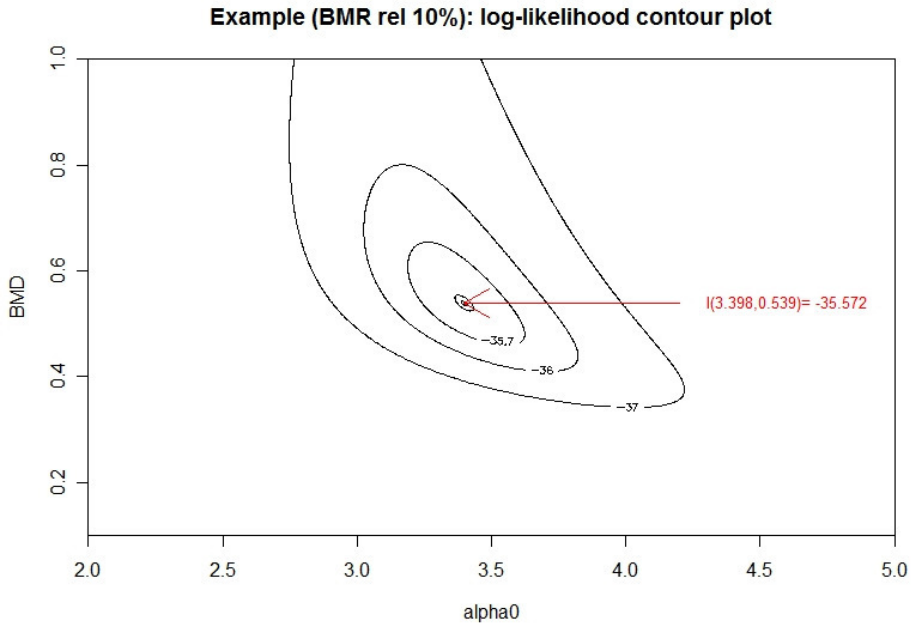


Fig.14. Contour plot of the log-likelihood function of the Exponential model for the Example data set, BMR(rel.) h=0.1. The BMR was defined as 10% loss in every quantile of survival.

The BMDL is estimated by $BMDL = 0.344$.

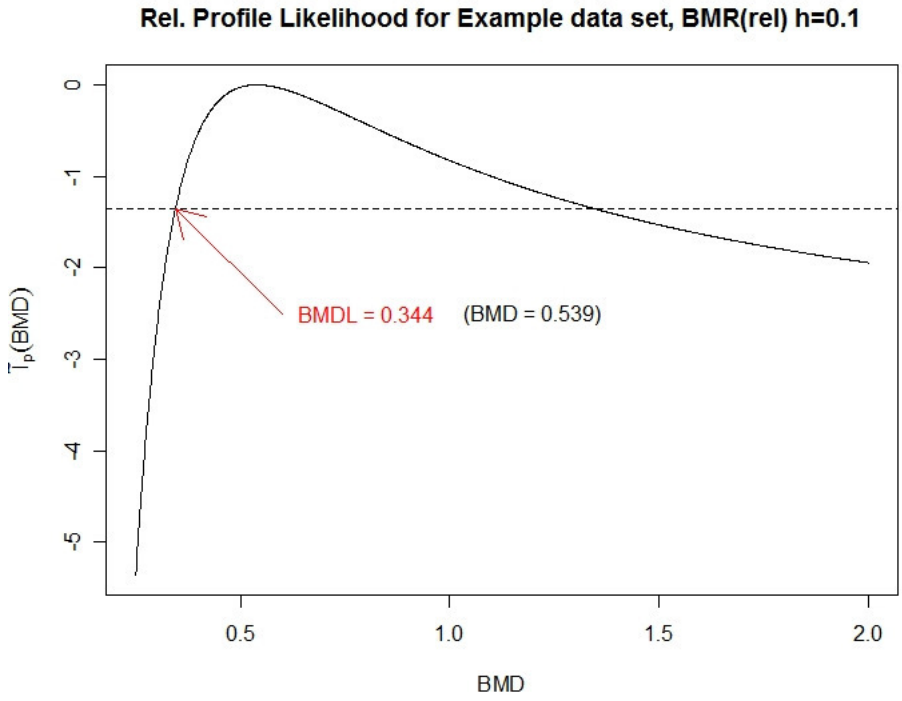


Fig.15. Relative Profile Likelihood for Example data set (Exponential), BMR(rel.) h=0.1: The BMDL for a BMR defined as 10% loss in every quantile of survival is given by $BMDL = 0.344$. *The R code can be found in the appendix.*

iii. BMDL for Weibull AFT models

Because the proceeding is analogous to the Exponential case, we will only list the formulas for α_1 for each specification of the BMR. This formula has to be plugged in in the log likelihood

$$l(p, \alpha_0, \alpha_1) = \left(\sum_{i=1}^{k} \delta_i\right)(-p\ \alpha_0) + \left(\sum_{i=1}^{k} d_i \delta_i\right)(-p\ \alpha_1)$$
$$- \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i} (x_{ij})^p\right) \exp(-p\ \alpha_0 - p\ \alpha_1\ d_i) + \left(\sum_{i=1}^{k} \delta_i\right) \log(p)$$
$$+ \quad (p-1) \sum_{i=1}^{k} \sum_{j=1}^{n_i} \delta_{ij} \log(x_{ij})$$

a) Absolute value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = S(t^*, 0) - h$

$$\alpha_1 = \frac{\log(-\log(\exp(-\exp(-p\,\alpha_0)\,(t^*)^p) - h)) + p\,\alpha_0 - p\log(t^*)}{-p\,d_{BMR}}$$

b) Relative value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = (1-h)\,S(t^*, 0)$

$$\alpha_1 = \frac{\log(-\log(1-h) + \exp(-p\,\alpha_0)\,(t^*)^p) - p\log(t^*) + p\,\alpha_0}{-p\,d_{BMR}}$$

c) Absolute value for the BMR as reduction in a specified quantile of survival, e.g. in median survival time: $t_{med}(d_{BMR}) = t_{med}(0) - h$

$$\alpha_1 = \frac{\log\left((-\log 0.5)^{1/p}\exp(\alpha_0) - h\right) - \frac{1}{\mathrm{p}}\log(-\log 0.5) - \alpha_0}{d_{BMR}}$$

d) Relative value for the BMR as reduction in a specified quantile of survival: $t_q(d_{BMR}) = (1-h)\,t_q(0)$

$$\alpha_1 = \frac{\log(1-h)}{d_{BMR}}$$

The re-parameterized Exponential and Weibull log likelihood functions for each discussed definition of the BMR can be found in the appendix.

Example: Again, we use the example dataset and state the BMR as 5 weeks loss in median survival. The ML estimates for α_0, BMD and p are determined via minimizing the negative re-parameterized log likelihood with the `nlm` procedure in R.

```
> rm(list=ls())
> library(splines)
> library(survival)
>
>
> bsp<-read.table("bsp.txt",header=T)
> attach(bsp)

> #Tools for loglikelihood
> delta<-sum(status)
> delta
[1] 10
>
> deltad<-sum(status*dose)
> deltad
```

```
[1] 43
> #BMR(absolute value)
> h<-5
> #x=(alpha0,BMD,p)
>
> f=function(x){delta*x[3]*x[1]+deltad*x[3]*((log(exp(x[1])*(-
log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2])+(sum(weeks[dose==0]^x[3]))*exp(-
x[3]*x[1])+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-
x[3]*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-
x[3]*10*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))-delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}
>
> nlm(f,c(4,1,1))
$minimum
[1] 35.55586

$estimate
[1] 3.3803322 1.4739423 0.9571154
```

$\Rightarrow \alpha_0 = 3.3803,\ BMD = 1.4794,\ p = 0.9571$

The relative profile likelihood which is used for the determination of the BMDL in that case is plotted in the following graphic. The BMDL is estimated by $BMDL = 0.393$.

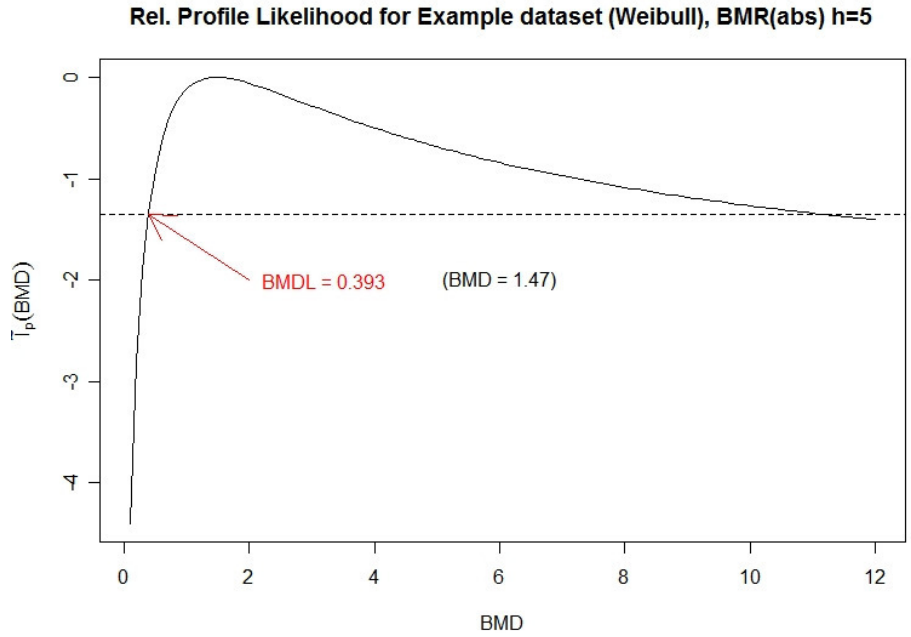


Fig.16. Relative Profile Likelihood for Example data set (Weibull), BMR(abs.) h=5: If a Weibull model is fitted to the example data set, we get the estimates $BMD = 1.47$ and $BMDL = 0.393$ for a BMR defined as 5 weeks loss in median survival. The R code can be found in the appendix.

If we define the BMR in relative terms, e.g. 10% loss in every quantale of survival, we get the following estimates: $\alpha_0 = 3.3803,\ \ BMD = 0.5409,\ p = 0.9571.$ The relative profile likelihood is shown in the following figure. The estimate for the BMDL is $BMDL = 0.33$.

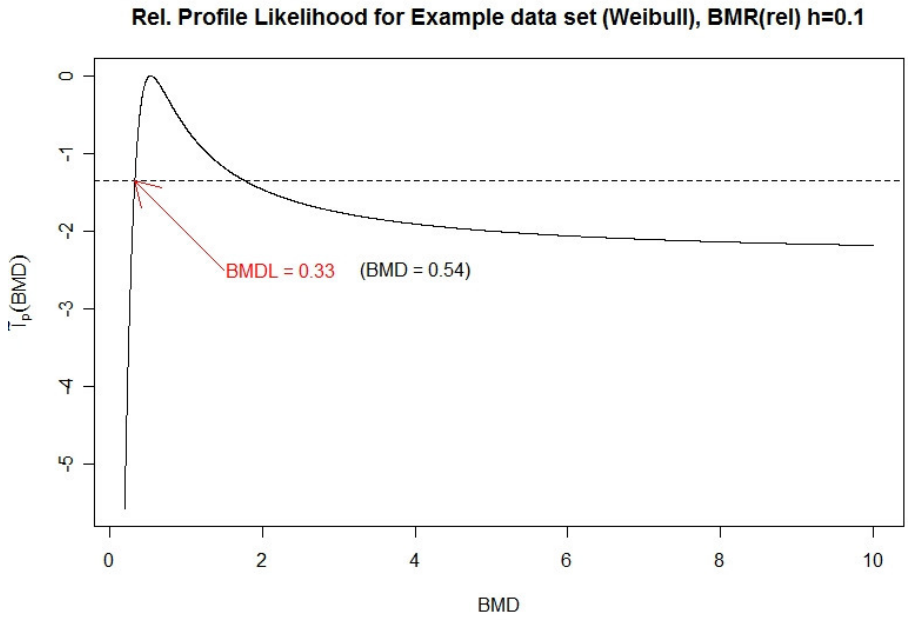


Fig.17. Relative Profile Likelihood for Example data set (Weibull), BMR(rel.) h=0.1: For a BMR defined as 10% loss in every quantile of survival we get the estimates $BMD = 0.54$ *and* $BMDL = 0.33$ *for a Weibull model with parameters* $\alpha_0 = 3.3803,\ BMD = 0.5409,\ p = 0.957$. *For the R code see the appendix.*

Part II: Application to experimental and simulated data

In this section the proceeding suggested in Part I will be applied to experimental data and afterwards the results will be compared with values obtained using dichotomous models. Furthermore, a simulation study will be presented that tries to evaluate the influence of censorings on the coverage probability of the one-sided profile likelihood confidence intervals. Finally, the estimates from the Urethane study are compared with those published in the literature.

4. The Urethane study

At first we apply the methods developed in Part II on data from a dose-response study in animals performed by Schmähl, Port and Wahrendorf [SCHMÄHL (1977)] when investigating urethane induced carcinogenesis. We used this experiment as application because the data were as individual data on hand, the investigation seemed to be appropriate for our approach and the study was to our knowledge totally completed with no ongoing follow up investigations after that publication.

Data source: Personal communication of the data by Dr. R. Port to Dr. L. Edler when Dr. Port was employed at the DKFZ in the department of Prof. Dr. W. Kunz.

4.1. Some background information on Urethane

Urethane (ethyl carbamate), the ethyl ester of carbamic acid, occurs in spirituous beverages and fermented food such as bread (<1 – 8 µg/l), beer (0.3 – 18 µg/l), soy sauce (<1-95 µg/l), wine (<1 – 110 µg/l) and yoghurt (0 – 3 µg/l) [RÖMPP (2007)]. Especially stone fruits liqueurs can have very high contents of ethyl carbamat (100 – 200000 µg/l) because cyanide, which is contained in the stones naturally, reacts under light and in presence of ethanol to form ethyl carbamate. [RÖMPP (2007)]. Therefore, an attempt to reduce urethane in these beverages is the exclusion of light in bottle spirits. Another source for urethane is diethylpyrocarbonate, an inhibitor of fermentation used as an antimicrobial agent for the preservation of soft drinks (and until 1973 also for wine), which can form ethyl carbamate during its decomposition in the presence of ammonia [JECFA (2005), RÖMPP (2007), SCHMÄHL (1977)].

Urethane has also been used for medical and industrial purposes but at the present time the major route of exposure to urethane in the human population is through consumption of comestibles listed above [JECFA (2005), NTP (2004)].

Urethane is listed as "*possibly carcinogenic to humans*" (Group 2B) by the International Agency for Research on Cancer [IARC (1987)] and as "*reasonably anticipated to be a human carcinogen*" in the Report on Carcinogens of the US National Toxicology Program [NTP (2004)]. These assessments are based on the results of various animal studies on Urethane which could prove the substance to be genotoxic and a multisite carcinogen in the species tested. Nevertheless, no adequate human studies have been reported which investigated the relationship between exposure and human cancer [JECFA (2005), NTP (2004)].

In 1986 the Federal Republic of Germany adopted a guideline value for urethane of max. 0.4 ml/l in stone fruits liqueurs. On the European scale does not exist a guideline at present [Positionspapier (2006)].

4.2. Description of the study and summary of the data

The study on urethane of SCHMÄHL ET AL. (1977) consisted of a rat and a mouse experiment, both with five identical dose groups: 0, 100, 500, 2500 and 12500 µg/kg BW/day . Planned were 40 female and 40 male animals in each group. The animals were chronically fed with urethane in the drinking water during their life-time. Throughout the study, they were kept until spontaneous death, or killed when they became moribund. At the end of the study, the animals still alive were sacrificed (8% of the total effective number in the rat and 13% in the mouse experiment).

The rat experiment ended after 670 days in the groups receiving the three highest dosages, after 730 days in the 100 µg/kg BW/day group and after 680 days in the control group. The mouse experiment ended in the 12500 µg/kg BW/day group after 660 days, in the 2500, 500 and 100 µg/kg BW/day groups after 730 days and in the control group after 760 days.

For our analysis, we will only consider the malignant tumors and ignore the benign ones. They will be treated as "no (malignant) tumors".

The following table shows the numbers of benign and malignant tumors in the mouse and rat experiments per dose groups and therefore prepares a rough summary of the data. The data we had at our disposal slightly differ from the published ones in the number of animals and

tumors [SCHMÄHL (1977)]. Following the paper, in the rat experiment about 50% of the total effective number was lost between 350 and 450 days, most of them probably due to an unspecified virus infection.

Experiment	**Dose group** [µg/kg BW/day]	**Number of animals**	Number of animals with benign tumors	**Number of animals with malignant tumors**
Mouse male	0	42	2 (4.8%)	4 (9.5%)
	100	39	0	6 (15.4%)
	500	41	7 (17.1%)	3 (7.3%)
	2500	40	8 (20%)	9 (22.5%)
	12500	40	8 (20%)	14 (35%)
Mouse female	0	38	2 (7.9%)	3 (5.3%)
	100	41	7 (17.1%)	7 (17.1%)
	500	39	5 (12.8%)	13 (33.3%)
	2500	40	5 (12.5%)	12 (30%)
	12500	40	5 (12.5%)	18 (45%)
Rat male	0	40	1 (2.5%)	0
	100	40	0	1 (2.5%)
	500	40	0	2 (5%)
	2500	40	0	2 (5%)
	12500	40	2 (5%)	2 (5%)
Rat female	0	38	0	2 (5%)
	100	41	3 (7.5%)	1 (2.5%)
	500	39	2 (5%)	2 (5%)
	2500	40	4 (10%)	6 (15%)
	12500	40	4 (10%)	12 (30%)

Tab.7. Numbers of animals such as benign and malignant tumors in the mouse and rat experiments of Schmähl et al. (1977) per dose groups.

We can observe the trend that the number of malignant tumors increases with increasing doses. As a kind of qualitative pre-test we investigate the interrelationship between the dose and the effect (i.e. incidence of malignant tumors) using the Cochran-Armitage test for (linear) trend in proportions. The Cochran-Armitage test is a modified chi squared test, which incorporates a suspected order in the effect of one explanatory variable with k categories (in our case the dose) on a response variable with only 2 categories (in our case the presence or absence of a tumor) and tests for a trend among binomial proportions. The following passage is written on the basis of AGRESTI (2002) and GART (1986).

Summarized, the Cochran-Armitage test analyzes a $2 \times k$ contingency table with ordered columns:

Response	**Dose** 1	2	...	k	**Sum**
1 (e.g. tumor)	n_{11}	n_{12}		n_{1k}	n_{1+}
0 (e.g. no tumor)	n_{01}	n_{02}		n_{0k}	n_{0+}
Sum	n_{+1}	n_{+2}		n_{+k}	n

Let $\pi_{1|i}$ denote the real underlying probability of response **1** in column i and let $p_{1|i}$ denote the observed sample proportion of response **1**, $i = 1, \dots, k$. Let $\{x_i\}$ be scores assigned to the columns. In our case, for each column the numeric value of the dose group can be taken as score. The Cochran-Armitage test can be interpreted as score test in a logistic regression model:

For the linear probability model

$$\pi_{1|i} = \alpha + \beta x_i$$

the hypotheses are

$H_0: \pi_{1|1} = \pi_{1|2} = \dots = \pi_{1|k} \quad (<=> \beta = 0)$

(i.e. no linear trend in binomial proportions of response across increasing levels of dose)

versus the one-sided alternative

$H_1: \pi_{1|1} \leq \pi_{1|2} \leq \dots \leq \pi_{1|k}$ with at least one strict inequality

(i.e. linear trend in binomial proportions of response across increasing levels of dose).

The prediction equation under ordinary least squares is

$$\hat{\pi}_{1|i} = p + b(x_i - \bar{x}),$$

where $p = \frac{n_{1+}}{n}$ denotes the overall proportion of 'successes' (response= **1**), $\bar{x} = \frac{\sum n_{+i} x_i}{n}$ and $b = \frac{\sum n_{+i}(p_{1|i} - p)(x_i - \bar{x})}{\sum n_{+i} (x_i - \bar{x})^2}$ (standard formula for weighted regressions).

It can be shown that the test statistic for the Cochran – Armitage trend test

$$z^2 = \left(\frac{b^2}{p_{1+}(1-p_{1+})}\right)\sum n_{+i}\,(x_i - \bar{x})^2$$

has an asymptotic $\chi^2(1)$ distribution.

Note, that the Cochran-Armitage test has the same null hypothesis as the chi square test of independence but a narrower alternative and therefore uses a different test statistic. This test statistic is based upon Pearson's test statistic, which is partitioned into the test statistic z^2 shown above, which includes the assumption of $\beta = 0$ and is $\chi^2(1)$ distributed, and a second statistic, which only accounts for the residual variance (independent of $\beta = 0$ or $\beta \neq 0$) and is $\chi^2(I-2)$ distributed. The second statistic has one degree of freedom less than the original Pearson statistic which is due to the fact that β is estimated. The Cochran-Armitage test uses only that part of the original Pearson test statistic that tests the suspected linear trend.

For more mathematical details see COCHRAN (1954) and ARMITAGE (1955). If no trend could be observed, it would not be meaningful to fit a dose-response model. For our data, the Cochran-Armitage tests (with SAS 9.1.) support the trend hypothesis for all experimental groups except for Rat male.

Experiment	Statistic (Z)	Asymptotic Test One sided	Exact Test One sided
Mouse male	-3.4100	0.0003	6.865E-04
Mouse female	-3.1916	0.0007	0.0011
Rat male	-0.7459	0.2279	0.1835
Rat female	-4.3353	< 0.0001	4.201E-05

Tab.8. Results of the Cochran-Armitage trend tests (calculated with the proc freq procedure in SAS 9.1.). The small right sided p-values for all experiments except Rat male indicate that the probability of a tumor increases as dose increases.

The Kaplan Meier point estimates for the quartiles of survival time are summarized in the following table in order to prepare an overview of the observed survival times in the different dose groups. In each experimental group, we would expect a reduction in each quantile of survival with increasing doses. Due to the low incidence in some doses groups and, in particular, in the control group not all quantiles could be calculated. The female mice seem to provide the most useful data for a dose-response analysis.

Experiment	Dose group [µg/kg BW/day]	75% quartile of survival	50% quartile of survival (median)	25% quartile of survival
Mouse male	0	-	-	-
	100	680	-	-
	500	-	-	-
	2500	595	729	729
	12500	547	662	662
Mouse female	0	-	-	-
	100	667	730	-
	500	555	729	-
	2500	667	729	729
	12500	492	565	639
Rat male	0	-	-	-
	100	-	-	-
	500	-	-	658
	2500	605	605	605
	12500	-	-	-
Rat female	0	-	-	-
	100	-	-	-
	500	-	-	-
	2500	-	-	648
	12500	-	646	577

Tab.9. Kaplan Meier point estimates of quartiles of survival time for the mouse and rat experiments per dose groups.

The expectation of a dose related reduction in each quantile of survival seems to hold but actually only a few point estimates can be calculated. The explanation for that unfavorable result is the huge percentage of censoring in each group not allowing the calculation of the quantiles chosen above. The number and percentages of censorings, i.e. of animals not facing the event during the study period are summarized in the following table:

Experiment	Dose group [µg/kg BW/day]	Number of events (malignant tumors)	Number of censorings	Percentage of censoring [%]
Mouse male	0	4	38	90.48
	100	6	33	84.62
	500	3	38	92.68
	2500	9	31	77.5
	12500	14	26	65
Mouse female	0	3	35	92.11
	100	7	34	82.93
	500	13	26	66.67
	2500	12	28	70
	12500	18	22	55

Rat male	0	0	40	100
	100	1	39	97.5
	500	2	38	95
	2500	2	38	95
	12500	2	38	95
Rat female	0	2	38	95
	100	1	39	97.5
	500	2	38	95
	2500	6	34	85
	12500	12	28	70

Tab.10. Numbers of events and censorings per dose group in the four experiments.

After having summarized the data we will present the results of the modeling as described in part II. We fitted both, the Exponential and Weibull AFT/PH models and calculated values for the BMDs and BMDLs. The analysis was done for each of the four sub-experiments irrespective how good a dose-response was expressed. By this way we could also learn about the robustness of the model given data of different quality.

4.3. Fitting an Exponential AFT model

We fitted Exponential AFT/PH models and defined the BMRs as 25%, 10% and 5% relative loss in each quantile of survival. According to section 3.2.2. defining the BMR in terms of a relative reduction in any (and thus because of the AFT property in each) quantile of survival is most appropriate. The BMDs are calculated as the Maximum Likelihood estimates and the BMDLs, the lower (one-sided) 95% confidence limits, are calculated using the profile likelihood method. The analysis was done for each experiment: mouse male, mouse female, rat male und rat female. We describe the proceeding in detail for mouse male and show only the results for the other experiments in order to avoid redundancy.

4.3.1. Mouse male

The Kaplan-Meier curves per dose groups and the Log-log-plot take the following forms:

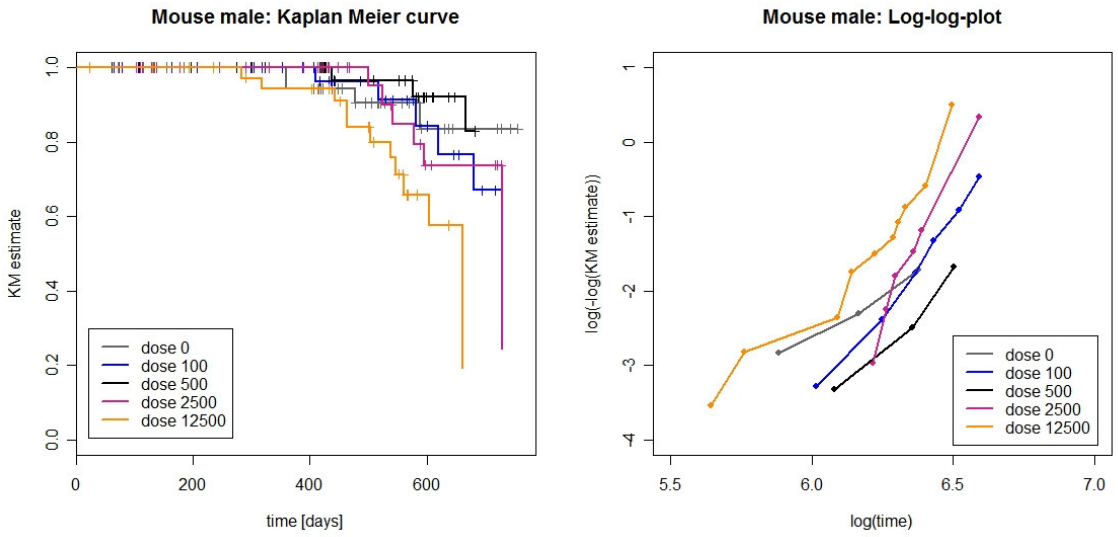


Fig.18. Kaplan-Meier curves and Log-log-plot for Mouse male.

The Weibull model assumes the Kaplan-Meier estimated survival curves to be of the same form and to be only 'stretched out' or contracted for different dose groups. The curves in the log-log plot should be parallel straight lines. The Exponential model assumes them additionally to have a slope equal to 1.

Although these assumptions seem not to be perfectly adequate they seem at least not fully unreasonable. The Kaplan-Meier estimated survival curves intersect but have rather similar forms. The figures which appear in the Log-log-plot are close to be parallel straight lines. One has to note that although the Cochran-Armitage trend test showed a significant dose-response

interrelationship, especially for the three lowest doses it seems not to be monotonic. But on the other hand, one should not forget that only a few events could be observed in these dose groups, which leads to additional uncertainty.

Thus it seems to be reasonable to apply a Weibull (and also an Exponential) model to these experimental data.

For an Exponential AFT/PH model we get the estimates $\alpha_0 = 8.274875$ and $\alpha_1 = -8.836129 * 10^{-05}$.

A comparison of the 'observed' (in the sense of estimated Kaplan-Meier survival curves) and 'expected' (in the sense of predicted by the fitted Exponential model) survival curves is performed graphically in the following figure. This model seems not to be appropriate because the fit is rather poor. (Therefore, we will also fit a Weibull model later.)

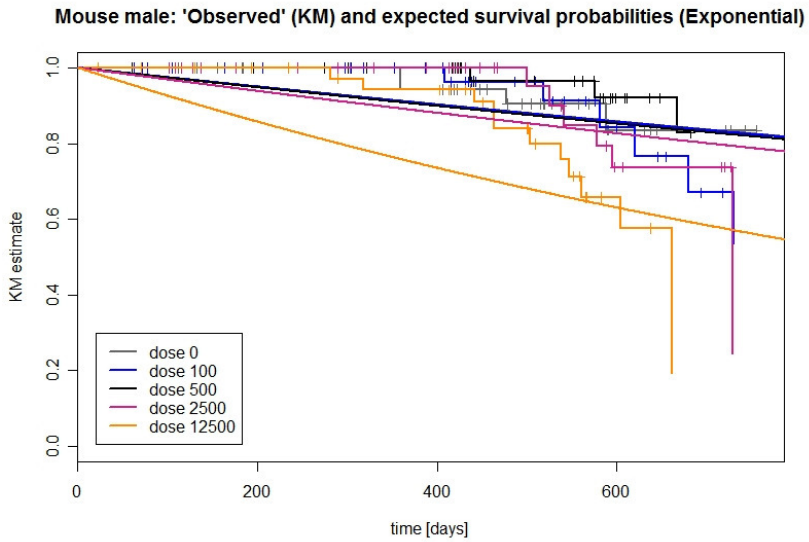


Fig.19. 'Observed' and expected survival probabilities (Exponential model) for Mouse male. The predicted survival curves nearly overlap for the three lowest dose groups. The estimates of survival probabilities are rough compared to the 'observed' ones.

Next we calculated the BMDs and BMDLs for three different (relative) BMRs: 25%, 10% and 5% loss in every quantile of survival (including the median). We used re-parameterized models with parameters α_0 and $d_{BMR} = BMD$. Additionally, we generated contour plots of the re-parameterized log-likelihood functions, which serve as a control of the ML estimates for α_0 and d_{BMR}. The profile likelihood function, which was used for the calculation of the BMDL, is also shown below. The BMDLs are determined with the formula

$$\min\left\{\theta: \tilde{l}_p(\theta) = -\frac{1}{2}\,\chi^2_{0.9}(1) \approx \text{-}1.352772\right\}.$$

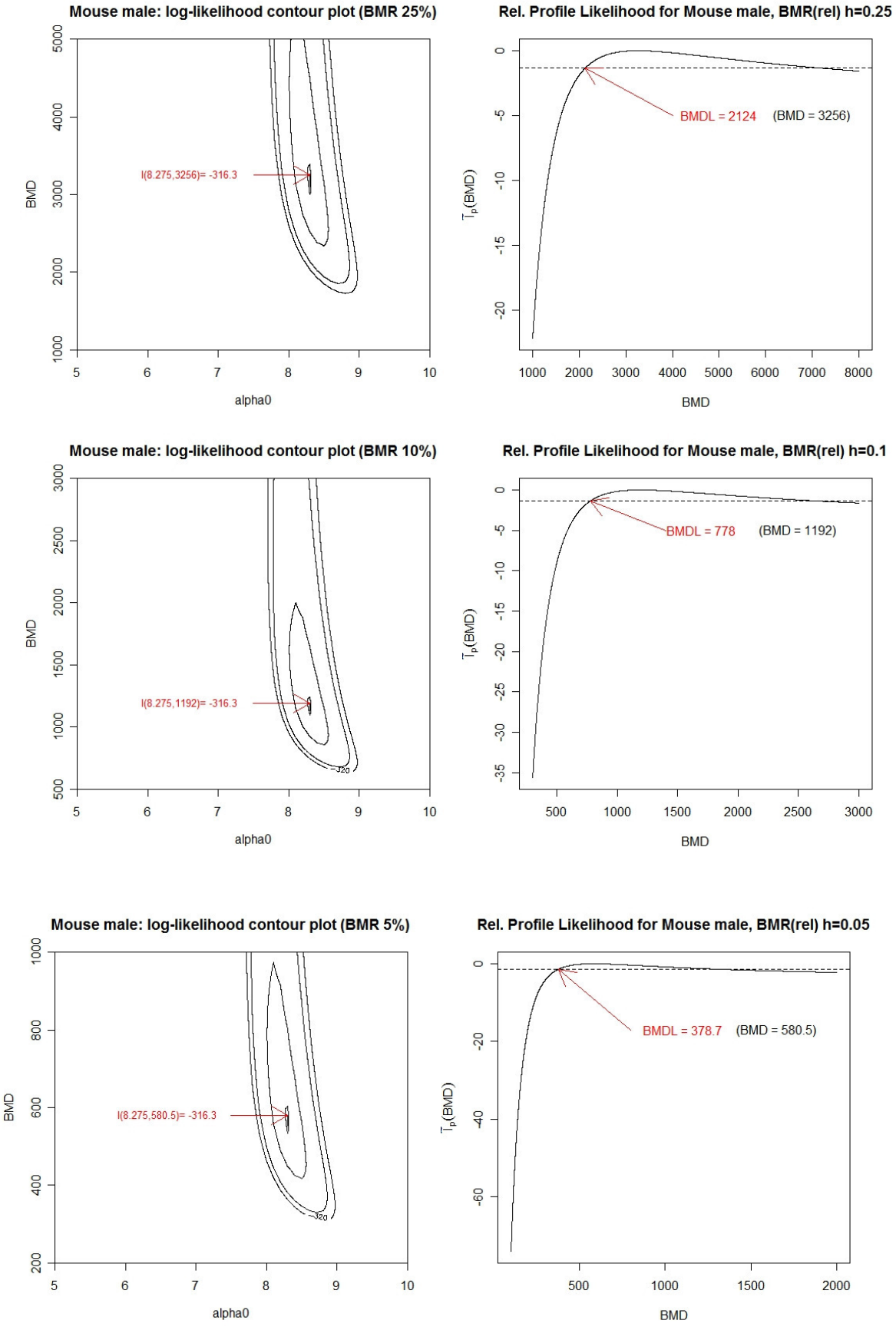


Fig.20. Log-likelihood contour plots and relative Profile Likelihood functions for different definitions of BMR for Mouse male. The dashed horizontal lines in the plots of the profile likelihood functions indicate the value of $-\frac{1}{2}\chi^2_{0.9}(1) \approx$ -1.35.

Note, that the shape of all curves shown above is identical, the different appearances are only caused by different scaling because of the different values for the BMR. We used the re-parameterized likelihood function for the calculations, which already includes the value chosen for the BMR and has the parameters α_0 and BMD. This explains the different regions for the "interesting" BMD-values in the contour plots. Of course, the maximum value of the Likelihood function is always the same.

The (rounded) estimates for the BMDs and BMDLs are summarized in the following table. The values for the BMDLs were about $2/3$ of the value for the BMDs:

BMR (rel.)	**BMD**	**BMDL**
25%	3256	2124
10%	1192	778
5%	580.5	378.7

Tab.11. Values for BMDs and BMDLs for different BMRs for the Mouse male experiment under fit of an Exponential model.

4.3.2. Mouse female

Because the proceeding is analogous to section 4.3.1. we summarize only the estimates for the BMDs and BMDLs for different specifications of the BMR. The estimates are a bit higher than those obtained for the Mouse male experiment but still in the same region of size.

BMR (rel.)	**BMD**	**BMDL**
25%	3721	2465
10%	1363	902.8
5%	663.4	439.5

Tab.12. Values for BMDs and BMDLs for different BMRs for the Mouse female experiment under fit of an Exponential model.

The Kaplan-Meier curves and Log-log-plot are shown in the following figure.

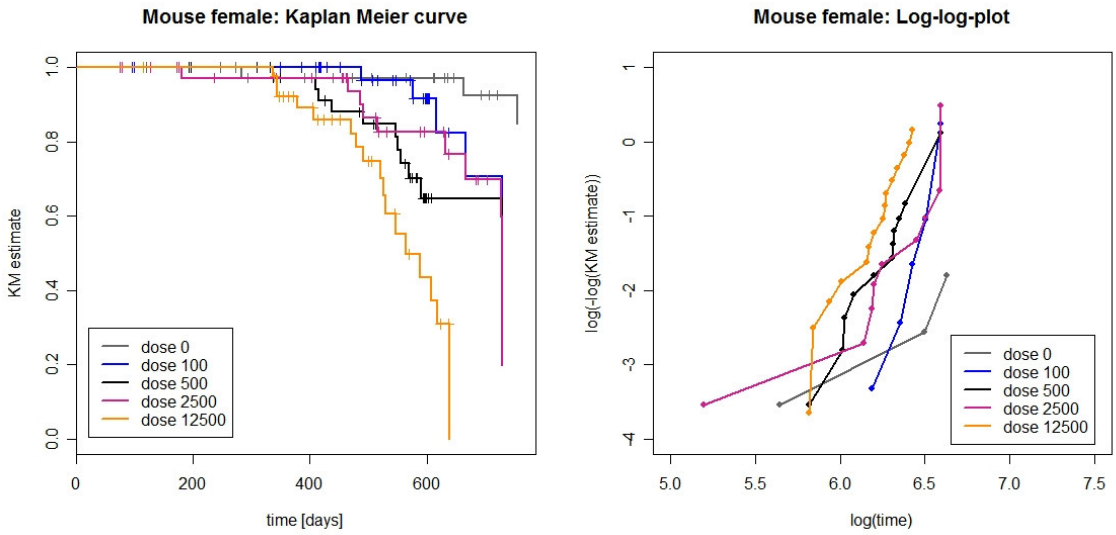


Fig.21. Kaplan-Meier curves and Log-log-plot for Mouse female. A Weibull (or Exponential) AFT/PH model seems not to be appropriate.

Fitting an Exponential model leads to the following estimates: $\alpha_0 = 7.884931$ and $\alpha_1 = -7.732023 * 10^{-05}$.

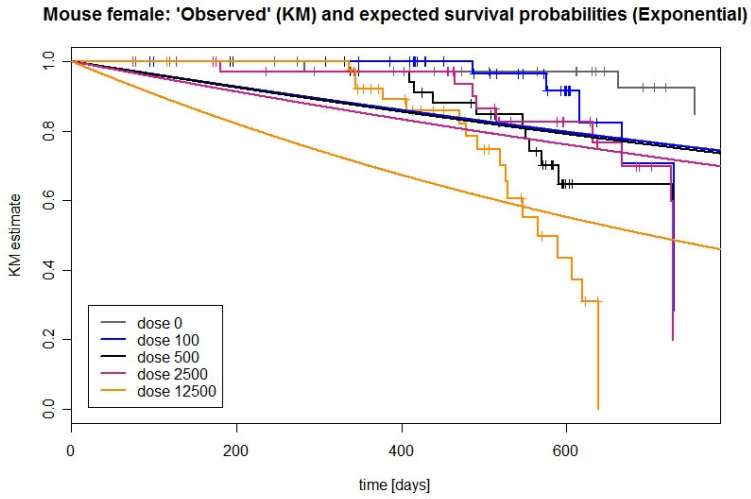


Fig.22. 'Observed' and expected survival probabilities (Exponential model) for Mouse female.

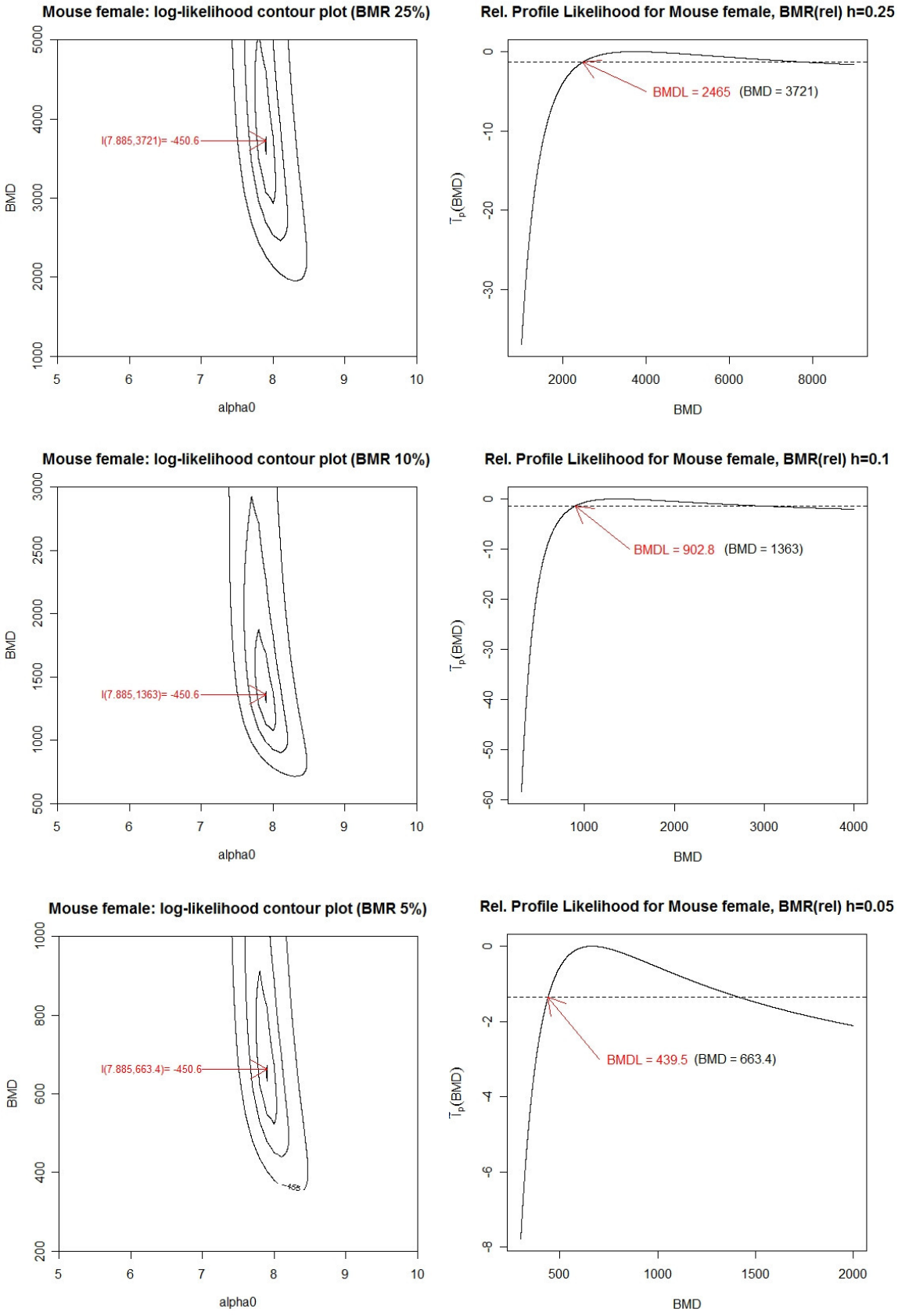


Fig.23. Log-likelihood contour plots and relative Profile Likelihood functions for different definitions of BMR for Mouse female.

4.3.2. Rat male

Kaplan-Meier curves and Log-log-plot:

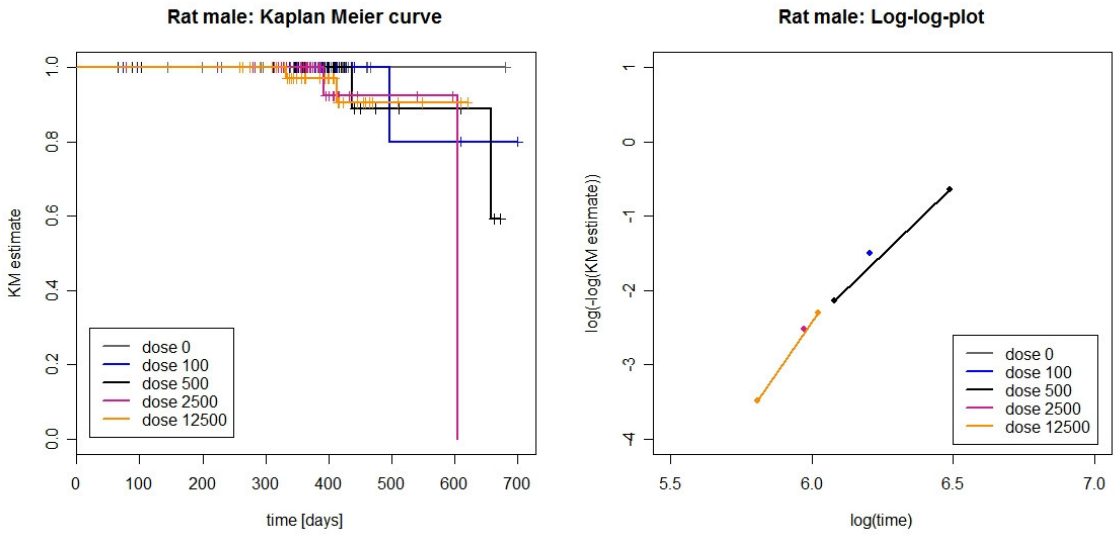


Fig.24. Kaplan-Meier curves and Log-log-plot for Rat male. A Weibull (or Expoential) AFT/PH model seems not to be appropriate.

Fitting an Exponential model leads to the following estimates: $\alpha_0 = 9.482464$ and $\alpha_1 = -4.793905 * 10^{-05}$.

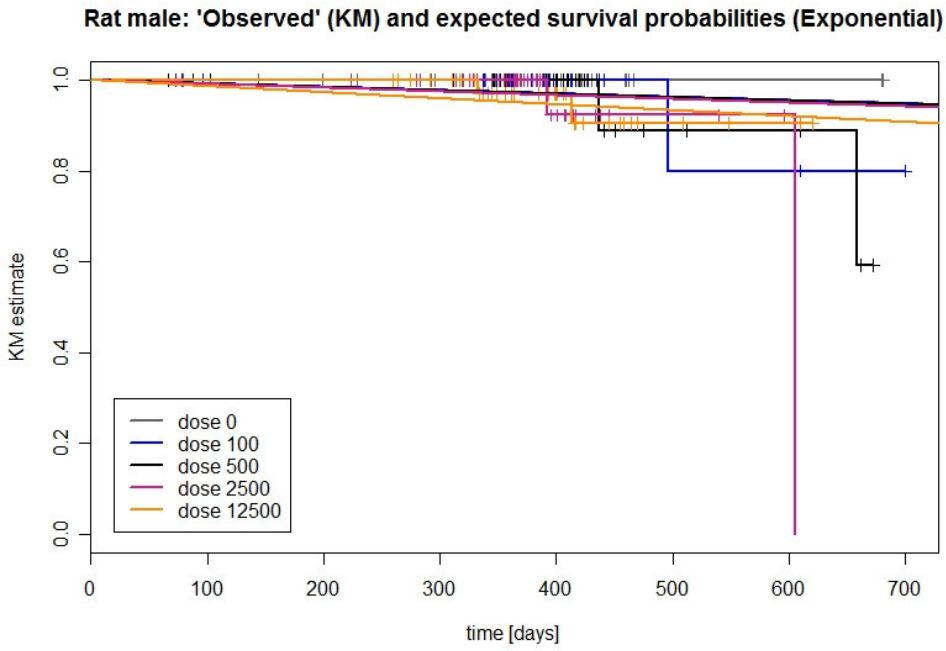


Fig.25. 'Observed' and expected survival probabilities (Exponential model) for Rat male.

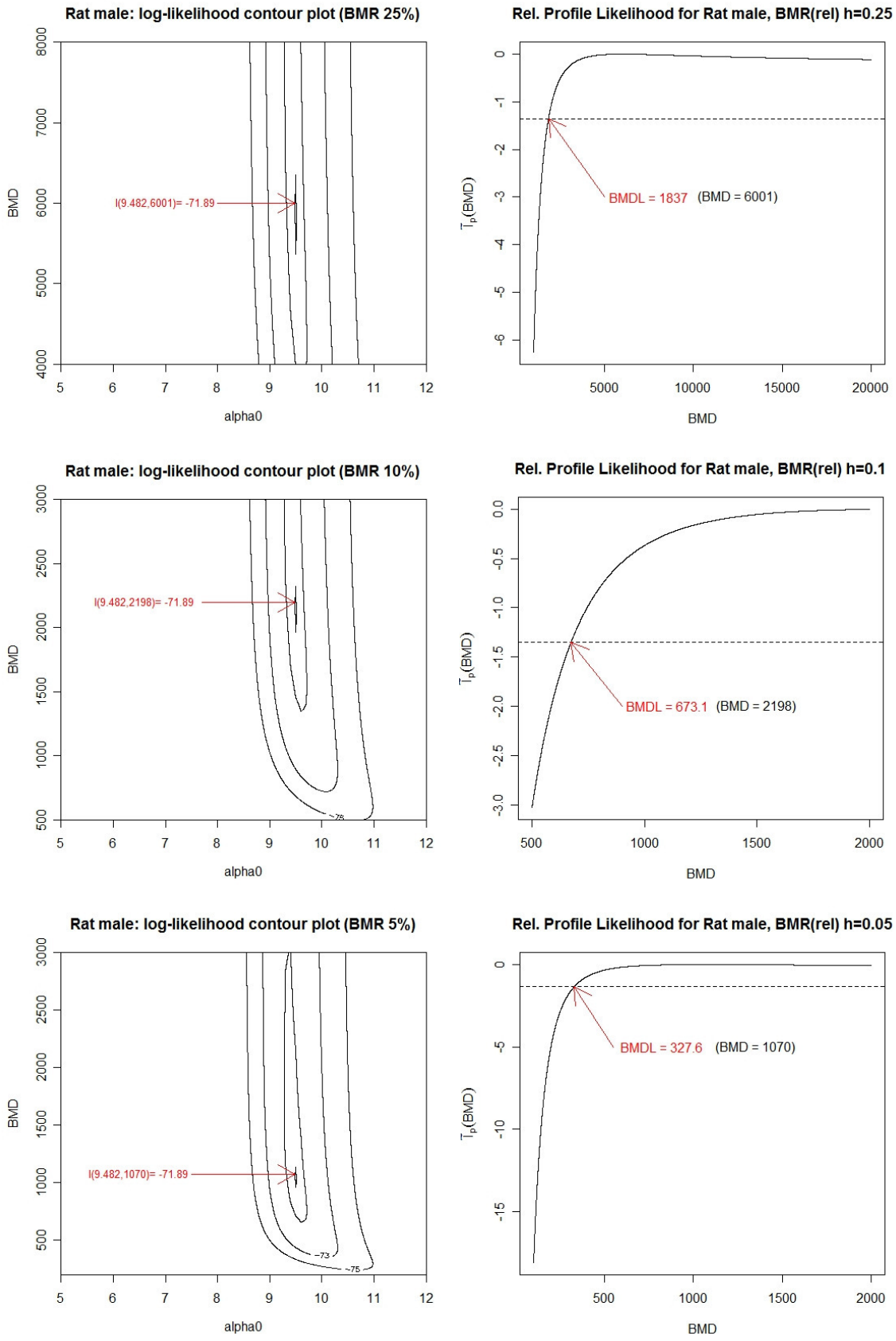


Fig.26. Log-likelihood contour plots and relative Profile Likelihood functions for different definitions of BMR for Rat male. The appearance of the relative profile likelihood function differs from those of the other experimental groups because no (finite) upper confidence limit for the BMD could be found. Anyway, the results of this experiment should be interpreted with caution because even the Cochran-Armitage test showed no significant dose-response interrelationship.

4.3.2. Rat female

Kaplan-Meier curves and Log-log-plot:

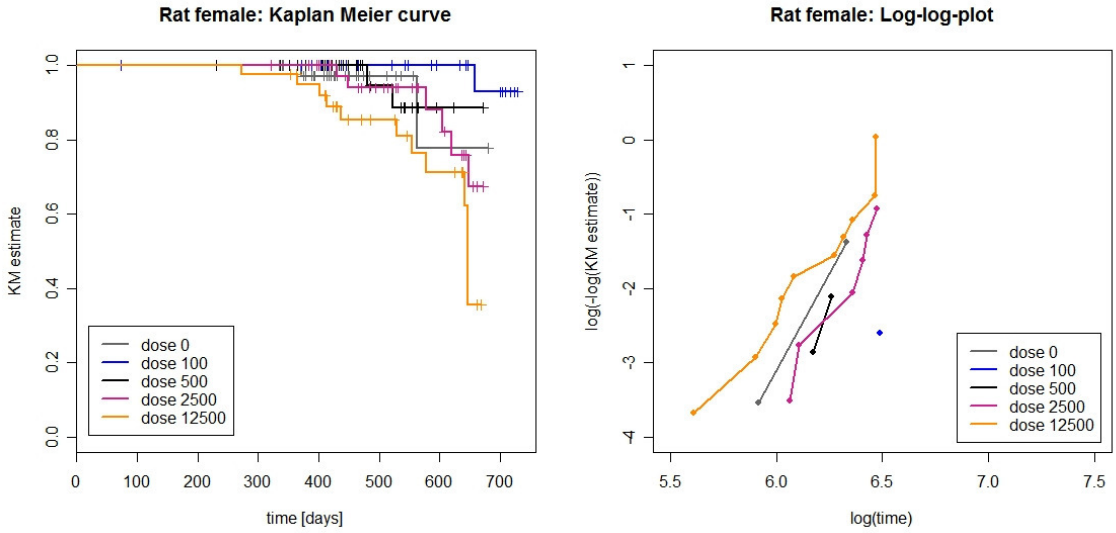


Fig. 27. Kaplan-Meier curves and Log-log-plot for Rat female. A Weibull (or Expoential) AFT/PH model seems not to be appropriate.

Fitting an Exponential model leads to the following estimates: $\alpha_0 = 9.0691749467$ and $\alpha_1 = -0.0001339523$.

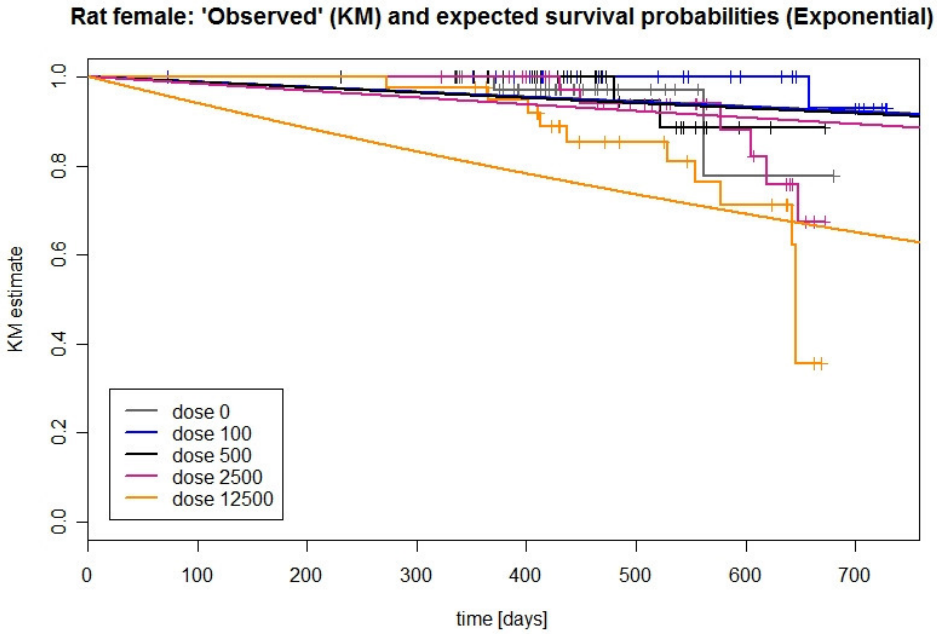


Fig. 28. 'Observed' and expected survival probabilities (Exponential model) for Rat female.

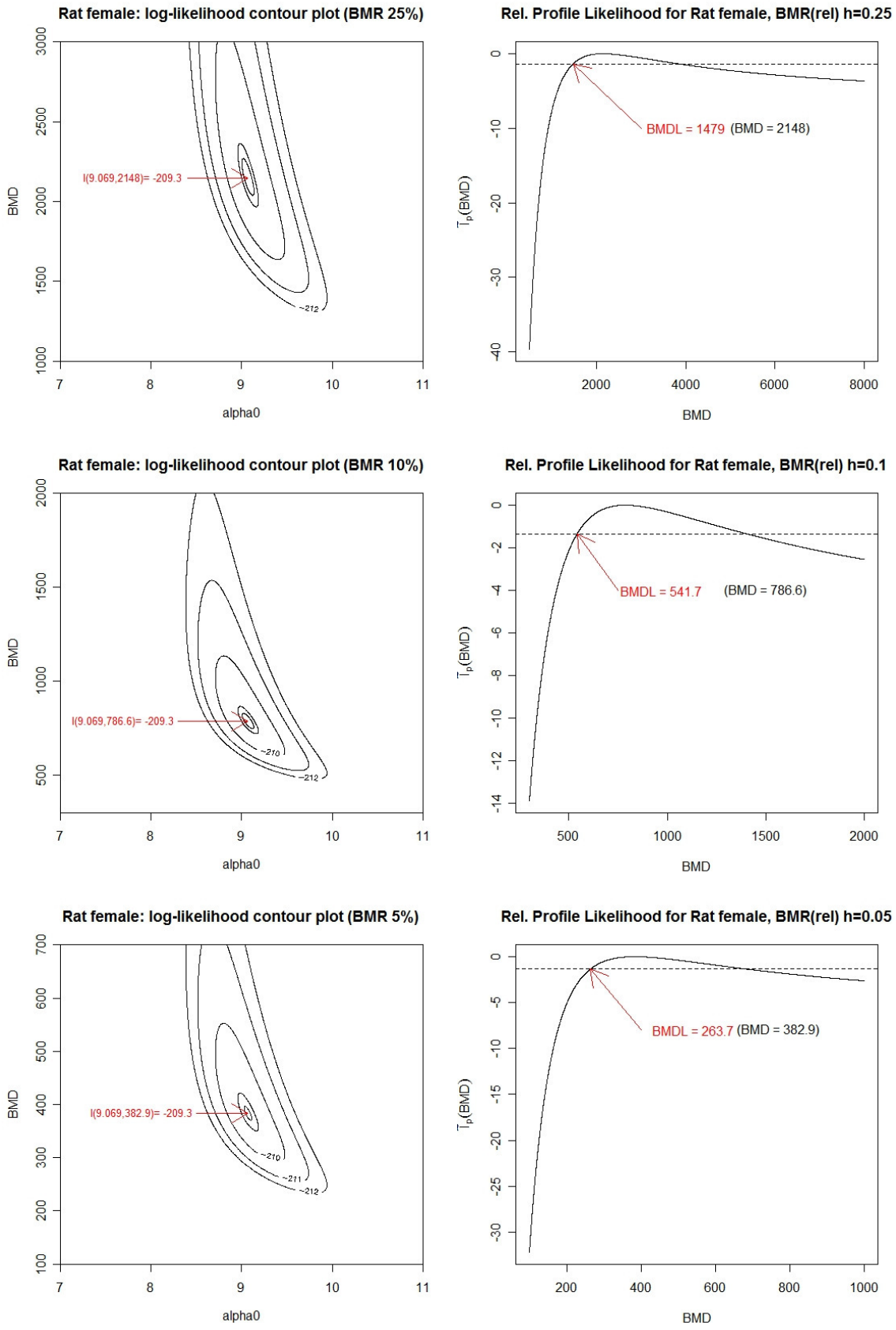


Fig.29. Log-likelihood contour plots and relative Profile Likelihood functions for different definitions of BMR for Rat female.

4.4. Fitting a Weibull AFT model

In this section, we summarize the results of fitting Weibull AFT/PH models. Again, the BMDs for three different BMRs are estimated via Maximum Likelihood: 25%, 10% and 5% reduction in each quantile of survival compared to the unexposed individuals. The BMDLs are determined with the profile likelihood method. Because the likelihood function of a Weibull model contains three parameters, no contour plots are plotted.

4.4.1. Mouse male

For the Mouse male experiment we get the estimates $p = 6.178721$, $\alpha_0 = 6.724810$ and $\alpha_1 = -1.991917 * 10^{-05}$.

A comparison of the 'observed' and expected survival probabilities shows that this model is much more appropriate than the Exponential one – even though the Kaplan-Meier curves and Log-logistic plot (*Fig.18.*) would also not suggest a Weibull AFT/PH model at once.

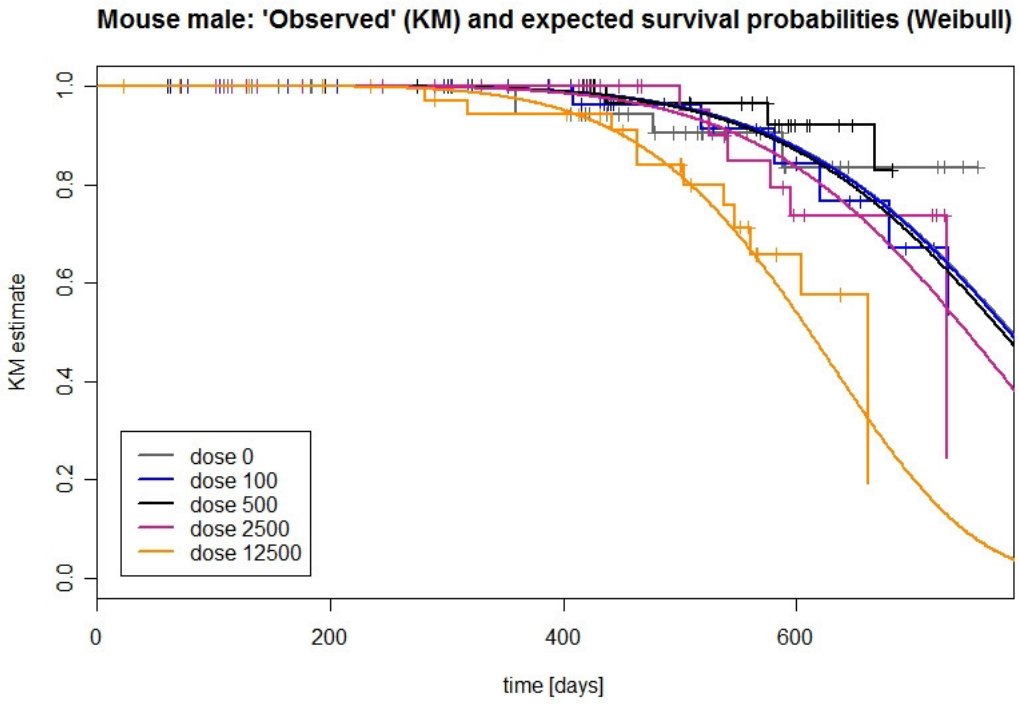


Fig.30. 'Observed' and expected survival probabilities (Weibull model) for Mouse male.

The following figure depicts the profile likelihood functions for each definition of BMR:

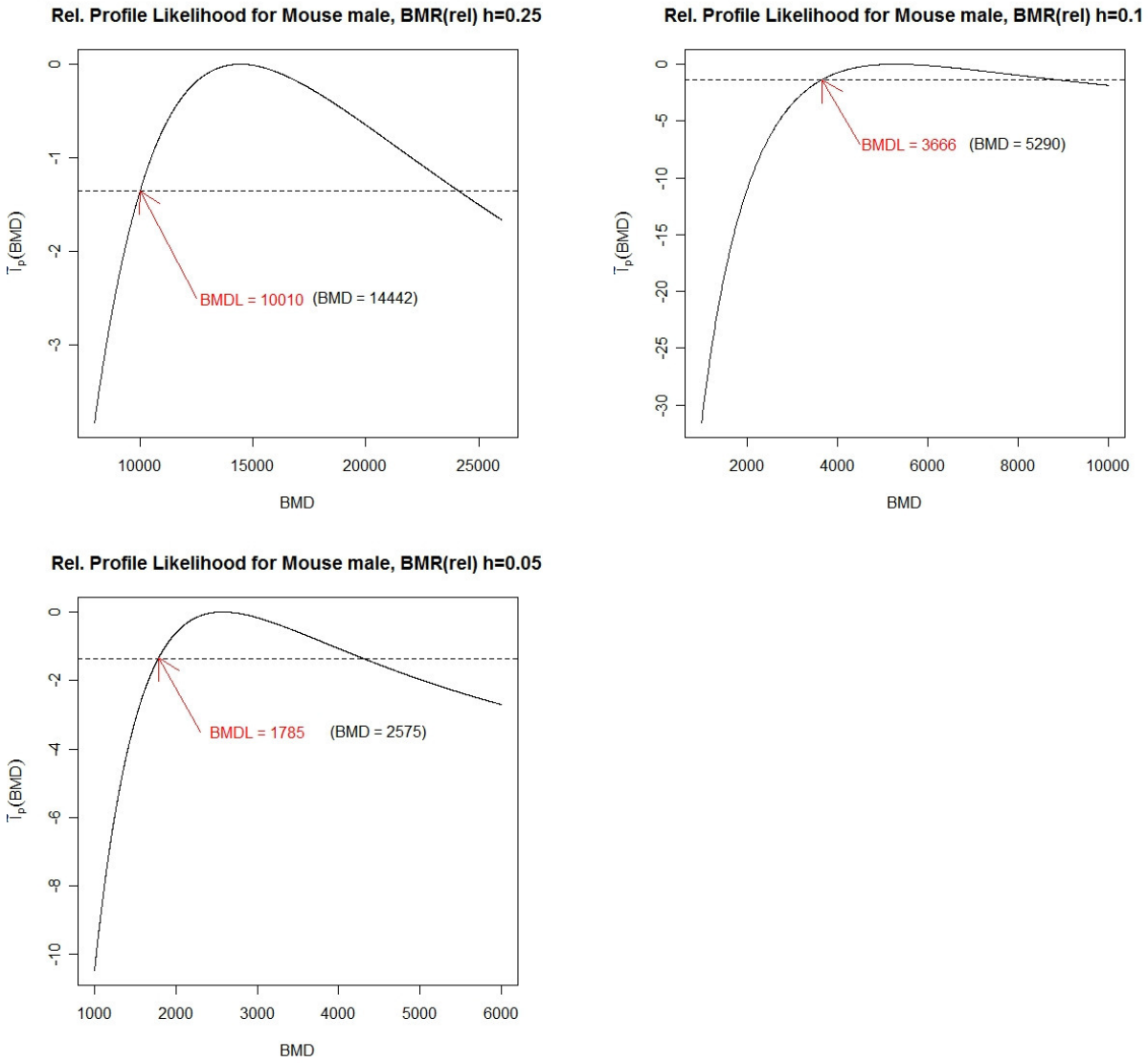


Fig.31. Relative Profile Likelihood functions for different definitions of BMR for Mouse male. The dashed horizontal lines in the plots of the profile likelihood functions indicate the value of $-\frac{1}{2}\chi^2_{0.9}(1) \approx -1.35$. *As in the Exponential case the different shapes only result from different scaling.*

The (rounded) estimates for the BMDs and BMDLs are:

BMR (rel.)	BMD	BMDL
25%	14442	10010
10%	5290	3666
5%	2575	1785

Tab.13. Values for BMDs and BMDLs for different BMRs for the Mouse male experiment under fit of an Weibull model.

4.4.2. Mouse female

The estimates for a Weibull AFT/PH model for the Mouse female experiment are: $p = 5.792518$, $\alpha_0 = 6.714603$ and $\alpha_1 = -2.597749 * 10^{-05}$.

The (rounded) estimates for the BMDs and BMDLs are a bit smaller than those obtained for the Mouse male experiment but still in the same region.

BMR (rel.)	BMD	BMDL
25%	11074	8578
10%	4056	3147
5%	1975	1529

Tab.14. Values for BMDs and BMDLs for different BMRs for the Mouse female experiment under fit of a Weibull model.

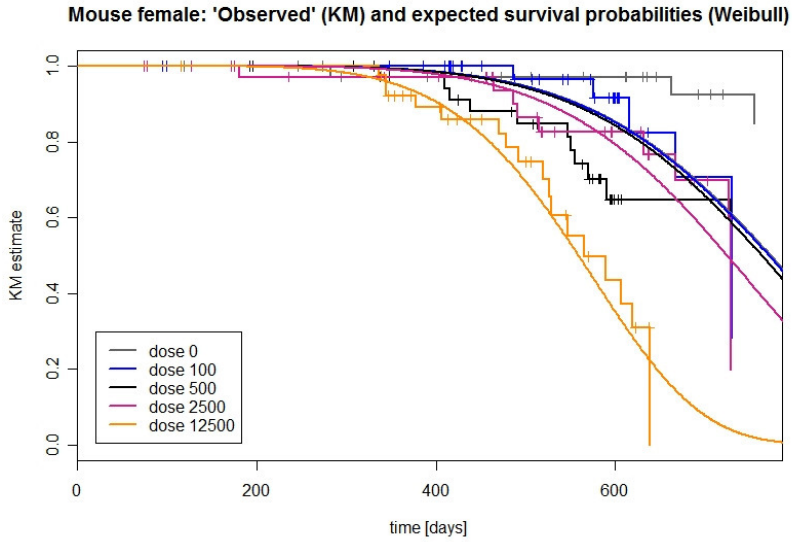


Fig.32. 'Observed' and expected survival probabilities (Weibull model) for Mouse female.

Profile likelihood functions for each definition of the BMR and estimates for the respective BMDs and BMDLs:

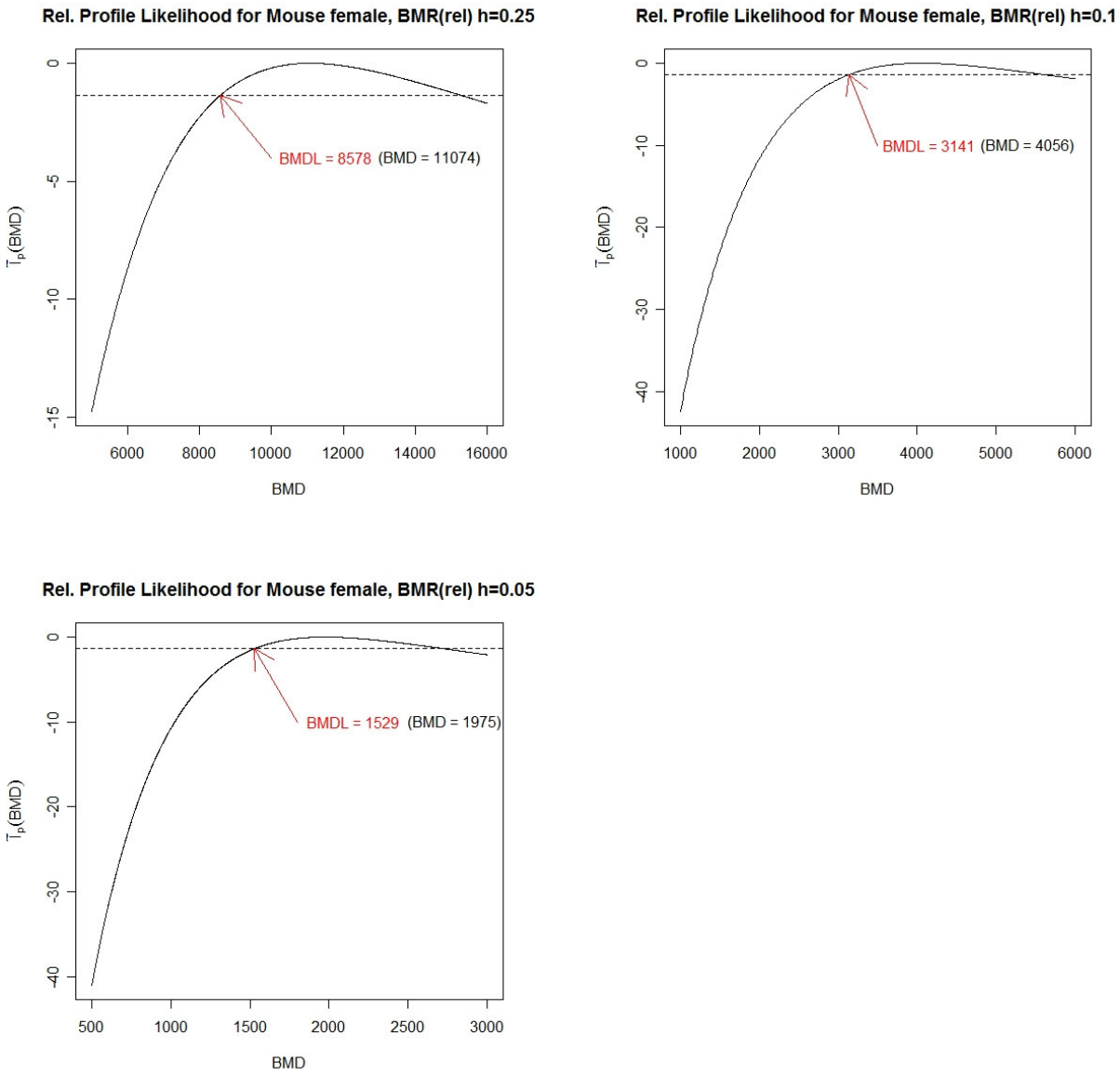


Fig.33. Relative Profile Likelihood functions for different definitions of BMR for Mouse female.

4.4.3. Rat male

The estimates for a Weibull AFT/PH model are: $p = 6.254374$, $\alpha_0 = 6.701951$ and $\alpha_1 = -1.236188 * 10^{-05}$.

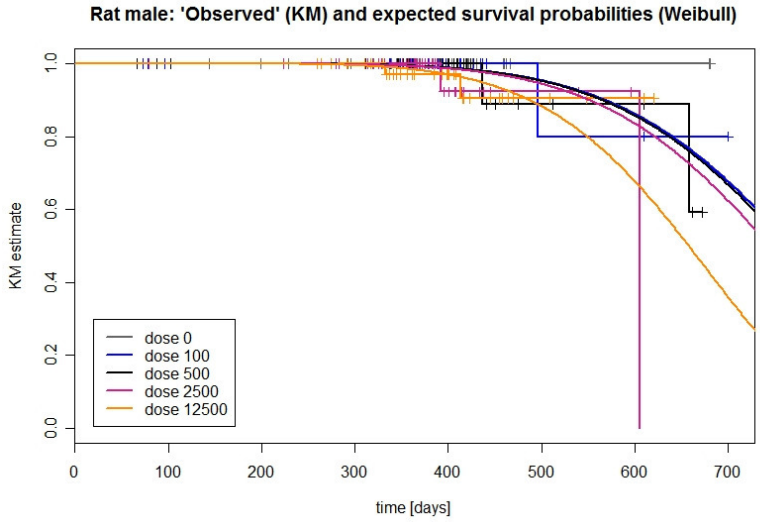
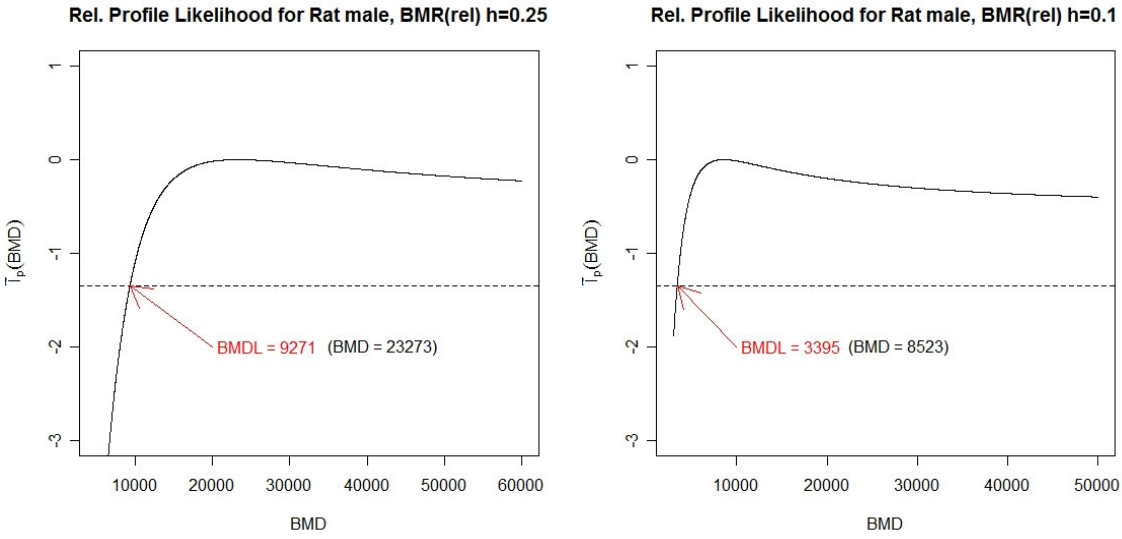


Fig.34. 'Observed' and expected survival probabilities (Weibull model) for Rat male.

Profile likelihood functions for each definition of the BMR and estimates for the respective BMDs and BMDLs:



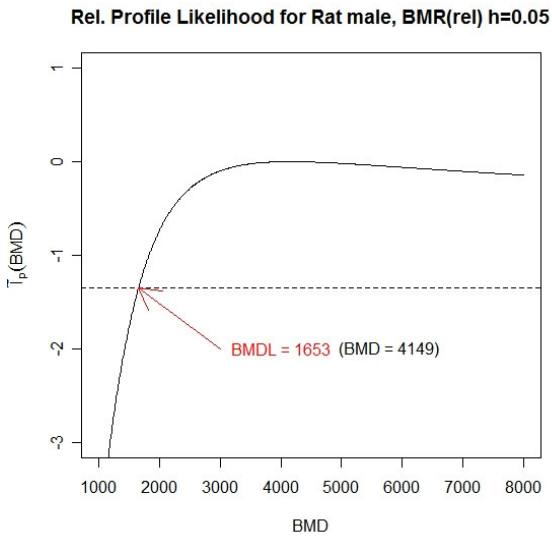


Fig.35. Relative Profile Likelihood functions for different definitions of BMR for Rat male.

4.4.4. Rat female

The estimates for a Weibull AFT/PH model are: $p = 5.470178$, $\alpha_0 = 6.859511$ and $\alpha_1 = -2.614513 * 10^{-05}$.

A graphical comparison of the Kaplan-Meier survival curves and the survival estimates predicted by the model is shown in the following figure:

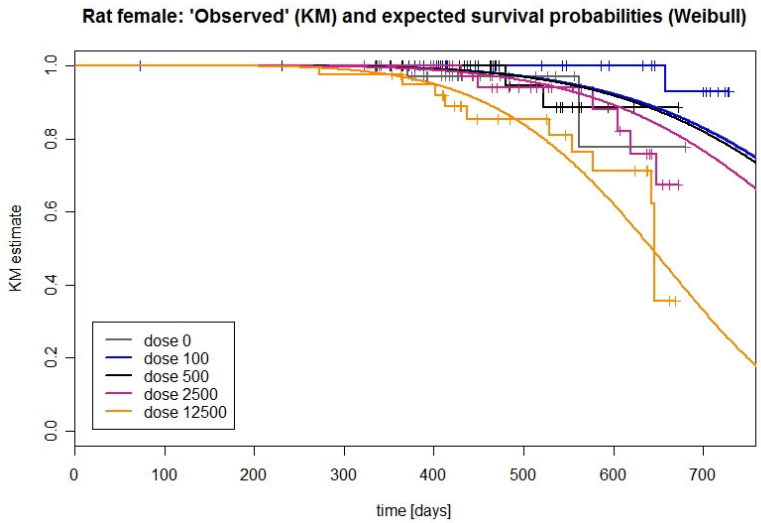


Fig.36. 'Observed' and expected survival probabilities (Weibull model) for Rat female.

Profile likelihood functions with estimates for BMDs and BMDLs:

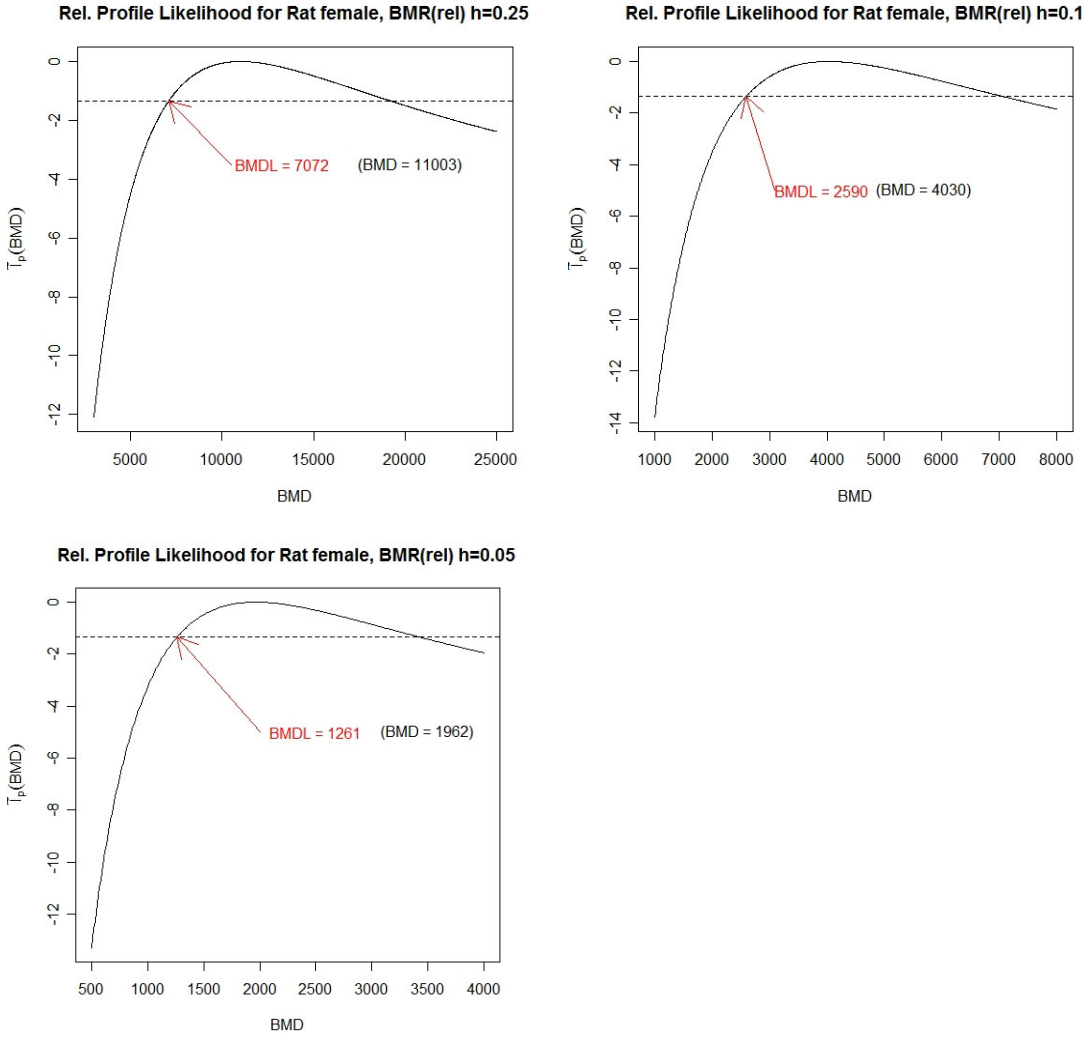


Fig.37. Relative Profile Likelihood functions for different definitions of BMR for Rat female.

4.5. Summary of the BMDs and BMDLs obtained with Exponential and Weibull models

Model (TTT)	Animals	BMD_{25}	$BMDL_{25}$	BMD_{10}	$BMDL_{10}$	BMD_5	$BMDL_5$
Exponential	**Mouse** ♂	3256	2124	1192	778	580.5	378.7
	Mouse ♀	3721	2465	1363	902.8	663.4	439.5
	Rat ♂	6001	1837	2198	673.1	1070	327.6
	Rat ♀	2148	1479	786.6	541.7	382.9	263.7
Weibull	**Mouse** ♂	14442	10010	5290	3666	2575	1785
	Mouse ♀	11074	8578	4056	3141	1975	1529
	Rat ♂	23273	9271	8523	3395	4149	1653
	Rat ♀	11003	7072	4030	2590	1962	1261

Tab.15. Summary of the estimates for the BMDs and BMDLs for Exponential and Weibull TTT models.

For the Exponential models, all estimates are lower than the respective ones obtained for the Weibull models. When interpreting this result, one should keep in mind, that in all experimental groups the Weibull models provide a statistically significant better fit than the nested Exponential models. We used the Likelihood ratio test statistic for a goodness of fit test:

$$W = -2\,\frac{\max L_{exp}(\alpha_0, \alpha_1)}{\max L_{weib}(\alpha_0, \alpha_1, p)} = -2\left(l_{exp}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML}) - l_{weib}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML}, \hat{p}_{ML})\right)$$

$$W \overset{a}{\sim} \chi^2(3-2) = \chi^2(1)$$

Experiment	$l_{exp}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML})$	$l_{weib}(\widehat{\alpha_0}_{ML}, \widehat{\alpha_1}_{ML}, \hat{p}_{ML})$	W	p-value
Mouse ♂	-316.3	-268.1	96.4	$< 5 * 10^{-23}$
Mouse ♀	-450.6	-385.9	129.4	$< 3 * 10^{-30}$
Rat ♂	-71.9	-59.3	25.2	$< 3 * 10^{-7}$
Rat ♀	-209.3	-182	54.6	$< 8 * 10^{-14}$

Tab.16. Comparison of goodness of fit for Exponential and Weibull models. For all experimental groups the difference between these nested models is highly significant.

4.6. Comparison with calculations for dichotomous models

An alternative approach of calculating BMDs and BMDLs for TTT data would be to reduce the information and analyze only tumor incidences. (This procedure especially is of interest if only summarized and no individual time to tumor data are available.) Then it is possible to fit a dichotomous (quantal) model with three underlying variables: dose group, total number of animals (per dose groups) and number of (malignant) tumors (per dose groups). This form of the data is reported in *Tab.7.*

We consider two dichotomous models, namely Weibull and Log-logistic, which have the following probability functions:

Weibull:

$$P(\mathrm{d}) = \mathrm{b} + (1 - \mathrm{b}) * (1 - \exp(-\mathrm{s} * \mathrm{d}^{\mathrm{p}}))$$

Log-logistic:

$$P(\mathrm{d}) = \frac{1 - \mathrm{b}}{1 + \exp\left(-\mathrm{c} + \mathrm{s} * \log\left(\mathrm{d}\right)\right)},$$

where d denotes the dose, b the background probability of an effect, p the power, c the intercept and s the slope.

The BMD analysis can be performed with the software BMDS 2.1., developed by US EPA.

To avoid having an infinite slope at zero dose, which would be biologically unrealistic and could lead to numerical problems when calculating confidence limits, the slope in the Log-logistic and respectively the power in the Weibull dichotomous model was restricted to be greater than or equal to 1 [BMDS Online-Tutorial].

We have defined the BMRs as 25%, 10% and 5% increase of the fraction affected (death with tumor) compared to the unexposed animals: 'extra risk' $\frac{P(d)-P(0)}{1-P(0)}$, where $P(d)$ is the risk at the BMD and $P(0)$ is the background risk at dose 0.

The estimated values for the respective BMDs and BMDLs are summarized in *Tab.17* and the following figure is an illustration of the proceeding (Weibull: Mouse male):

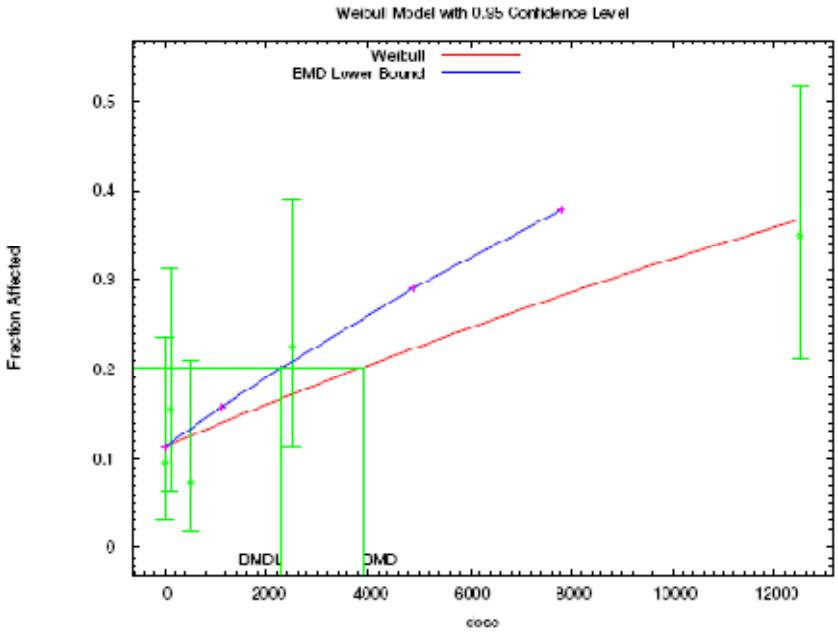


Fig.38. BMD analysis of Mouse male with BMDS 2.1. A dichotomous Weibull model is fitted with restricted power (red line). The BMD estimation is shown graphically (green lines) and the BMDL (blue line).

Model (dichotomous)	**Animals**	**BMD_{25}**	**$BMDL_{25}$**	**BMD_{10}**	**$BMDL_{10}$**	**BMD_5**	**$BMDL_5$**
Weibull	**Mouse ♂**	10618	6300	3889	2307	1893	1123
	Mouse ♀	8356	4943	3060	1810	1490	881.3
	Rat ♂	115994	13251	42482	10178	20682	4955

Log-logistic	**Rat** ♀	10785	6792	3950	2487	1923	1211
	Mouse ♂	10195	5455	3398	1818	1610	861.3
	Mouse ♀	7289	3700	2430	1233	1151	584.2
	Rat ♂	131081	13323	43694	9970	20697	4723
	Rat ♀	10599	6159	3533	2053	1674	972.4

Tab.17. Summary of the (rounded) estimates for the BMDs and BMDLs Weibull and Log-logistic dichotomous models, software: BMDS 2.1. The whole output including graphs for the default BMR of 10% extra risk can be found in the appendix.

All estimates are higher than the corresponding ones for the Exponential TTT models. In comparison to the Weibull TTT models, all corresponding estimates for the dichotomous models are lower but more or less in the same region of size (except for rat male). The estimates for the $BMDL_{10}$ are compared in the following graphic.

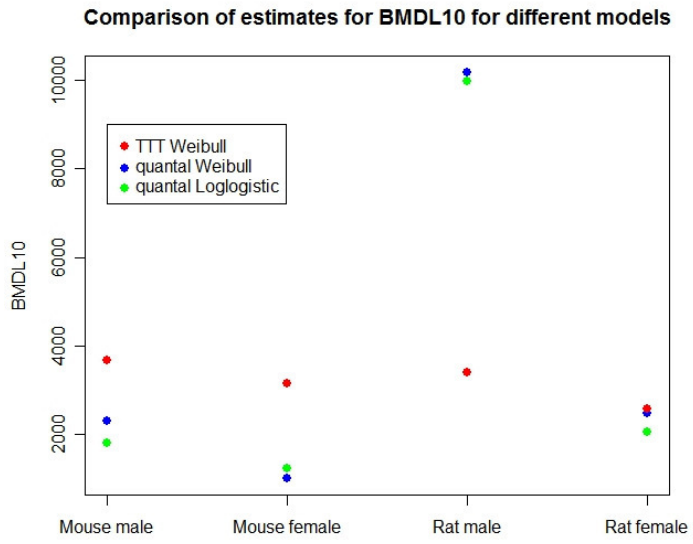


Fig.39. Comparison of the estimates for the $BMDL_{10}$ for the Weibull TTT model and the quantal models Weibull and Log-logistic. The values are lower for the quantal models than for the Weibull TTT model in all experiments except for rat male. The Weibull TTT model leads to estimates for the $BMDL_{10}$ that are in the same region of size for all experiments.

In contrast to the quantal models, the Weibull TTT model leads to estimates for the $BMDL_{10}$ which are in the same region of size for all experiments.

5. Simulation study to the coverage evaluation of Confidence Intervals for Exponential TTT models

In order to get an idea of the reliability of the BMDLs we have calculated with the profile likelihood method, a simulation study was done. We were especially interested in the effect of the censoring percentage.

Therefore, we defined an Exponential model with survival function

$$S(t) = \exp\left(-\exp\left(-5 + 0.0001 * dose\right)\right)$$

and sampled data sets of 40 individuals per group for dosages of 0, 100, 500, 2500 and 12500 with censoring percentages in the range of 0% and 90%. (Details can be found in the appendix.) For each dose 1000 samples were drawn. In each case we estimated the BMDs and BMDLs for a relative BMR of 10% loss in each quantile of survival (AFT property).

The 'true' BMD of the underlying model is $BMD = 1053.605$. In order to evaluate the coverage of the BMDLs, we compared the estimated BMDLs with that value. Approximately, it should hold

$$P(BMDL < 1053.605) = 0.95$$

Actually, the coverage was quite good because for all censoring percentages the coverage probability estimated from the simulations was either larger than or reasonably close to 0.95 as compared to its standard error:

Censoring	0%	10%	20%	30%	40%	50%	60%	70%	80%	90%
Coverage	**0.955**	**0.957**	**0.955**	**0.95**	**0.956**	**0.936**	**0.957**	**0.955**	**0.952**	**0.949**
std error	**0.0066**	**0.0064**	**0.0066**	**0.0069**	**0.0065**	**0.0077**	**0.0064**	**0.0066**	**0.0068**	**0.0070**

Tab.18. Coverage probability. In about 95% the estimated BMDL was smaller than the 'real' BMD for all percentages of censoring investigated. The coverage probability can be interpreted as a binomial rate p and standard error $\sqrt{\frac{p(1-p)}{1000}}$ *gives the precision of the estimates. Thus, a 95% confidence interval of the coverage probability is given by [coverage prob.* $\pm 1.96\ std\ error$*].*

Nevertheless, the percentage of censoring had an influence on the estimated BMDLs. As one might expect, the variability of the values for the BMDLs increased especially for high

censoring percentages. The median BMDLs show a trend to become smaller for higher percentages of censoring:

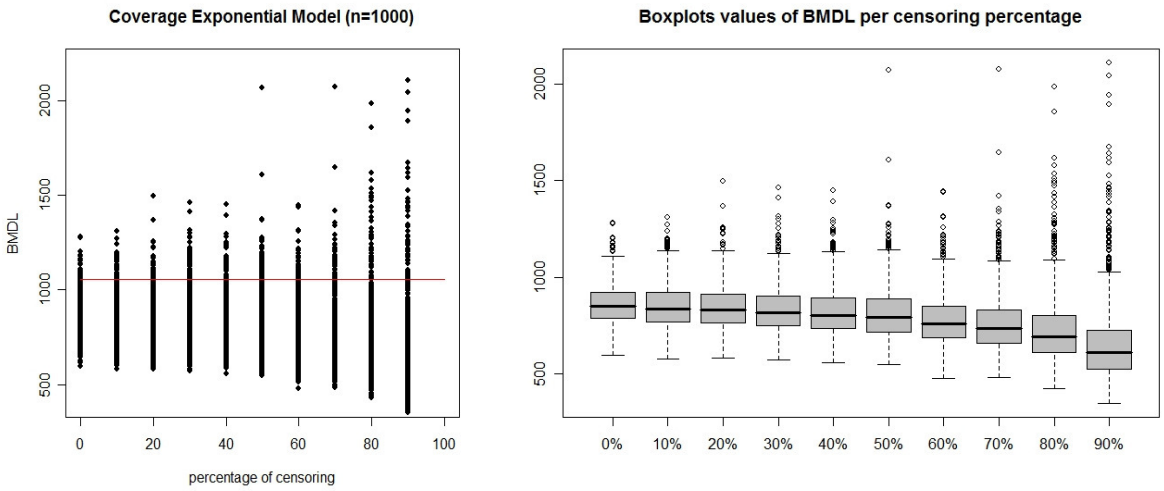


Fig.40. Dependence of the estimated BMDLs on the percentage of censoring. In the left figure, the red horizontal line indicates the value of the 'true' BMD for the underlying Exponential model. The boxplots depicted in the right figure show the tendency of the estimated BMDLs to show a greater variability especially for high censoring percentages, whereby the median tends to become even smaller.

Of course, it would be also interesting to evaluate this relationship for Weibull models. But in the case of Weibull data it is technically much more complicated to control the censoring percentages in the simulations and thus the investigation of the influence of censorings on BMD calculations for Weibull models was beyond the scope of present work.

6. Comparison with BMDs and BMDLs for Urethane from other studies

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) has provided a summary of values for BMDs and BMDLs from different studies on Urethane. In all cases, the analysis was done for the tumor incidences (dichotomous models) and the BMR was defined as 10% extra risk of tumors. The BMD values range from 500 to 630 µg/kg BW/day for lung adenoma or carcinoma and 470 to 760 µg/kg BW/day for harderian gland adenoma or carcinoma. The corresponding BMDLs range from 260 to 510 respective 280 to 610 µg/kg BW/day. [JECFA (2005)]

These values are smaller than the values we got for dichotomous models and the ones we calculated with our TTT models. But one has to keep in mind that this result also depends on the different definitions of the endpoint: We analyzed only the malignant tumors, whereas the studies quoted included also the benign ones.

7. Discussion

In this thesis, we suggested a statistical method on how the BMD approach can be extended to continuous incomplete (here right censored) time-to-tumor data obtained in animal carcinogenesis studies. For this aim the four steps of the

1. identification of the statistical distribution model for the endpoint variable and of the dose-response model,
2. definition of the *Benchmark Response (BMR)* level,
3. estimation of the *Benchmark Dose (BMD)* and
4. calculation of the (one-sided) lower 95% confidence bound as so called *BMDL (Benchmark Dose Lower Limit)*

were investigated for ways to adjust them to this type of data. Whereas by now those data were only analyzed in their aggregate form of tumor incidences in the risk assessment, the methods developed in this thesis allow the analysis of the original time-to-tumor data. The former quantal dose response models for dichotomous data (tumor present or absent) were thus replaced by dose-time response models which are more realistic since they account for the timely occurrence of the endpoint tumor. This approach could not be done without making assumptions at the first two definition steps of the approach listed above. This will be discussed next under "methods".

Methods. The distribution model chosen for the time-to-tumor data was the Weibull survival time distribution which has been often used as parametric survival model for data of animal experiments. Whereas this model is often used as a sort of "black box" model in standard software it was developed in this thesis analytically in detail (also its special case of the Exponential distribution) since we had to understand the analytical form of the distribution for two specific reasons

a) for the implementation of the dose model in that survival distribution

b) for the explicit definition of the form of the BMR.

The dose model chosen was in essence a linear dose-response relationship postulated for a distribution parameter. It should be not too difficult to extend this assumption to more complicated functional forms. The most natural extension would be to use polynomials, starting with a quadratic form $\beta_0 + \beta_1\, dose + \beta_2\, (dose)^2$. Actually, it would be also possible to extend this model to non-monotone functions from a mathematical perspective. Ideas of

fractional polynomials [e.g. ROYSTON (1999)] as they are used for other biomedical problems when covariates have to be analyzed could be used. However, there is one hurdle in stepping into this direction when applying such models in risk assessment. One should be able to have an answer to the question: Why will this model be chosen? It would be hard if not impossible to justify more complicated models only for mathematical reasons without support from toxicological arguments. Since in most applications not much is known on the mode of action of a carcinogen, the principle to use at first the simplest model and increase the complexity of the models only when those simple models do not fit, may hold also in our case. Therefore our choice of the linear form can be considered as the first but also very natural step in model development.

In this thesis, we focused on Weibull and Exponential regression models with a linear dose-response relationship assumed for the distribution parameter λ. The Weibull distribution seems to be appropriate for time-to-tumor data since it has been used in the past. Some arguments for its appropriateness for this type of data were summarized by PETO ET AL. (1972). Exponential models have also been used in the literature. However, we found in our examples that this class of distributions is insufficient for TTT data which is quite obvious when one notes the restriction of the hazard function to be constant over time. There are also biological arguments against such a rigid assumption: one has to expect the risk of a tumor increasing over time due to its development process [DOLL (1971)].

Our choice of the BMR functional seems to be quite natural since it relates to the descriptive parametric Kaplan-Meier estimate for censored survival data describing the timely occurrence of tumors. The median TTT is a common measure in clinical trials to characterize the survival of a group of patients. Another common measure for clinicians to assess the prognosis of a patient by an easy comprehensible quantitative number is the 1-year or the 2-year survival probability, which we had in mind when suggesting defining the BMR as loss in survival probability at a specific time point. Nevertheless, we concluded that the BMR should be stated in terms of a relative loss in median survival time and thereby quasi automatically in all quantiles of the TTT distribution because this definition makes use of the AFT property of Weibull models. Thus the crucial decision, which quantile or time point is most appropriate for the definition of the BMR is omitted, but it may become an issue when generalizing the approach to other distributions. Then, one may no more avoid the inclusion of toxicological knowledge for the definition of the BMR.

We like to make some further comments on the calculation of the BMDLs. An important methodological challenge on this work was the calculation of the confidence limits. In order to obtain estimates for the BMDLs we used the profile likelihood method, requiring a re-parameterization of the model, which includes the BMD as a parameter itself. Although the application of this method has been used for the BMD approach, see e.g. the BMDS software (http://www.epa.gov/ncea/bmds), it was not at all easy to find this method well described in text books and surprisingly almost no reasonable explanation could be found on BMD related literature, in spite of its common use in the software. Therefore the profile likelihood method was applied to the special functions used in the thesis and it was implemented in R. We found it also important to illustrate this method beyond its formal derivation. The graphs used in the example were given in detail in order to communicate this method to non-statistical user of BMD software.

Results. The evaluation of Moerbeek and colleagues [MOERBEEK (2004)], which suggested to use the profile likelihood ratio method for the estimation of the BMDL, is compatible with the results of our simulation study we performed to evaluate the coverage probability of the BMDLs for different percentages of censoring. Indeed, the profile likelihood ratio method seems to be an appropriate way of estimating BMDLs for Exponential models. Of course, this hint should be investigated further e.g. in larger simulation studies where the simulation parameters are varied at a wider scope. The findings obtained in our simulation study were very intuitive: The coverage probability was found to be very close to the approximate value for all censoring percentages but the variability of the BMDLs increased especially for higher censoring percentages, whereby the median of the estimated BMDLs decreased.

Note, high censoring percentages are a systematic problem in these dose-response experiments with data coming from animal experiments where the experimental doses are rather low. Thus, the overall incidence can be very low and the amount of censorings high. This is ultimately a question of the design of such experiments and the endpoint. The BMD is a tool in risk assessment which was particularly developed for dealing with the challenges of investigating the effect of substances at low doses. The underlying idea of the BMD approach of modeling the dose-response relationship instead of searching for a dose which leads to no statistically significant effect compared to the control group (NOAEL). The BMD has been shown to be useful for these settings. The important advantage of the BMD approach is that it not crucially dependent on the number of animals on a given dose. That, however would strongly influences the power of statistical tests, which could have been applied also for TTT

data using methods for censored survival data, e.g. the well known log-rank test. In contrast the BMD can interpolate between applied doses while the NOAEL approach is restricted to these doses. As with respect to human health especially low doses are of concern, it is very important to have an approach on hand that is powerful for investigating these low doses. Therefore, the methods developed in this work can be very valuable in risk assessment.

We want to stress here the importance of the calculation of the one-sided lower 95% confidence bound. It is actually the starting point of extrapolating the results of benchmark dose analyses of animal experiments to humans. The BMDL is used as reference point because this value also accounts for the quality of the study as increasing the number of animals in the study leads to less conservative lower confidence limits. However, a purely numerical increase of the number may not suffice to increase the precision of the BMD and as such lead to BMDL values close to the value of the BMD. This is a very difficult statistical problem of optimal design since we deal in all the BMD application mostly with non-linear models, i.e. we encounter here the problem of optimal design in non-linear regression which, however, was not considered here further.

Application. The data used as example in this thesis were data on the carcinogenicity of Urethane from an animal study conducted by Schmähl and colleagues at the DKFZ (1977). One should remark here, that the endpoint we analyzed was not the actual TTT (time-to-tumor) but the time-to-death-with-tumor. This is the typical endpoint in animal studies which investigate occult (internal) tumors. For details how handling this complication see GART (1986). They discuss primarily the statistical testing for differences between dose groups. Accounting for the difference between TTT and time-to death with tumor in dose-response modeling has so far not investigated in toxicology. It would need the use of other modeling, e.g. competing risk models, which have been developed for clinical applications [e.g. FINE (1999)].

We applied our new BMD approach to these experimental tumor data. The Exponential TTT models, which are less flexible than the Weibull models, led to estimates which are much more conservative, i.e. higher BMDs and BMDLs than those obtained with the dichotomous and Weibull TTT models. An explanation for the remarkable differences of the estimates could be found in the diagnostic the Log-log plots of the four experiments suggested. Actually the Exponential model type appears less appropriate for our data than the Weibull models and

the Likelihood ratio tests showed the Weibull models to have a significantly better fit. Generally, these experimental data had the constraint that, although the Cochran-Armitage trend test showed a significant interrelationship, the dose-response was not strictly monotonic for the lower doses.

The estimates for the BMDs and BMDLs for Weibull TTT models were compared with the results for dichotomous models (loglogistic and Weibull), which investigate only the tumor incidence. A comparison of these different model types led to the observation that with the dichotomous models, which use only a smaller amount of information, lower estimates for the BMD and BMDL were obtained. Of course, one has to keep in mind that the underlying BMRs for dichotomous and TTT models might not be equivalent. For example, 10% loss in median tumor-free survival time is a different way of stating the risk of a substance than 10% increase of tumor incidence. But nevertheless, this observation could be a hint that TTT models, which take more of the available information into account, could lead to more appropriate, less conservative estimates (Fig. 39).

Exceptions of this observation are the estimates for the experiment Rat male. Here, the estimates for the BMDs and BMDLs are much higher for the dichotomous models. The reason might be the fact that we cannot find a significant dose-related trend in the selected endpoint. Furthermore, as mentioned in the paper, this experiment has to be interpreted with extended caution because about half of the animals died due to an unspecified virus infection [SCHMÄHL (1977)] and thus the low incidence of tumors could also be caused by the fact that many animals were censored after a respectively short observation period. Nevertheless, even in that case the TTT models seem to be more appropriate as they lead to estimates for the BMDLs that are more or less in the same region of size for each experiment and thus more consistent than the values obtained with the quantal models.

Finally the estimates for the BMDs and BMDLs obtained for the data of the urethane study of Schmähl and colleagues were compared to those cited in a JECFA report [JECFA (2005)]. In all reported calculations the analysis was performed for the tumor incidences (dichotomous models) and the BMR was defined as 10% extra risk of tumors. The estimates reported by JECFA, based on other studies, were even lower than the estimates gained with the dichotomous models for our data. This result could be explained with the fact that the assessments reported by JECFA included also benign tumors in the endpoint – whereas we investigated only the occurrence of malign tumors. The question how to deal with the occurrence of benign tumors leads however to a more complex problem as it seems. Of

course, treating animals with benign tumors as censored is a questionable decision because we cannot exclude the possibility that the benign tumors are competing risks for malignant tumors, e.g. an animal may be sacrificed because of a benign tumor. On the other hand, benign and malignant tumors are so different types of tumors that they should not be combined. They differ significantly in their pathway of etiology and health consequences. From a methodological point of view one would have to look out for models for competing risks, mentioned above. Form a toxicological point of view, as open question in this context remains how the findings should be extrapolated to humans.

Outlook. Finally, we would like to provide an outlook on how the approach could be further extended and which steps could be done so that this approach gets accepted by practical working researchers in risk assessment.

As we concentrated only on Exponential and Weibull models, it could be profitable to extend the approach suggested in this thesis to other distributions, which could be appropriate for modeling TTT data. Candidates which commonly appear in literature for survival analysis would be for example the lognormal and gamma distribution.

In order to make the approach attractive also for practical working researchers in risk assessment, further simulation studies should be done, which evaluate the coverage of the BMDLs for different percentages of censoring. Especially, it would be important, to investigate the coverage probability for underlying Weibull distributions and evaluate, whether the trend we observed for underlying Exponential distributions also holds for the Weibull case.

In principle, the Weibull TTT model would be also applicable to epidemiological studies with a larger sample size, since often a large number of humans investigated there. Human data have two other beneficial facts: the extrapolation from animal to human is avoided through this data type, and individual data cover mostly the whole dose range of interest. But as observational data they lead also to some methodological challenges. One is the need to systematically detect confounding factors and include them appropriately in the model. For further details on the BMD approach for epidemiological data see e.g. BUDTZ-JØRGENSEN (2001).

All models used in this thesis are parametric models. One should, however, note that the Weibull model is a special parametric family since it combines in its definition the PH as well as the AFT property. As such one could think of extensions to semi-parametric models. For this purpose especially the Cox semi-parametric PH models [e.g. Cox (1972), Cox (1984)] could be of interest and the work of Nikulin [NIKULIN (2005), NIKULIN (2006)] on generalizations of AFT models could be also a source for further work.

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Software BMDS (with Online Tutorial):

http://www.epa.gov/ncea/bmds (last visit: 30-03-2010)

Appendix

Table of re-parameterized Log-likelihood functions

	BMR specification	Log likelihood function
Exponential AFT model	**Absolute value for the BMR at a fixed time point t^*:** $S(t^*, d_{BMR}) = S(t^*, 0) - h$	$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i\delta_i\right)\frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}} - \sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}} d_i\right)$
	Relative value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = (1-h)\, S(t^*, 0)$	$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i\delta_i\right)\frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}} - \sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(-\log(1-h) + \exp(-\alpha_0)\, t^*) - \log(t^*) + \alpha_0}{-d_{BMR}} d_i\right)$
	Absolute value for the BMR as reduction in a specified quantile of survival, e.g. in median survival time: $t_{med}(d_{BMR}) = t_{med}(0) - h$	$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i\delta_i\right)\frac{\log\left((-\log 0.5)\exp(\alpha_0) - h\right) - \log(-\log 0.5) - \alpha_0}{d_{BMR}} - \sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log\left((-\log 0.5)\exp(\alpha_0) - h\right) - \log(-\log 0.5) - \alpha_0}{d_{BMR}} d_i\right)$
	Relative value for the BMR as reduction in a specified quantile of survival: $t_q(d_{BMR}) = (1-h)\, t_q(0)$	$l(\alpha_0, d_{BMR}) = -\left(\sum_{i=1}^{m} \delta_i\right)\alpha_0 - \left(\sum_{i=1}^{m} d_i\delta_i\right)\frac{\log(1-h)}{d_{BMR}} - \sum_{i=1}^{m} x_i \exp\left(-\alpha_0 - \frac{\log(1-h)}{d_{BMR}} d_i\right)$

Weibull AFT model	**Absolute value for the BMR at a fixed time point t^*:** $S(t^*, d_{BMR}) = S(t^*, 0) - h$	$$l(p, \alpha_0, \alpha_1) = \left(\sum_{i=1}^{k} \delta_i\right)(-p\,\alpha_0) + \left(\sum_{i=1}^{k} d_i \delta_i\right) \frac{\log(-\log(\exp(-\exp(-p\,\alpha_0)\,(t^*)^p) - h)) + p\,\alpha_0 - p\log(t^*)}{d_{BMR}} - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp\left(-p\,\alpha_0 + \frac{\log(-\log(\exp(-\exp(-p\,\alpha_0)\,(t^*)^p) - h)) + p\,\alpha_0 - p\log(t^*)}{d_{BMR}}\, d_i\right) + \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$
	Relative value for the BMR at a fixed time point t^*: $S(t^*, d_{BMR}) = (1-h)\,S(t^*, 0)$	$$l(p, \alpha_0, \alpha_1) = \left(\sum_{i=1}^{k} \delta_i\right)(-p\,\alpha_0) + \left(\sum_{i=1}^{k} d_i \delta_i\right)\left(\frac{\log(-\log(1-h) + \exp(-p\,\alpha_0)\,(t^*)^p) - p\log(t^*) + p\,\alpha_0}{d_{BMR}}\right) - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp\left(-p\,\alpha_0 + \frac{\log(-\log(1-h) + \exp(-p\,\alpha_0)\,(t^*)^p) - p\log(t^*) + p\,\alpha_0}{d_{BMR}}\, d_i\right) + \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$

Weibull AFT model	**Absolute value for the BMR as reduction in a specified quantile of survival, e.g. median survival time:** $t_{med}(d_{BMR}) = t_{med}(0) - h$	$$l(p,\alpha_0,\alpha_1) = \left(\sum_{i=1}^{k}\delta_i\right)(-p\,\alpha_0) + \left(\sum_{i=1}^{k} d_i\delta_i\right)\left(-p\,\frac{\log\left((-\log 0.5)^{1/p}\exp(\alpha_0) - h\right) - \frac{1}{\mathrm{p}}\log(-\log 0.5) - \alpha_0}{d_{BMR}}\right) - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp\left(-p\,\alpha_0 - p\,\frac{\log\left((-\log 0.5)^{1/p}\exp(\alpha_0) - h\right) - \frac{1}{\mathrm{p}}\log(-\log 0.5) - \alpha_0}{d_{BMR}}\,d_i\right) + \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$
	Relative value for the BMR as reduction in a specified quantile of survival: $t_q(d_{BMR}) = (1-h)\,t_q(0)$	$$l(p,\alpha_0,\alpha_1) = \left(\sum_{i=1}^{k}\delta_i\right)(-p\,\alpha_0) + \left(\sum_{i=1}^{k} d_i\delta_i\right)\left(-p\,\frac{\log(1-h)}{d_{BMR}}\right) - \sum_{i=1}^{k}\left(\sum_{j=1}^{n_i}(x_{ij})^p\right)\exp\left(-p\,\alpha_0 - p\,\frac{\log(1-h)}{d_{BMR}}\,d_i\right) + \left(\sum_{i=1}^{k}\delta_i\right)\log(p) + \quad (p-1)\sum_{i=1}^{k}\sum_{j=1}^{n_i}\delta_{ij}\log(x_{ij})$$

Selected R code

Fig. 12.

```
#Example: assumption Exponential distribution
#MLE re-parameterized, BMR(abs) in median surv
#profile likelihood contour plot

# …

#Tools for log likelihood
delta<-sum(status)
deltad<-sum(status*dose)
t0<-sum(weeks[dose==0])
t1<-sum(weeks[dose==1])
t10<-sum(weeks[dose==10])

#BMR(absolute value)
h<-5

#contour plot
l<-function(x){
alpha0<-x[1]
BMD<-x[2]
return(-(delta*x[1]+deltad*(log((-log(0.5))*exp(x[1])-h)-log(-log(0.5))-
x[1])/x[2]+t0*exp(-x[1])+t1*exp(-x[1]-(log((-log(0.5))*exp(x[1])-h)-log(-
log(0.5))-x[1])/x[2]*1)+t10*exp(-x[1]-(log((-log(0.5))*exp(x[1])-h)-log(-
log(0.5))-x[1])/x[2]*10)))
}

BMDgrid<-seq(from=0.8, to=3, by=0.01)
alpha0grid<-seq(from=3, to=4, by=0.01)

grid<-expand.grid(alpha0=alpha0grid, BMD=BMDgrid)
werte<-matrix(data=apply(grid,1,FUN=l), nrow=length(alpha0grid))

contour(alpha0grid,BMDgrid,werte,main="Example: log-likelihood contour
plot",xlab=expression(alpha0),ylab=expression(BMD),levels=-
c(36,35.8,35.7,35.6,35.58,35.572),xaxs="i",yaxs="i",ylim=c(0.9,3))

arrows(3.6,1.41,3.398365,1.41,col="red")
text(3.73,1.41,"l(3.398,1.41)= -35.57",col="red",cex=0.8)
```

Fig. 13.

```
#Example: assumption Exponential distribution, prof. lik. CI, one-sided

# …
#re-parameterized log-likelihood
#BMR(absolute value)
h<-5
#x=(alpha0,BMD)
f=function(x){delta*x[1]+deltad*(log((-log(0.5))*exp(x[1])-h)-log(-
log(0.5))-x[1])/x[2]+t0*exp(-x[1])+t1*exp(-x[1]-(log((-log(0.5))*exp(x[1])-
h)-log(-log(0.5))-x[1])/x[2]*1)+t10*exp(-x[1]-(log((-log(0.5))*exp(x[1])-
h)-log(-log(0.5))-x[1])/x[2]*10)}

nlm<-nlm(f,c(5,3))
esta0<-nlm$est[1]
estBMD<-nlm$est[2]
```



```
# profile likelihood CI
start<-0.1
end<-7
step<-0.001
BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)

Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x+deltad*(log((-log(0.5))*exp(x)-h)-log(-
log(0.5))-x)/BMDgrid[i]+t0*exp(-x)+t1*exp(-x-(log((-log(0.5))*exp(x)-h)-
log(-log(0.5))-x)/BMDgrid[i]*1)+t10*exp(-x-(log((-log(0.5))*exp(x)-h)-log(-
log(0.5))-x)/BMDgrid[i]*10)}
       nlmResult<-nlm(f,5)
       res[i]<-nlmResult$estimate
       }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),BMDgrid)

profilLoglik<-c(1:k)
func<-function(x){delta*x[1]+deltad*(log((-log(0.5))*exp(x[1])-h)-log(-
log(0.5))-x[1])/x[2]+t0*exp(-x[1])+t1*exp(-x[1]-(log((-log(0.5))*exp(x[1])-
h)-log(-log(0.5))-x[1])/x[2]*1)+t10*exp(-x[1]-(log((-log(0.5))*exp(x[1])-
h)-log(-log(0.5))-x[1])/x[2]*10)}


for(i in 1:k)
      {x<-c(y[1,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)

for(i in 1:k)
      {x<-c(esta0,estBMD)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML

plot(BMDgrid, relProfvals, type="l", main="Rel. Profile Likelihood for
Example data set, BMR(abs) h=5",xlab=expression(BMD),
ylab=expression(tilde(l)[p](BMD)))
abline(h=-1/2*qchisq(0.9,1),lty=2)

# Find BMDL
a<-relProfvals+1/2*qchisq(0.9,1)
index=1
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }

BMDL<-start+(index-1)*step
BMDL
# …
```

Fig. 15.

```
#Example: assumption Exponential distribution, prof. lik. CI, one-sided

# …
##Re-parameterized log-likelihood
#BMR(rel): 10% loss in median survival

h<-0.10
#x=(alpha0,BMD)
f=function(x){delta*x[1]+deltad*(log(1-h)/x[2])+t0*exp(-x[1])+t1*exp(-x[1]-
(log(1-h)/x[2]))+t10*exp(-x[1]-10*(log(1-h)/x[2]))}

nlm<-nlm(f,c(5,50))
esta0<-nlm$est[1]
estBMD<-nlm$est[2]

# profile likelihood CI
start<-0.25
end<-2
step<-0.001
BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)
Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x+deltad*(log(1-h)/BMDgrid[i])+t0*exp(-
x)+t1*exp(-x-(log(1-h)/BMDgrid[i]))+t10*exp(-x-10*(log(1-h)/BMDgrid[i]))}
       nlmResult<-nlm(f,5)
       res[i]<-nlmResult$estimate
       }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),BMDgrid)

profilLoglik<-c(1:k)
func<-function(x){delta*x[1]+deltad*(log(1-h)/x[2])+t0*exp(-x[1])+t1*exp(-
x[1]-(log(1-h)/x[2]))+t10*exp(-x[1]-10*(log(1-h)/x[2]))}

for(i in 1:k)
      {x<-c(y[1,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)
for(i in 1:k)
      {x<-c(esta0,estBMD)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML

plot(BMDgrid, relProfvals, type="l", main="Rel. Profile Likelihood for
Example data set, BMR(rel) h=0.1",xlab=expression(BMD),
ylab=expression(tilde(l)[p](BMD)))
abline(h=-1/2*qchisq(0.9,1),lty=2)

# Find BMDL
a<-relProfvals+1/2*qchisq(0.9,1)
```



```
index=1
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }

BMDL<-start+(index-1)*step
BMDL

# …
```

Fig. 16.

```
#Example: assumption Weibull distribution
#MLE re-parameterized, BMR(abs) in median surv
#profile likelihood CI, one-sided

# …
#Tools for loglikelihood
delta<-sum(status)
deltad<-sum(status*dose)

#re-parameterized log-likelihood

#BMR(absolute value)
h<-5
#x=(alpha0,BMD,p)
f=function(x){delta*x[3]*x[1]+deltad*x[3]*((log(exp(x[1])*(-
log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2])+(sum(weeks[dose==0]^x[3]))*exp(-
x[3]*x[1])+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-
x[3]*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-
x[3]*10*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))-delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}

nlm<-nlm(f,c(4,1,1))
esta0<-nlm$est[1]
estBMD<-nlm$est[2]
estp<-nlm$est[3]

# profile likelihood CI
start<-0.1
end<-12
step<-0.001
BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)

#x=(alpha0,p)
Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x[2]*x[1]+deltad*x[2]*((log(exp(x[1])*(-
log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i])+(sum(weeks[dose==0]^x[2]))*exp(-
x[2]*x[1])+(sum(weeks[dose==1]^x[2]))*exp(-x[2]*x[1]-
x[2]*((log(exp(x[1])*(-log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i]))+(sum(weeks[dose==10]^x[2]))*exp(-x[2]*x[1]-
```

```
x[2]*10*((log(exp(x[1])*(-log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i]))-delta*log(x[2])-(x[2]-1)*sum(status*log(weeks))}
       nlmResult<-nlm(f,c(4,1))
       res[i]<-nlmResult$estimate[1]
      }
return(res)
}

Wertp<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x[2]*x[1]+deltad*x[2]*((log(exp(x[1])*(-
log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i])+(sum(weeks[dose==0]^x[2]))*exp(-
x[2]*x[1])+(sum(weeks[dose==1]^x[2]))*exp(-x[2]*x[1]-
x[2]*((log(exp(x[1])*(-log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i]))+(sum(weeks[dose==10]^x[2]))*exp(-x[2]*x[1]-
x[2]*10*((log(exp(x[1])*(-log(0.5))^(1/x[2])-h)-(1/x[2])*log(-log(0.5))-
x[1])/BMDgrid[i]))-delta*log(x[2])-(x[2]-1)*sum(status*log(weeks))}
       nlmResult<-nlm(f,c(4,1))
       res[i]<-nlmResult$estimate[2]
      }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),Wertp(BMDgrid),BMDgrid)


profilLoglik<-c(1:k)
func<-function(x){delta*x[3]*x[1]+deltad*x[3]*((log(exp(x[1])*(-
log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2])+(sum(weeks[dose==0]^x[3]))*exp(-
x[3]*x[1])+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-
x[3]*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-
x[3]*10*((log(exp(x[1])*(-log(0.5))^(1/x[3])-h)-(1/x[3])*log(-log(0.5))-
x[1])/x[2]))-delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}

for(i in 1:k)
      {x<-c(y[1,i],y[3,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)
for(i in 1:k)
      {x<-c(esta0,estBMD,estp)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML

plot(BMDgrid, relProfvals, type="l", main="Rel. Profile Likelihood for
Example dataset(Weibull), BMR(abs) h=5",xlab=expression(BMD),
ylab=expression(tilde(l)[p](BMD)))
abline(h=-1/2*qchisq(0.9,1),lty=2)

# Find BMDL
a<-relProfvals+1/2*qchisq(0.9,1)

index=1
```



```
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }

BMDL<-start+(index-1)*step
BMDL

# …
```

Fig. 17.

```
#Example: assumption Weibull distribution
#MLE re-parameterized, BMR(rel) in every quantale of surv 10%
#profile likelihood CI, one-sided

# …
##Re-parameterized log likelihood
#BMR(rel): 10% loss in median survival
h<-0.1
#x=(alpha0,BMD,p)

f=function(x){delta*x[3]*x[1]+deltad*x[3]*(log(1-
h)/x[2])+(sum(weeks[dose==0]^x[3]))*exp(-
x[3]*x[1])+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-1*x[3]*(log(1-
h)/x[2]))+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-10*x[3]*(log(1-
h)/x[2]))-delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}

nlm<-nlm(f,c(3,2,1))
esta0<-nlm$est[1]
estBMD<-nlm$est[2]
estp<-nlm$est[3]

# profile likelihood CI
start<-0.2
end<-10
step<-0.001
BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)

#x=(alpha0,p)
Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x[2]*x[1]+deltad*x[2]*(log(1-
h)/BMDgrid[i])+(sum(weeks[dose==0]^x[2]))*exp(-
x[2]*x[1])+(sum(weeks[dose==1]^x[2]))*exp(-x[2]*x[1]-1* x[2]*(log(1-
h)/BMDgrid[i]))+(sum(weeks[dose==10]^x[2]))*exp(-x[2]*x[1]-10*x[2]*(log(1-
h)/BMDgrid[i]))-delta*log(x[2])-(x[2]-1)*sum(status*log(weeks))}
       nlmResult<-nlm(f,c(3,1))
       res[i]<-nlmResult$estimate[1]
       }
return(res)
}

Wertp<-function(BMD)
{
res<-BMD
for (i in 1:k)
```

```
      {
       f=function(x){delta*x[2]*x[1]+deltad*x[2]*(log(1-
h)/BMDgrid[i])+(sum(weeks[dose==0]^x[2]))*exp(-
x[2]*x[1])+(sum(weeks[dose==1]^x[2]))*exp(-x[2]*x[1]-1* x[2]*(log(1-
h)/BMDgrid[i]))+(sum(weeks[dose==10]^x[2]))*exp(-x[2]*x[1]-10*x[2]*(log(1-
h)/BMDgrid[i]))-delta*log(x[2])-(x[2]-1)*sum(status*log(weeks))}
       nlmResult<-nlm(f,c(3,1))
       res[i]<-nlmResult$estimate[2]
       }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),Wertp(BMDgrid),BMDgrid)


profilLoglik<-c(1:k)
func<-function(x){delta*x[3]*x[1]+deltad*x[3]*(log(1-
h)/x[2])+(sum(weeks[dose==0]^x[3]))*exp(-
x[3]*x[1])+(sum(weeks[dose==1]^x[3]))*exp(-x[3]*x[1]-1* x[3]*(log(1-
h)/x[2]))+(sum(weeks[dose==10]^x[3]))*exp(-x[3]*x[1]-10*x[3]*(log(1-
h)/x[2]))-delta*log(x[3])-(x[3]-1)*sum(status*log(weeks))}

for(i in 1:k)
      {x<-c(y[1,i],y[3,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)
for(i in 1:k)
      {x<-c(esta0,estBMD,estp)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML

plot(BMDgrid, relProfvals, type="l", main="Rel. Profile Likelihood for
Example data set (Weibull), BMR(rel) h=0.1",xlab=expression(BMD),
ylab=expression(tilde(l)[p](BMD)))
abline(h=-1/2*qchisq(0.9,1),lty=2)

# Find BMDL

a<-relProfvals+1/2*qchisq(0.9,1)

index=1
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }

BMDL<-start+(index-1)*step
BMDL
# …
```

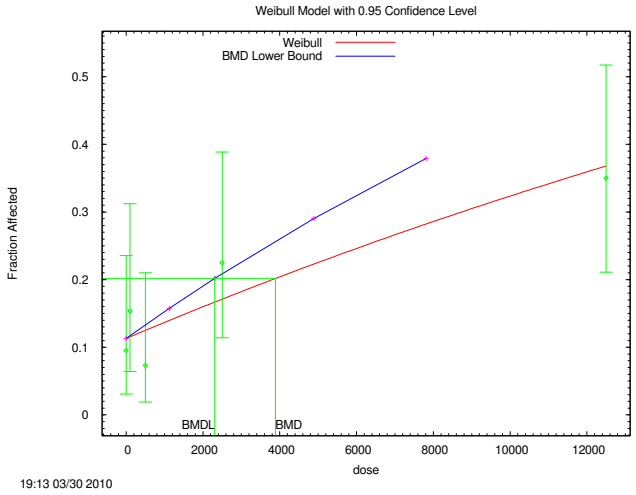
Mouse male (dichotomous model: Weibull), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weimousemaleSetting.plt
                                      Tue Mar 30 20:16:59 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =     0.104651
                        Slope = 2.60708e-005
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             Background        Slope

Background            1        -0.42

     Slope        -0.42            1


                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background         0.113047        0.0287171           0.0567621
0.169331
          Slope     2.70943e-005     1.01445e-005        7.21144e-006
4.69771e-005
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -87.909         5
   Fitted model         -89.2243        2       2.63049      3
0.4522
  Reduced model         -94.6734        1       13.5288      4
0.008961

           AIC:         182.449


                                Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected    Observed      Size        Residual
  ----------------------------------------------------------------------
     0.0000      0.1130         4.748      4.000          42       -0.364
   100.0000      0.1154         4.502      6.000          39        0.750
   500.0000      0.1250         5.124      3.000          41       -1.003
  2500.0000      0.1711         6.845      9.000          40        0.905
 12500.0000      0.3679        14.714     14.000          40       -0.234

 Chi^2 = 2.58      d.f. = 3        P-value = 0.4618


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10617.8

            BMDL =       6300.18
```

BMR=10%



```
 ================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weimousemaleSetting.plt
                                      Tue Mar 30 20:13:12 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =     0.104651
                         Slope = 2.60708e-005
                         Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.42

     Slope        -0.42            1


                                Parameter Estimates

                                                        95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
    Background         0.113047        0.0287171           0.0567621
0.169331
         Slope     2.70943e-005     1.01445e-005        7.21144e-006
4.69771e-005
         Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                       Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -87.909         5
   Fitted model        -89.2243         2       2.63049      3
0.4522
  Reduced model        -94.6734         1       13.5288      4
0.008961

           AIC:         182.449


                                  Goodness  of  Fit
                                                                      Scaled
     Dose      Est._Prob.     Expected    Observed      Size       Residual
   ---------------------------------------------------------------------------
    0.0000      0.1130         4.748      4.000          42       -0.364
  100.0000      0.1154         4.502      6.000          39        0.750
  500.0000      0.1250         5.124      3.000          41       -1.003
 2500.0000      0.1711         6.845      9.000          40        0.905
12500.0000      0.3679        14.714     14.000          40       -0.234

 Chi^2 = 2.58      d.f. = 3        P-value = 0.4618


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3888.66

            BMDL =       2307.37
```

BMR=5%

```
 ================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weimousemaleSetting.plt
                                      Tue Mar 30 20:15:39 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                 Default Initial (and Specified) Parameter Values
                    Background =     0.104651
                         Slope = 2.60708e-005
                         Power =            1
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.42
Slope	-0.42	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.113047	0.0287171	0.0567621	0.169331
Slope	2.70943e-005	1.01445e-005	7.21144e-006	4.69771e-005
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-87.909	5			
Fitted model	-89.2243	2	2.63049	3	0.4522
Reduced model	-94.6734	1	13.5288	4	0.008961

AIC: 182.449

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1130	4.748	4.000	42	-0.364
100.0000	0.1154	4.502	6.000	39	0.750
500.0000	0.1250	5.124	3.000	41	-1.003
2500.0000	0.1711	6.845	9.000	40	0.905
12500.0000	0.3679	14.714	14.000	40	-0.234

Chi^2 = 2.58 d.f. = 3 P-value = 0.4618

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1893.14

BMDL = 1123.31

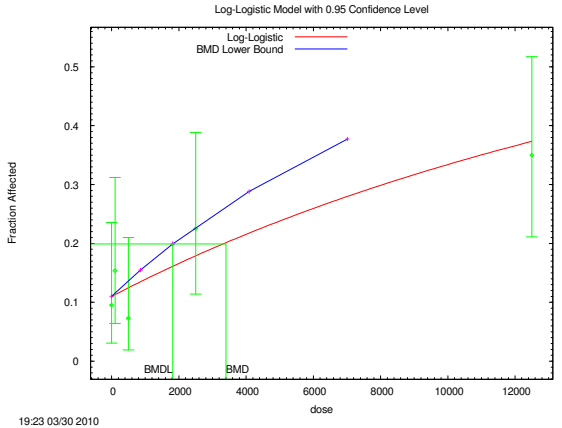
Mouse male (dichotomous model: Log-logistic), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlmousemaleSetting.plt
                                          Tue Mar 30 20:21:14 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0952381
                    intercept =      -10.356
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             background    intercept

background            1        -0.46

 intercept        -0.46            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background          0.11033            *               *
*
      intercept         -10.3283            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -87.909         5
   Fitted model         -89.1439        2       2.46976      3
0.4808
  Reduced model         -94.6734        1       13.5288      4
0.008961

           AIC:         182.288


                              Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected     Observed      Size       Residual
  ----------------------------------------------------------------------
     0.0000      0.1103         4.634      4.000           42       -0.312
   100.0000      0.1132         4.416      6.000           39        0.800
   500.0000      0.1246         5.110      3.000           41       -0.998
  2500.0000      0.1776         7.102      9.000           40        0.785
 12500.0000      0.3684        14.738     14.000           40       -0.242

 Chi^2 = 2.41      d.f. = 3        P-value = 0.4920


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10195.1

            BMDL =        5454.64
```

BMR=10%




```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousemaleSetting.(d)
```

```
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlmousemaleSetting.plt
                                            Tue Mar 30 20:23:06 2010
 ========================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0952381
                     intercept =      -10.356
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.46

 intercept        -0.46            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background          0.11033            *               *
*
      intercept         -10.3283            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -87.909         5
   Fitted model        -89.1439         2       2.46976      3
0.4808
  Reduced model        -94.6734         1       13.5288      4
```

```
0.008961

           AIC:         182.288

                                  Goodness  of  Fit
                                                                    Scaled
     Dose      Est._Prob.     Expected    Observed      Size       Residual
  ---------------------------------------------------------------------------
    0.0000      0.1103          4.634      4.000          42       -0.312
  100.0000      0.1132          4.416      6.000          39        0.800
  500.0000      0.1246          5.110      3.000          41       -0.998
 2500.0000      0.1776          7.102      9.000          40        0.785
12500.0000      0.3684         14.738     14.000          40       -0.242

 Chi^2 = 2.41      d.f. = 3        P-value = 0.4920


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3398.36

            BMDL =        1818.21
```

BMR=5%

```
 ========================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlmousemaleSetting.plt
                                            Tue Mar 30 20:23:53 2010
 ========================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                 Default Initial Parameter Values
                    background =    0.0952381
                     intercept =      -10.356
                         slope =            1
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been
specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.46
intercept	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Lower Conf. Limit	Upper Conf. Limit
background	0.11033	*	*	*
intercept	-10.3283	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-87.909	5			
Fitted model	-89.1439	2	2.46976	3	0.4808
Reduced model	-94.6734	1	13.5288	4	0.008961

AIC: 182.288

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1103	4.634	4.000	42	-0.312
100.0000	0.1132	4.416	6.000	39	0.800
500.0000	0.1246	5.110	3.000	41	-0.998
2500.0000	0.1776	7.102	9.000	40	0.785
12500.0000	0.3684	14.738	14.000	40	-0.242

Chi^2 = 2.41 d.f. = 3 P-value = 0.4920

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1609.75

BMDL = 861.258

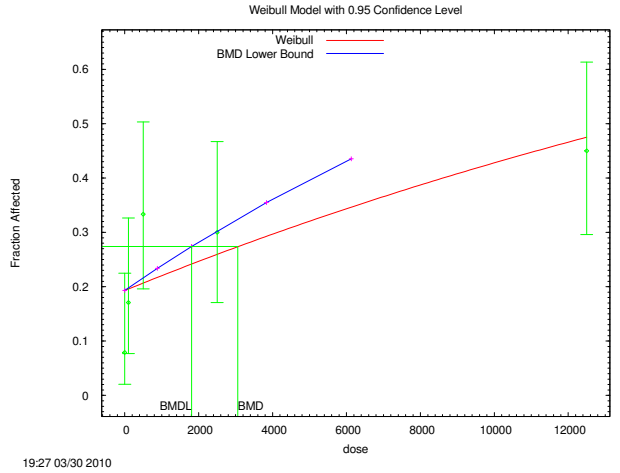
Mouse female (dichotomous model: Weibull), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\weimousefemaleSetting.plt
                                        Tue Mar 30 20:26:30 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0897436
                        Slope = 4.04822e-005
                        Power =           1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
               have been estimated at a boundary point, or have been
specified by the user,
               and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.46

     Slope        -0.46            1


                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background         0.193359        0.0363137            0.122186
0.264533
          Slope     3.44303e-005     1.30704e-005        8.81282e-006
6.00477e-005
          Power                1              NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -106.018         5
   Fitted model        -109.952         2       7.86813      3
0.04882
  Reduced model        -115.025         1       18.0135      4
0.001227

           AIC:         223.905


                                Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected    Observed      Size        Residual
  ---------------------------------------------------------------------------
     0.0000      0.1934         7.348      3.000           38       -1.786
   100.0000      0.1961         8.041      7.000           41       -0.410
   500.0000      0.2071         8.078     13.000           39        1.945
  2500.0000      0.2599        10.395     12.000           40        0.578
 12500.0000      0.4755        19.019     18.000           40       -0.323

 Chi^2 = 7.58      d.f. = 3        P-value = 0.0556


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         8355.5

            BMDL =        4942.66
```

BMR=10%




```
 ======================================================================
```

```
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\weimousefemaleSetting.plt
                                                Tue Mar 30 20:27:45 2010
 ================================================================

 BMDS Model Run
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   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0897436
                        Slope = 4.04822e-005
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.46

     Slope        -0.46            1


                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background         0.193359        0.0363137            0.122186
0.264533
          Slope     3.44303e-005     1.30704e-005        8.81282e-006
6.00477e-005
          Power                1              NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -106.018         5
   Fitted model        -109.952         2       7.86813      3
0.04882
  Reduced model        -115.025         1       18.0135      4
0.001227

           AIC:         223.905


                                Goodness  of  Fit
                                                                    Scaled
     Dose      Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.1934         7.348     3.000          38       -1.786
  100.0000      0.1961         8.041     7.000          41       -0.410
  500.0000      0.2071         8.078    13.000          39        1.945
 2500.0000      0.2599        10.395    12.000          40        0.578
12500.0000      0.4755        19.019    18.000          40       -0.323

 Chi^2 = 7.58      d.f. = 3        P-value = 0.0556


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3060.11

            BMDL =        1810.2
```

BMR=5%

```
 ================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weimousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\weimousefemaleSetting.plt
                                                Tue Mar 30 20:28:30 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0897436
                        Slope = 4.04822e-005
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates
```

(*** The model parameter(s) -Power
have been estimated at a boundary point, or have been
specified by the user,
and do not appear in the correlation matrix)

	Background	Slope
Background	1	-0.46
Slope	-0.46	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.193359	0.0363137	0.122186	0.264533
Slope	3.44303e-005	1.30704e-005	8.81282e-006	6.00477e-005
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-106.018	5			
Fitted model	-109.952	2	7.86813	3	0.04882
Reduced model	-115.025	1	18.0135	4	0.001227

AIC: 223.905

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.1934	7.348	3.000	38	-1.786
100.0000	0.1961	8.041	7.000	41	-0.410
500.0000	0.2071	8.078	13.000	39	1.945
2500.0000	0.2599	10.395	12.000	40	0.578
12500.0000	0.4755	19.019	18.000	40	-0.323

Chi^2 = 7.58 d.f. = 3 P-value = 0.0556

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1489.77

BMDL = 881.269

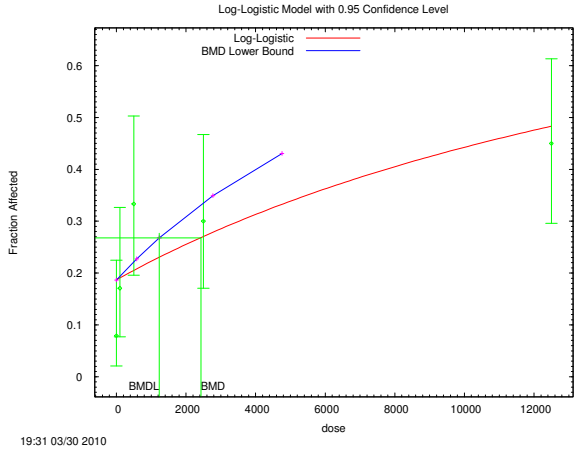
Mouse female (dichotomous model: Log-logistic), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.plt
                                        Tue Mar 30 20:30:13 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =    0.0789474
                     intercept =     -9.35842
                         slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.52

 intercept        -0.52            1


                                Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background         0.186714            *               *
*
      intercept         -9.99274            *               *
*
          slope                1            *               *
*
```

```
* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -106.018         5
   Fitted model        -109.763         2        7.4894      3
0.05783
  Reduced model        -115.025         1       18.0135      4
0.001227

           AIC:         223.526


                                Goodness  of  Fit
                                                                  Scaled
    Dose      Est._Prob.     Expected    Observed      Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.1867         7.095      3.000          38       -1.705
  100.0000      0.1904         7.807      7.000          41       -0.321
  500.0000      0.2049         7.991     13.000          39        1.987
 2500.0000      0.2702        10.806     12.000          40        0.425
12500.0000      0.4825        19.301     18.000          40       -0.412

 Chi^2 = 7.31      d.f. = 3        P-value = 0.0627


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        7289.02

            BMDL =        3699.77
```

BMR=10%



```
=====================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.plt
                                          Tue Mar 30 20:31:18 2010
 =====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0789474
                    intercept =     -9.35842
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.52

 intercept        -0.52            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background         0.186714            *               *
*
      intercept         -9.99274            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -106.018         5
   Fitted model        -109.763         2        7.4894      3
0.05783
  Reduced model        -115.025         1       18.0135      4
0.001227

           AIC:         223.526


                                Goodness  of  Fit
                                                                    Scaled
     Dose      Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
     0.0000      0.1867         7.095     3.000          38       -1.705
   100.0000      0.1904         7.807     7.000          41       -0.321
   500.0000      0.2049         7.991    13.000          39        1.987
  2500.0000      0.2702        10.806    12.000          40        0.425
 12500.0000      0.4825        19.301    18.000          40       -0.412

 Chi^2 = 7.31      d.f. = 3        P-value = 0.0627


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        2429.67

            BMDL =        1233.26
```

BMR=5%

```
 =====================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.(d)
        Gnuplot Plotting File:
C:\USEPA\BMDS21\Data\lnlmousefemaleSetting.plt
                                          Tue Mar 30 20:32:03 2010
 =====================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0789474
```

```
                    intercept =     -9.35842
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

           ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             background    intercept

background            1        -0.52

 intercept        -0.52            1


                                  Parameter Estimates

                                                          95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background         0.186714            *                *
*
      intercept         -9.99274            *                *
*
          slope                1            *                *
*

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -106.018         5
   Fitted model        -109.763         2        7.4894      3
0.05783
  Reduced model        -115.025         1       18.0135      4
0.001227

           AIC:         223.526


                                  Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.1867         7.095     3.000          38       -1.705
  100.0000     0.1904         7.807     7.000          41       -0.321
  500.0000     0.2049         7.991    13.000          39        1.987
 2500.0000     0.2702        10.806    12.000          40        0.425
12500.0000     0.4825        19.301    18.000          40       -0.412

 Chi^2 = 7.31      d.f. = 3        P-value = 0.0627


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         1150.9

            BMDL =        584.175
```

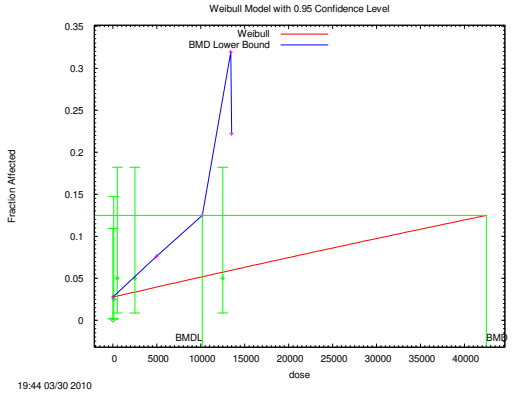
Rat male (dichotomous model: Weibull), BMDS 2.1.

BMR=25%

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratmaleSetting.plt
                                          Tue Mar 30 20:43:17 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



                Default Initial (and Specified) Parameter Values
                   Background =    0.0121951
                        Slope =  4.0515e-006
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.52

     Slope        -0.52            1



                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background        0.0275621         0.014861        -0.00156489
0.056689
          Slope     2.48015e-006     3.68816e-006       -4.74852e-006
9.70881e-006
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.4981         5
   Fitted model        -30.0531         2       3.11003      3
0.375
  Reduced model        -30.3429         1       3.68959      4
0.4496

           AIC:         64.1062


                                Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected     Observed      Size       Residual
  ----------------------------------------------------------------------
    0.0000      0.0276         1.102      0.000          40       -1.065
  100.0000      0.0278         1.112      1.000          40       -0.108
  500.0000      0.0288         1.151      2.000          40        0.803
 2500.0000      0.0336         1.343      2.000          40        0.577
12500.0000      0.0572         2.290      2.000          40       -0.197

 Chi^2 = 2.16      d.f. = 3        P-value = 0.5394


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         115994

            BMDL =       13250.6
```

BMR=10%




```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratmaleSetting.plt
                                          Tue Mar 30 20:44:50 2010
 ======================================================================
```

```
 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0121951
                        Slope =  4.0515e-006
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.52

     Slope        -0.52            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
      Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
    Background        0.0275621         0.014861        -0.00156489
0.056689
         Slope     2.48015e-006     3.68816e-006       -4.74852e-006
9.70881e-006
         Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                     Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model         -28.4981          5
   Fitted model         -30.0531          2       3.11003      3
0.375
  Reduced model         -30.3429          1       3.68959      4
0.4496

          AIC:          64.1062
```

```
                                Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.    Expected    Observed     Size        Residual
  ---------------------------------------------------------------------------
     0.0000      0.0276         1.102       0.000       40        -1.065
   100.0000      0.0278         1.112       1.000       40        -0.108
   500.0000      0.0288         1.151       2.000       40         0.803
  2500.0000      0.0336         1.343       2.000       40         0.577
 12500.0000      0.0572         2.290       2.000       40        -0.197

 Chi^2 = 2.16      d.f. = 3        P-value = 0.5394


   Benchmark Dose Computation

Specified effect =           0.1

Risk Type        =      Extra risk

Confidence level =          0.95

             BMD =       42481.6

            BMDL =       10178.3
```

BMR=5%

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratmaleSetting.plt
                                                  Tue Mar 30 20:45:33 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0121951
                        Slope =  4.0515e-006
                        Power =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.52
```

Slope -0.52 1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0275621	0.014861	-0.00156489	0.056689
Slope	2.48015e-006	3.68816e-006	-4.74852e-006	9.70881e-006
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.4981	5			
Fitted model	-30.0531	2	3.11003	3	0.375
Reduced model	-30.3429	1	3.68959	4	0.4496

AIC: 64.1062

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0276	1.102	0.000	40	-1.065
100.0000	0.0278	1.112	1.000	40	-0.108
500.0000	0.0288	1.151	2.000	40	0.803
2500.0000	0.0336	1.343	2.000	40	0.577
12500.0000	0.0572	2.290	2.000	40	-0.197

Chi^2 = 2.16 d.f. = 3 P-value = 0.5394

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 20681.6

BMDL = 4955.17

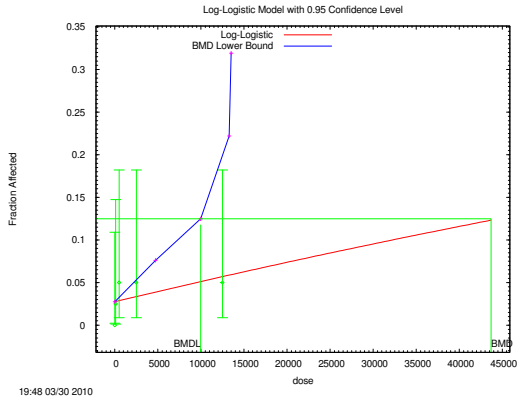
Rat male (dichotomous model: Log-logistic), BMDS 2.1.

BMR=25%

```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratmaleSetting.plt
                                          Tue Mar 30 20:47:10 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model

                 Default Initial Parameter Values
                    background =           0
                     intercept =     -11.6339
                         slope =           1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.53

 intercept        -0.53            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background        0.0274826            *               *
*
      intercept         -12.8822            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.4981         5
   Fitted model        -30.0511         2        3.1059      3
0.3756
  Reduced model        -30.3429         1       3.68959      4
0.4496

           AIC:         64.1021


                          Goodness  of  Fit
                                                                  Scaled
     Dose      Est._Prob.    Expected    Observed      Size       Residual
  ---------------------------------------------------------------------
    0.0000      0.0275         1.099      0.000          40       -1.063
  100.0000      0.0277         1.109      1.000          40       -0.105
  500.0000      0.0287         1.149      2.000          40        0.806
 2500.0000      0.0336         1.345      2.000          40        0.574
12500.0000      0.0574         2.298      2.000          40       -0.202

 Chi^2 = 2.16      d.f. = 3        P-value = 0.5395


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =         131081

            BMDL =        13322.5
```

BMR=10%




```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
```

```
        Input Data File: C:\USEPA\BMDS21\Data\lnlratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratmaleSetting.plt
                                          Tue Mar 30 20:48:24 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =           0
                    intercept =     -11.6339
                        slope =           1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.53

 intercept        -0.53            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background        0.0274826            *               *
*
      intercept         -12.8822            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -28.4981         5
   Fitted model        -30.0511         2        3.1059      3
0.3756
  Reduced model        -30.3429         1       3.68959      4
0.4496

           AIC:         64.1021


                                  Goodness  of  Fit
                                                                      Scaled
    Dose      Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
     0.0000      0.0275         1.099      0.000          40       -1.063
   100.0000      0.0277         1.109      1.000          40       -0.105
   500.0000      0.0287         1.149      2.000          40        0.806
  2500.0000      0.0336         1.345      2.000          40        0.574
 12500.0000      0.0574         2.298      2.000          40       -0.202

 Chi^2 = 2.16      d.f. = 3        P-value = 0.5395


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        43693.6

            BMDL =         9970.4
```

BMR=5%

```
 ================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlratmaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratmaleSetting.plt
                                          Tue Mar 30 20:49:07 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =           0
                    intercept =     -11.6339
                        slope =           1
```

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -slope
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	background	intercept
background	1	-0.53
intercept	-0.53	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Lower Conf. Limit	Upper Conf. Limit
background	0.0274826	*	*	*
intercept	-12.8822	*	*	*
slope	1	*	*	*

* - Indicates that this value is not calculated.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-28.4981	5			
Fitted model	-30.0511	2	3.1059	3	0.3756
Reduced model	-30.3429	1	3.68959	4	0.4496

AIC: 64.1021

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0275	1.099	0.000	40	-1.063
100.0000	0.0277	1.109	1.000	40	-0.105
500.0000	0.0287	1.149	2.000	40	0.806
2500.0000	0.0336	1.345	2.000	40	0.574
12500.0000	0.0574	2.298	2.000	40	-0.202

Chi^2 = 2.16 d.f. = 3 P-value = 0.5395

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 20696.9

BMDL = 4722.82

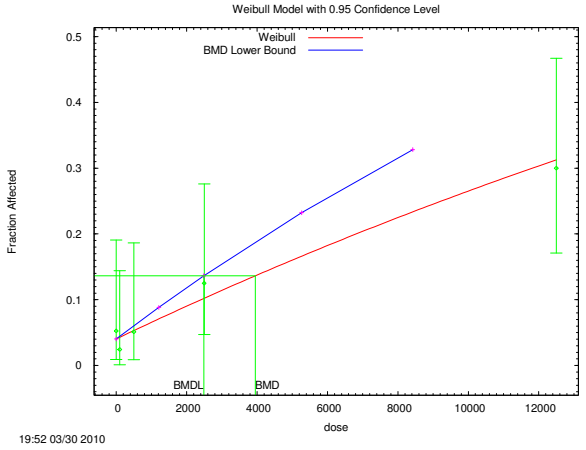
Rat female (dichotomous model: Weibull), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratfemaleSetting.plt
                                      Tue Mar 30 20:51:52 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0641026
                        Slope = 1.92824e-005
                        Power =      1.02228


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             Background        Slope

Background            1        -0.37

     Slope        -0.37            1


                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background        0.0403044        0.0192118          0.00264992
0.077959
          Slope     2.66754e-005     8.49096e-006        1.00334e-005
4.33174e-005
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -59.9306         5
   Fitted model        -60.3218         2      0.782315      3
0.8537
  Reduced model        -69.0688         1       18.2763      4
0.00109

          AIC:         124.644


                                Goodness  of  Fit
                                                                    Scaled
    Dose      Est._Prob.     Expected     Observed      Size       Residual
  ---------------------------------------------------------------------------
    0.0000      0.0403         1.532      2.000          38         0.386
  100.0000      0.0429         1.757      1.000          41        -0.584
  500.0000      0.0530         2.068      2.000          39        -0.048
 2500.0000      0.1022         4.089      5.000          40         0.476
12500.0000      0.3124        12.497     12.000          40        -0.170

 Chi^2 = 0.75      d.f. = 3        P-value = 0.8620


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10784.5

            BMDL =        6791.52
```

BMR=10%




```
 ======================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratfemaleSetting.plt
                                      Tue Mar 30 20:52:50 2010
```

```
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0641026
                        Slope = 1.92824e-005
                        Power =      1.02228


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope

Background            1        -0.37

     Slope        -0.37            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     Background        0.0403044        0.0192118          0.00264992
0.077959
          Slope     2.66754e-005     8.49096e-006        1.00334e-005
4.33174e-005
          Power                1               NA

NA - Indicates that this parameter has hit a bound
     implied by some inequality constraint and thus
     has no standard error.


                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -59.9306         5
   Fitted model        -60.3218         2      0.782315      3
0.8537
  Reduced model        -69.0688         1       18.2763      4
0.00109

           AIC:         124.644
```

```
                                  Goodness  of  Fit
                                                                    Scaled
     Dose      Est._Prob.    Expected    Observed      Size       Residual
 ----------------------------------------------------------------------
      0.0000      0.0403       1.532       2.000          38        0.386
    100.0000      0.0429       1.757       1.000          41       -0.584
    500.0000      0.0530       2.068       2.000          39       -0.048
   2500.0000      0.1022       4.089       5.000          40        0.476
  12500.0000      0.3124      12.497      12.000          40       -0.170

 Chi^2 = 0.75      d.f. = 3        P-value = 0.8620


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3949.72

            BMDL =       2487.32
```

BMR=5%

```
 ================================================================
        Weibull Model using Weibull Model (Version: 2.12;  Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\weiratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\weiratfemaleSetting.plt
                                            Tue Mar 30 20:53:32 2010
 ================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background + (1-background)*[1-EXP(-slope*dose^power)]


   Dependent variable = resp
   Independent variable = dose
   Power parameter is restricted as power >=1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


                Default Initial (and Specified) Parameter Values
                   Background =    0.0641026
                        Slope = 1.92824e-005
                        Power =      1.02228


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -Power
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            Background        Slope
```

Background	1	-0.37
Slope	-0.37	1

Parameter Estimates

Variable	Estimate	Std. Err.	95.0% Wald Confidence Interval Lower Conf. Limit	Upper Conf. Limit
Background	0.0403044	0.0192118	0.00264992	0.077959
Slope	2.66754e-005	8.49096e-006	1.00334e-005	4.33174e-005
Power	1	NA		

NA - Indicates that this parameter has hit a bound
implied by some inequality constraint and thus
has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	# Param's	Deviance	Test d.f.	P-value
Full model	-59.9306	5			
Fitted model	-60.3218	2	0.782315	3	0.8537
Reduced model	-69.0688	1	18.2763	4	0.00109

AIC: 124.644

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Scaled Residual
0.0000	0.0403	1.532	2.000	38	0.386
100.0000	0.0429	1.757	1.000	41	-0.584
500.0000	0.0530	2.068	2.000	39	-0.048
2500.0000	0.1022	4.089	5.000	40	0.476
12500.0000	0.3124	12.497	12.000	40	-0.170

Chi^2 = 0.75 d.f. = 3 P-value = 0.8620

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 1922.87

BMDL = 1210.92

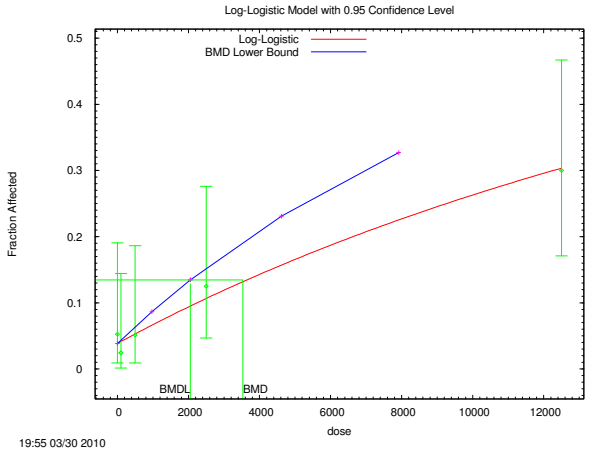
Rat female (dichotomous model: Log-logistic), software: BMDS 2.1.

BMR=25%

```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratfemaleSetting.plt
                                          Tue Mar 30 20:54:58 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0526316
                    intercept =      -10.536
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             background    intercept

background            1        -0.39

 intercept        -0.39            1


                              Parameter Estimates

                                                    95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background        0.0385894            *               *
*
      intercept         -10.3671            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.
```

```
                        Analysis of Deviance Table

      Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -59.9306         5
   Fitted model        -60.2635         2      0.665837      3
0.8812
  Reduced model        -69.0688         1       18.2763      4
0.00109

           AIC:         124.527


                          Goodness  of  Fit
                                                                  Scaled
    Dose      Est._Prob.     Expected     Observed      Size      Residual
  ----------------------------------------------------------------------
     0.0000      0.0386        1.466      2.000          38         0.449
   100.0000      0.0416        1.706      1.000          41        -0.552
   500.0000      0.0535        2.085      2.000          39        -0.061
  2500.0000      0.1087        4.347      5.000          40         0.332
 12500.0000      0.3099       12.396     12.000          40        -0.135

 Chi^2 = 0.64      d.f. = 3        P-value = 0.8875


   Benchmark Dose Computation

Specified effect =           0.25

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        10598.7

            BMDL =        6158.49
```

BMR=10%



```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratfemaleSetting.plt
                                        Tue Mar 30 20:55:59 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0526316
                    intercept =      -10.536
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

             background    intercept

background            1        -0.39

 intercept        -0.39            1


                                Parameter Estimates

                                                      95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background        0.0385894            *               *
*
      intercept         -10.3671            *               *
*
          slope                1            *               *
*

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
```

```
     Full model        -59.9306         5
   Fitted model        -60.2635         2      0.665837      3
0.8812
  Reduced model        -69.0688         1       18.2763      4
0.00109

           AIC:         124.527


                                Goodness  of  Fit
                                                                    Scaled
     Dose      Est._Prob.    Expected    Observed     Size        Residual
  ----------------------------------------------------------------------
    0.0000      0.0386         1.466      2.000          38         0.449
  100.0000      0.0416         1.706      1.000          41        -0.552
  500.0000      0.0535         2.085      2.000          39        -0.061
 2500.0000      0.1087         4.347      5.000          40         0.332
12500.0000      0.3099        12.396     12.000          40        -0.135

 Chi^2 = 0.64      d.f. = 3        P-value = 0.8875


   Benchmark Dose Computation

Specified effect =            0.1

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        3532.91

            BMDL =        2052.83
```

BMR=5%

```
 ======================================================================
        Logistic Model. (Version: 2.12; Date: 05/16/2008)
        Input Data File: C:\USEPA\BMDS21\Data\lnlratfemaleSetting.(d)
        Gnuplot Plotting File:  C:\USEPA\BMDS21\Data\lnlratfemaleSetting.plt
                                        Tue Mar 30 20:56:48 2010
 ======================================================================

 BMDS Model Run
~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

   The form of the probability function is:

   P[response] = background+(1-background)/[1+EXP(-intercept-
slope*Log(dose))]


   Dependent variable = resp
   Independent variable = dose
   Slope parameter is restricted as slope >= 1

   Total number of observations = 5
   Total number of records with missing values = 0
   Maximum number of iterations = 250
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008



   User has chosen the log transformed model


                Default Initial Parameter Values
                   background =    0.0526316
```

```
                    intercept =      -10.536
                        slope =            1


          Asymptotic Correlation Matrix of Parameter Estimates

          ( *** The model parameter(s)  -slope
                have been estimated at a boundary point, or have been
specified by the user,
                and do not appear in the correlation matrix )

            background    intercept

background            1        -0.39

 intercept        -0.39            1


                                Parameter Estimates

                                                        95.0% Wald
Confidence Interval
       Variable         Estimate        Std. Err.     Lower Conf. Limit
Upper Conf. Limit
     background        0.0385894            *                *
*
      intercept         -10.3671            *                *
*
          slope                1            *                *
*

* - Indicates that this value is not calculated.


                        Analysis of Deviance Table

       Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
     Full model        -59.9306         5
   Fitted model        -60.2635         2      0.665837      3
0.8812
  Reduced model        -69.0688         1       18.2763      4
0.00109

           AIC:         124.527


                                Goodness  of  Fit
                                                                    Scaled
     Dose     Est._Prob.    Expected    Observed     Size       Residual
  ---------------------------------------------------------------------
    0.0000     0.0386         1.466     2.000          38        0.449
  100.0000     0.0416         1.706     1.000          41       -0.552
  500.0000     0.0535         2.085     2.000          39       -0.061
 2500.0000     0.1087         4.347     5.000          40        0.332
12500.0000     0.3099        12.396    12.000          40       -0.135

 Chi^2 = 0.64      d.f. = 3        P-value = 0.8875


   Benchmark Dose Computation

Specified effect =           0.05

Risk Type        =      Extra risk

Confidence level =           0.95

             BMD =        1673.49

            BMDL =        972.393
```

Simulation study

Idea: Simulate two independent random variables '$etime$' and '$censtime$', both from an Exponential distribution but with different rates λ and δ. The random variable '$etime$' gives the time of the event and '$censtime$' gives a time point of censoring for one individual. Then, the observed time of this individual is $\min(etime,\ censtime)$.

To get data sets with a pre-specified fraction x of censorings, make use of the equation: $x = \frac{\delta}{\delta+\lambda} \Leftrightarrow \delta = \frac{x\,\lambda}{1-x}$.

The rates are re-parameterized as usual to get an Exponential regression model with predictor variable '$dose$'.

```
rm(list=ls())
library(survival)
library(splines)

sim<-function() {

#Dosisvektor
dose<-c(rep(0,40),rep(100,40),rep(500,40),rep(2500,40),rep(12500,40))

##################
#censoring 0%
#event
a0<-5
a1<--0.0001
etime0<-c(rexp(40,rate=exp(-a0-a1*0)),rexp(40,rate=exp(-a0-
a1*100)),rexp(40,rate=exp(-a0-a1*500)), rexp(40,rate=exp(-a0-a1*2500)),
rexp(40,rate=exp(-a0-a1*12500)))

ctime0<-etime0
obs0<-etime0
for (i in 1:200)
      {
       if (etime0[i]>ctime0[i]) {obs0[i]<-ctime0[i]}
      }

tumor0<-c(rep(1,200))

for (i in 1:200)
      {
       if (etime0[i]>ctime0[i]) {tumor0[i]<-0}
      }

x<-matrix(c(dose, tumor0, obs0),ncol=3)

write.table(x,file="expsim0.txt",sep=" ",row.names=FALSE,
col.names=c("UDosis","Tumor","Time"))

b<-read.table("expsim0.txt",header=T)
attach(b)

#Tools for log likelihood
delta<-sum(Tumor)
deltad<-sum(Tumor*UDosis)
```



```
t0<-sum(Time[UDosis==0])
t100<-sum(Time[UDosis==100])
t500<-sum(Time[UDosis==500])
t2500<-sum(Time[UDosis==2500])
t12500<-sum(Time[UDosis==12500])

#BMR(rel): 10% loss in median survival
h<-0.1
#x=(alpha0,BMD)
f=function(x){delta*x[1]+deltad*(log(1-h)/x[2])+t0*exp(-x[1])+t100*exp(-
x[1]-100*(log(1-h)/x[2]))+t500*exp(-x[1]-500*(log(1-h)/x[2]))+t2500*exp(-
x[1]-2500*(log(1-h)/x[2]))+t12500*exp(-x[1]-12500*(log(1-h)/x[2]))}
nlm<-nlm(f,c(5,500))
esta0<-nlm$est[1]
estBMD<-nlm$est[2]

start<-101
end<-50001
step<-100

BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)

Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x+deltad*(log(1-h)/BMDgrid[i])+t0*exp(-
x)+t100*exp(-x-100*(log(1-h)/BMDgrid[i]))+t500*exp(-x-500*(log(1-
h)/BMDgrid[i]))+t2500*exp(-x-2500*(log(1-h)/BMDgrid[i]))+t12500*exp(-x-
12500*(log(1-h)/BMDgrid[i]))}
       nlmResult<-nlm(f,5)
       res[i]<-nlmResult$estimate
       }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),BMDgrid)

profilLoglik<-c(1:k)
func<-function(x){delta*x[1]+deltad*(log(1-h)/x[2])+t0*exp(-
x[1])+t100*exp(-x[1]-100*(log(1-h)/x[2]))+t500*exp(-x[1]-500*(log(1-
h)/x[2]))+t2500*exp(-x[1]-2500*(log(1-h)/x[2]))+t12500*exp(-x[1]-
12500*(log(1-h)/x[2]))}

for(i in 1:k)
      {x<-c(y[1,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)

for(i in 1:k)
      {x<-c(esta0,estBMD)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML
# Find BMDL (rough estimate)
a<-relProfvals+1/2*qchisq(0.9,1)
index=1
```

```
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }
BMDL<-start+(index-1)*step

#smaller interval
# profile likelihood CI
start<-BMDL-100
end<-BMDL+100
step<-1
BMDgrid<-seq(from=start, to=end, by=step)
k<-length(BMDgrid)

Wertalpha0<-function(BMD)
{
res<-BMD
for (i in 1:k)
      {
       f=function(x){delta*x+deltad*(log(1-h)/BMDgrid[i])+t0*exp(-
x)+t100*exp(-x-100*(log(1-h)/BMDgrid[i]))+t500*exp(-x-500*(log(1-
h)/BMDgrid[i]))+t2500*exp(-x-2500*(log(1-h)/BMDgrid[i]))+t12500*exp(-x-
12500*(log(1-h)/BMDgrid[i]))}
       nlmResult<-nlm(f,5)
       res[i]<-nlmResult$estimate
       }
return(res)
}

y<-rbind(Wertalpha0(BMDgrid),BMDgrid)

profilLoglik<-c(1:k)
func<-function(x){delta*x[1]+deltad*(log(1-h)/x[2])+t0*exp(-
x[1])+t100*exp(-x[1]-100*(log(1-h)/x[2]))+t500*exp(-x[1]-500*(log(1-
h)/x[2]))+t2500*exp(-x[1]-2500*(log(1-h)/x[2]))+t12500*exp(-x[1]-
12500*(log(1-h)/x[2]))}

for(i in 1:k)
      {x<-c(y[1,i],y[2,i])
       profilLoglik[i]<-func(x)
      }

ProfilML<-c(1:k)

for(i in 1:k)
      {x<-c(esta0,estBMD)
       ProfilML[i]<-func(x)
      }

relProfvals<--profilLoglik+ProfilML


# Find BMDL
a<-relProfvals+1/2*qchisq(0.9,1)
index=1
for (i in 1:length(a))
      {if (a[i]>=0) {index<-i-1
                     break}
      }
BMDL<-start+(index-1)*step
BMD0<-estBMD
BMDL0<-BMDL
```



```
#########################
#censoring 10%
#event
a0<-5
a1<--0.0001
etime10<-c(rexp(40,rate=exp(-a0-a1*0)),rexp(40,rate=exp(-a0-
a1*100)),rexp(40,rate=exp(-a0-a1*500)), rexp(40,rate=exp(-a0-a1*2500)),
rexp(40,rate=exp(-a0-a1*12500)))

a0<-7.197225
a1<--0.0001003592
ctime10<-c(rexp(40,rate=exp(-a0-a1*0)),rexp(40,rate=exp(-a0-
a1*100)),rexp(40,rate=exp(-a0-a1*500)), rexp(40,rate=exp(-a0-a1*2500)),
rexp(40,rate=exp(-a0-a1*12500)))

obs10<-etime10
for (i in 1:200)
      {
       if (etime10[i]>ctime10[i]) {obs10[i]<-ctime10[i]}
      }

tumor10<-c(rep(1,200))

for (i in 1:200)
      {
       if (etime10[i]>ctime10[i]) {tumor10[i]<-0}
      }

x<-matrix(c(dose, tumor10, obs10),ncol=3)

write.table(x,file="expsim10.txt",sep=" ",row.names=FALSE,
col.names=c("UDosis","Tumor","Time"))

b<-read.table("expsim10.txt",header=T)
attach(b)

# …

BMD10<-estBMD
BMDL10<-BMDL

# …

BMD<-c(BMD0,BMD10,BMD20,BMD30,BMD40,BMD50,BMD60,BMD70,BMD80,BMD90)
BMDL<-
c(BMDL0,BMDL10,BMDL20,BMDL30,BMDL40,BMDL50,BMDL60,BMDL70,BMDL80,BMDL90)

result<-c(BMD,BMDL)
result
}

ausg<-matrix(c(rep(0,20000)),nrow=1000)
for (i in 1:1000)
      {
       ausg[i,]<-sim()
      }
# …
```