2011
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FACULTY OF SCIENCES

Master of Statistics: Biostatistics

Masterproef

Dose response assessment in a behavior learning curve experiment

Promotor :
dr. Herbert THIJS

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Master Thesis nominated to obtain the degree of Master of Statistics , specialization Biostatistics

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KNOWLEDGE IN ACTION

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At this moment I am really happy to be in the position to write the final part of my master thesis in statistics. This thesis is not a sole individual project. Some people played an import role to make this thesis what it is right now. I would like to express my sincere gratitude to those people.

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Sarah

Abstract

A new animal model of diseases affecting cognitive functioning, like schizophrenia and dementia, is designed to test the efficacy of new candidate treatments. A pharmacological deficit is induced by the administration of compound X. Subsequently, a candidate treatment is added to assess the potential for reversal of the degrading effect induced by compound X. A good understanding of the effect of compound X on cognitive functioning is therefore crucial.

Two dose response studies with compound X were pooled and a meta-analysis was performed. Longitudinal data was available on the proportion of correctly executed trails from an animal behavior experiment from 96 male wistar rats during a period of 14 days. The aim of the report is to study and quantify the dose effect of compound X on learning behavior.

First, the complete profiles were modeled by a non-linear model. The average proportions were modeled by a Weibull learning curve (Gallistel et al, 2004) and the dose effect was included in the parameters of the Weibull function while taking into account the heterogeneity between animals. The model revealed that the time until proportion 0.7 was reached (*T70*) was the most important characteristic of learning behavior to study the dose effect of compound X. *T70* prolongs with increasing dose level. Secondly, a time-to-event analysis was performed to quantify the dose effect on *T70* and was compared with the results of the non-linear model. *T70* was estimated at different dose levels with less precision compared to the non-linear model, making it less suitable to answer the research question in this report. Additionally, the results based on the time-to-event analysis are sensitive on how *T70* was defined.

1 Introduction

To test candidate treatments against diseases affecting cognitive functioning in humans, like schizophrenia and dementia, a new behavior model is set up to assess the cognitive functioning in animals. A pharmacological deficit is induced by the administration of compound X. Subsequently, a candidate treatment is added to assess the potential for reversal of the degrading cognitive functioning. A good understanding of the pharmacological deficit induced by compound X is therefore crucial. If the dose is too low, the deficit is insufficient to show beneficial effect of the candidate treatment, whereas a very high dose might lead to a non-reversible deficit.

To understand better the dose effect of compound X on cognitive functioning, two dose response studies with compound X were pooled and a meta-analysis is performed. The variable of interest is a proportion of correctly executed trials from an animal behavior experiment measured in a longitudinal fashion.

The aim of this report is to study and quantify the dose effect of compound X on learning behavior. To put it explicitly, we raise the following research questions: How does compound X affects learning behavior? Which characteristics of learning behavior are sensitive to the dose effect? How to quantify the dose effect on these characteristics?

2 Methods

The methodology of this report will be explained by four main parts. The first part describes the experimental setup in detail. The second and third part explains the methodology of the non-linear model and the time-to-event analysis. The last part explains how the non-linear model will be compared with the time-to-event analysis.

2.1 Experimental setup

Male wistar rats were trained to perform an action (choosing the correct image between two different images) by the use of an operant conditioning chamber. An operant conditioning chamber is an experimental box used to study animal behavior through reward/punishment mechanisms.

The animal was placed in the box at the start of a new session. It contains three touchscreens on one side of the wall and a food dispenser with food pellets on the opposite wall. An image of an experimental box is shown in Figure 1.

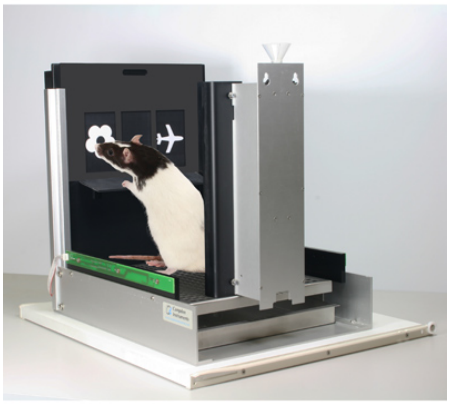

Figure 1: An illustration of an operant conditioning chamber. (Retrieved from www.campden-inst.com on 12/08/2012, URL: http://www.campden-inst.com/product_detail.asp?ItemID=1975&cat=2)

One training session exist of multiple trials. All animals were trained in advance to initialize a new trial by noise-poking the food magazine. After initialization of the first trial, two different images appear on the touchscreens (e.g., an image of a spider and an image of a plane). The animal has to choose the correct image by noise-poking the visual stimuli on the touchscreen. If the animal chooses the correct image, a food pellet is dispensed into the food magazine as a reward. If the rat chooses the wrong image, no food pellets are dispensed. After a few seconds the animal can initialize the next trial. The same two images will appear on the screen. In case of a previous correct choice, the two images will appear at different positions; in case of a previous wrong choice, the two images will appear at the same position until the correct answer is given.

One training session ends after 48 trials or after 30 minutes maximally. The proportion of successfully executed trials (e.g., choosing the correct image between two images) in one session were recorded. To control for unknown effects, most features of the experiment were randomized. The correct image for an animal stays the same during the whole study period in the current setting.

Two dose-response studies were performed in identical conditions. In study 1, 48 animals were equally randomized over four dose levels of compound X: vehicle dose (0mg), 0.5mg, 0.75mg and 1mg. The animals were daily injected with compound X before every session (including the first session) during the whole study period. The sessions were performed daily during a period of 14 days.
In study 2, 48 animals were equally randomized over four dose levels of compound X: vehicle dose (0mg), 0.25mg, 0.5mg and 1mg. The sessions were performed daily during a period of 15 days. The two studies were pooled together during the analysis. The total amount of animals is 96. An overview is provided in Table 1.

Table 1: Overview of the total amount of animals used in the study.

Dose level compound X:	0mg	0.25mg	0.5mg	0.75mg	1mg	Total # of animals
Study 1	12		12	12	12	48
Study 2	12	12	12		12	48
Total # of animals	24	12	24	12	24	96

2.2 Non-linear model

In this section, the dataset and the exploratory data analysis will be explained first. Next, the different parts of the non-linear model will gradually be introduced and illustrated to the reader and finally, the model building procedures will be explained.

2.2.1 Data

The response variable of interest is the proportion of correctly executed trials during one session. Daily data on the response variable during a period of 15 days was available for 96 animals. Note that data from study 1 were only available for 14 days. Therefore no data was available at dose level 0.75mg at day 15. This will have no implication in the analysis because the missing data is a consequence of the study setup and is not related to the response variable itself. A summary of the variables in the dataset are provided in Table 2.

Table 2: A summary of the variables in the dataset.

Variable	
Animal ID	A numerical indicator for the identification of the animal.
Dose	Dose level of compound X in mg: 0, 0.25, 0.5, 0.75, 1.
Day	Day when the session was performed. Ranging from 0 to 14, where day 0 refers to the 1st day of the study period and day 14 refers to the 15th day of the study period.
Response	The proportion of correctly executed trials per session, the variable of interest.

To avoid numerical problems in the analyses, the response variable was transformed to restrict the response to the interval]0, 1[:

$$transformed\ response = \frac{response + 0.01}{1.02} \qquad (1)$$

In the remaining of the report we will refer to the transformed response variable by the response variable.

2.2.2 Exploratory data analysis

Animal specific profiles were plotted separately and per dose level to explore the individual profiles. The average profiles (based on the arithmetic mean) per dose level were plotted to get an idea about the dose effect on the profiles.

2.2.3 Model

Since the response variable is a proportion, it is no longer expected to follow a classical symmetric distribution. The response is therefore assumed to follow a beta distribution. The beta distribution is very flexible for modeling proportions since its density can have quite different shapes depending on the value of the two parameters that index the distribution (i.e., α and β). When modeling, it is more appealing to model the mean of the response instead of the less meaningful parameters α and β to allow a direct interpretation of the modeling exercise.

Therefore, we shall work with a different parameterization of the beta density as described in Ferrari and Cribari-Neto (2004):

$$response_{ij} \sim Beta(\alpha, \beta) \qquad (2)$$

$$where:$$

$$\alpha = \mu_{ij}\phi$$

$$\beta = (1-\mu_{ij})\phi$$

$$E(response_{ij}) = \mu_{ij}$$

$$\mathrm{var}(response_{ij}) = \frac{V(\mu_{ij})}{1+\phi}$$

$$V(\mu_{ij}) = \mu_{ij}(1-\mu_{ij})$$

$Response_{ij}$ are the observed proportions at day j for animal i. The parameters of the beta distribution, α and β are expressed in terms of μ_{ij} and Φ where μ_{ij} is the average response variable at day j for animal i and Φ can be interpreted as a precision parameter in the sense that, for fixed μ_{ij}, the larger the value of Φ, the smaller the variance of the response. Given the natural boundaries 0-1 for mu, the logit link function will be used. For ease of notation, mu will denote the combination of the average proportion and the link function.

The response variable in function of days, increases on average from 0.5 up to 0.8-0.9 in an S-shaped way. Profiles from different animals show a lot of variety in their form. Therefore, the average response variable (μ_{ij}) is modeled as a Weibull learning curve as described in Gallistel et al (2004). The cumulative distribution function of a Weibull distribution is a monotonic increasing function that can take widely different forms depending on its scale and shape parameter. Because of its flexibility, the Weibull learning curve is capable to describe different forms of learning behavior. This is illustrated in Figure 2.

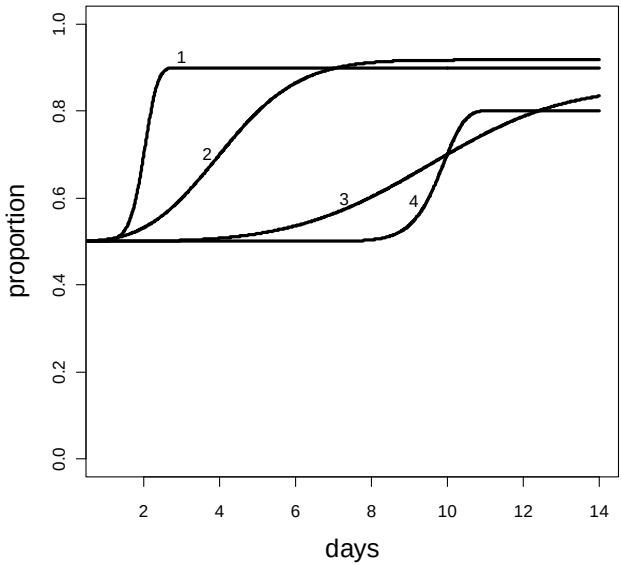

Figure 2: An illustration of the flexibility of the Weibull learning curve. Four hypothetical profiles are shown by the use of the cumulative distribution function of a Weibull distribution based on different parameters.

Different hypothetical profiles are plotted in Figure 2, by the use of the Weibull function. The Y-axis represents the proportion of correctly executed trials within one training session, the X-axis the day when the session was performed.

Profile 1 illustrates a quick learner. The task is understood immediately and the time to learn is very short. Profile 2 illustrates the profile of a learner who immediately understands the task, but the learning process is rather gradual. Profile 3 illustrates a slow learner. It takes time before the task is understood. Ones the task is understood, it takes time to learn the task. Profile 4 illustrates the profile of a learner who needs a lot of time to understand the task but once the task is understood, the time to learn is very short.

Besides the shape (*S*) and scale (*L*) parameter of the Weibull function, an intercept (*I*) and an asymptote (*A*) is added to the function:

$$\mu_{ij} = I + (A - I) * (1 - e^{-[(day_{ij} / L)^S]}) \qquad (3)$$

The function contains four parameters, describing the learning behavior in an individual animal. *I* indicates the proportion of successful executed trials at the start of the experiment. It is expected to be around 0.5 since the animals will choose the correct image in a random fashion at the beginning of the

study period because they have not learned the correct image yet. *A* indicates the asymptotic level (i.e., the maximum learning capacity). *S* and *L* describe how the asymptotic level is reached. *S* describes the abruptness of the rise. Roughly speaking, the higher the value of *S*, the more abrupt the rise. However it is important to bear in mind that this measure of abruptness depends on the value of *L*, because *S* is the power to which the ratio day_{ij}/L is raised. When the onset *L* is short, low values of *S* may be found in data that show rapid initial rise in the level of performance.

L and *S* are the scale and shape parameter, respectively, however, their biological interpretation is questionable. To get a more meaningful interpretation for the scale parameter, *L* is reparameterized as *T70*. *T70* is the time when proportion 0.7 is reached:

$$\mu_{ij} = I + (A - I) * (1 - e^{-[(day_{ij}/L)^S]}) \qquad (3)$$

$$where \;\; L = \frac{T70}{\left(-\ln\left(\frac{A - 0.847}{A - I}\right)\right)^{(1/S)}} \qquad (4)$$

The value 0.847 is the logit of 0.7 and is a constant.

A graphical presentation of the Weibull learning curve is presented in Figure 3. Panel A shows a Weibull learning curve with (*I*= 0.5), (*A*= 0.9), (*T70*= 5 days) and (*S*= 3). The upper and the lower horizontal dotted lines show the asymptotic level and the intercept, respectively. The horizontal solid line represents the level at proportion 0.7, the vertical dotted line represents the time when this proportion is reached (*T70*).

Panel B visualizes the influence of *S* on the form of the curve. Six curves are presented with *S* ranging from 1 to 3.5. The remaining parameters were kept constant (*I*= 0.5, *A*= 0.9 and *T70*= 5 days). Note that for a constant value of *T70*, the higher the value of *S*, the more abrupt the function increases towards the asymptotic level.

Panel C visualizes the influence of *T70* on the form of the curve. Six curves are presented with *T70* ranging from 1 to 11 days. The remaining parameters were kept constant (*I*= 0.5, *A*= 0.9 and *S*= 3). Note the change in abruptness with a constant value for *S* at different levels of *T70*. When *T70* is small, *S* equal to 3 generates a curve that shows an abrupt rise, whereas for a large value of *T70*, *S* equal to 3 generates a curve with a slow rise. This illustrates that caution must be taken when direct interpreting the parameter *S*.

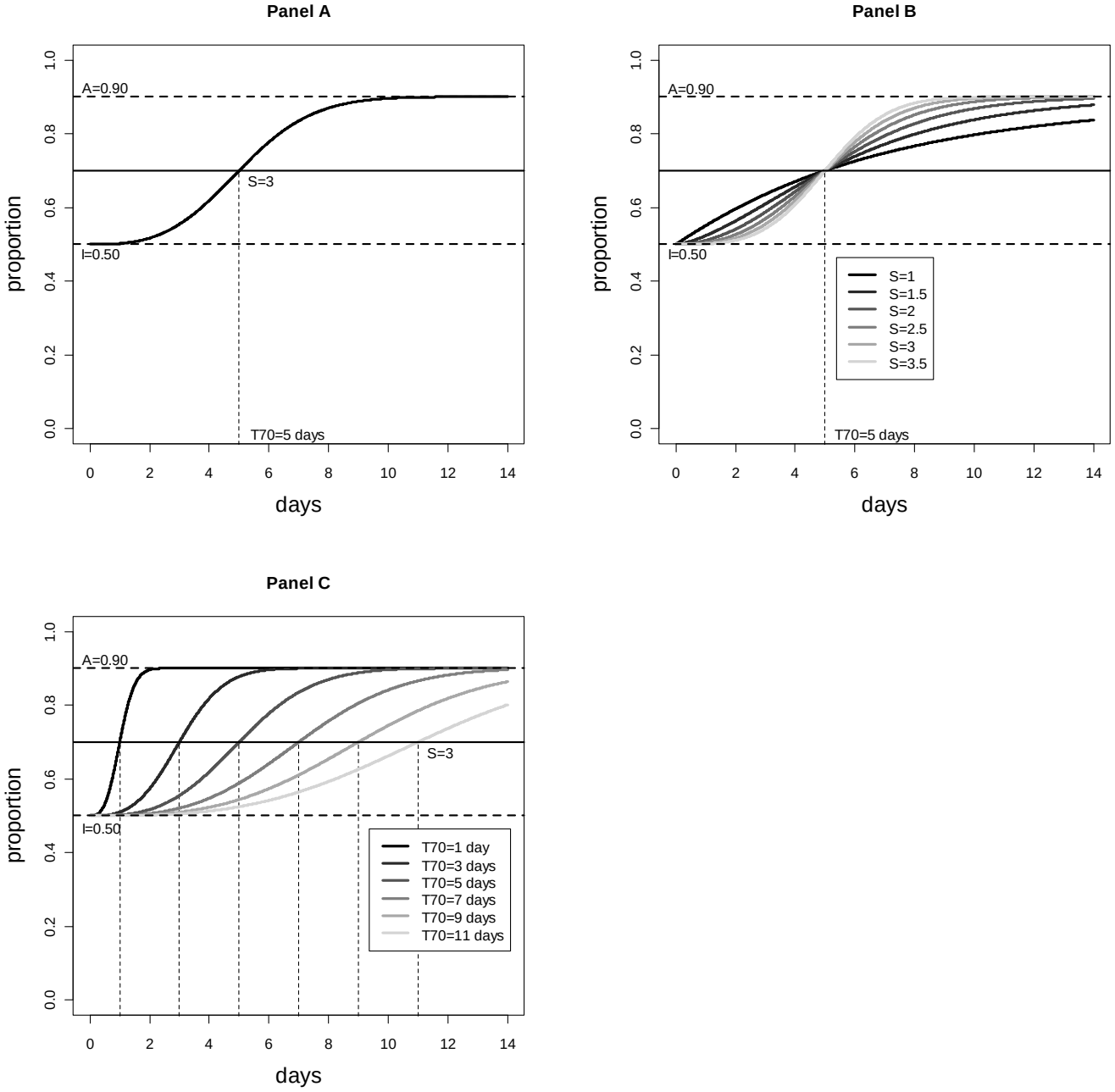

Figure 3: A graphical presentation of the Weibull learning curve. Panel A: A Weibull learning curve with parameters (*A*= 0.90), (*I*= 0.50), (*T70*= 5 days) and (*S*= 3). Panel B: Six Weibull learning curves with parameter *S* ranging from 1 to 3.5 and remaining parameters kept constant. Panel C: Six Weibull learning curves with parameter *T70* ranging from 1 to 11 days and remaining parameters kept constant. The upper and the lower horizontal dotted lines show the asymptotic level and the intercept, respectively. The horizontal solid line represents the level at proportion 0.7, the vertical dotted lines represents the time when this proportion was reached (*T70*).

To fit the above model, a number of reparameterizations were required to increase numerical stability. In the model, a logit link function was used to restrict the predicted values within the interval]0, 1[. *T70* and *S* were log-transformed to preserve the restrictions for the scale and shape parameter of the Weibull function (i.e., *L*>0, S>0). Because of these transformations, the parameters were no longer estimated on their original scale. An asterisk (*) is used in the model formulation to indicate parameters expressed on their transformed scale.

Response variables at different time points from the same animal are correlated. To take the correlated nature of the data into account, random effects were added to the model. The model without the inclusion of the covariate *dose* is extended as follow:

$$T70^{*} = \left(T70\_\text{int} + t_i\right) \qquad (5)$$

$$S^{*} = \left(S\_\text{int} + s_i\right) \qquad (6)$$

$$I^{*} = \left(I\_\text{int} + i_i\right) \qquad (7)$$

$$A^{*} = A\_\text{int} \qquad (8)$$

T70_int, *S_int*, *I_int* and *A_int* are fixed effects parameters. They represent the time until proportion 0.7 is reached, the abruptness, the intercept and the asymptotic level, respectively for an average animal (i.e., an animal with all random effects equal to zero). $b_i = (t_i, i_i, s_i)$ is a vector of random effects. t_i is the animal specific *T70*, i_i is the animal specific intercept and s_i is the animal specific abruptness. The random effects were assumed to follow a three-variate normal distribution with zero mean vector and an unstructured 3x3 variance-covariance matrix.

To model the effect of compound X on learning behavior, the covariate *dose* is included in the model by allowing the parameters *T70*, *S* and *A* to change in function of dose level. The parameter *I* is not considered since there is no dose effect expected during the first learning session at day 0. Randomization is assumed to provide equal groups at day 0.

The model with the inclusion of covariate *dose* will be an extension of the previous model as follow:

$$T70^{*} = \left(T70\_\text{int} + t_i\right) + T70\_slope*dose_i \qquad (9)$$

$$S^{*} = \left(S\_\text{int} + s_i\right) + S\_slope*dose_i \qquad (10)$$

$$I^{*} = \left(I\_\text{int} + i_i\right) \qquad (11)$$

$$A^{*} = A\_\text{int} + A\_slope*dose_i \qquad (12)$$

$Dose_i$ is the second covariate added to the model. (the first covariate in the model is day_{ij}) $Dose_i$ represents the dose level of compound X for animal i. Note that there is only one dose level per animal. *T70_slope*, *S_slope* and *A_slope* are fixed effects and represent the dose effect on *T70*, the abruptness and the asymptotic level, respectively for an average animal. Note that due to the log-transformation of *T70* and *S* and the use of the logit link function, dose effect can no longer be interpreted as a slope on the original scale of the parameters, however, a multiplicative interpretation is attained.

Different functional forms can be used to describe the dose-response relationship between the parameters and dose level. For now, we will focus on the dose-response relationship of *T70* only. Dose effect as introduced in (*9*) assumes a log-linear relationship between *T70* and dose level. An example of a log-linear dose-response curve is shown in Figure 4, right panel. *T70* increases exponentially with increasing dose levels.

If an hyperbolic dose-response relationship is expected, an Emax model (Gabrielson and Weiner, 2000) can be used. The inclusion of a dose effect on *T70* by an Emax model is as follow:

$$T70^{*} = E_0 + \frac{dose_i * E_{\max}}{dose_i + ED_{50}} \qquad (13)$$

E_0 is the basal effect, corresponding to the response when the dose level is zero (i.e., *T70* at dose level 0mg), E_{max} is the maximum effect attributable to the drug and ED_{50} is the dose level, which produces half of the E_{max}. An illustration of the Emax dose-response curve is shown in Figure 4, left panel.

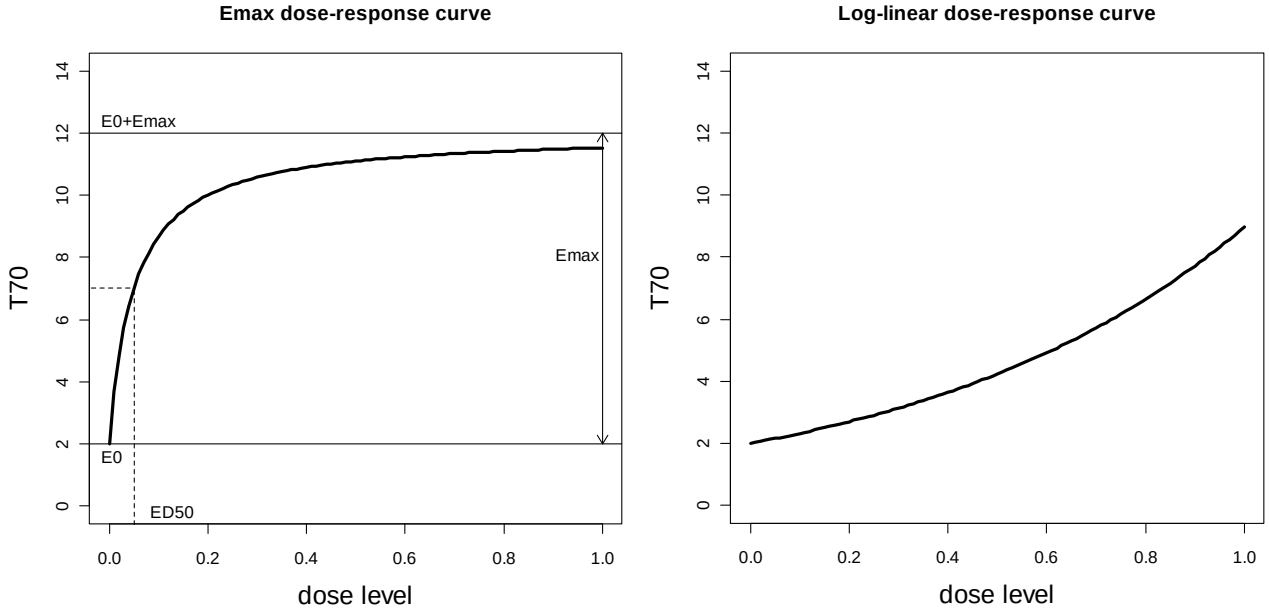

Figure 4: An example of Emax dose-response curve (left panel) and log-linear dose-response curve (right panel).

2.2.4 Model building

In the first phase of the model building, attention was paid to the random effects structure. A model without dose effects was fitted and random effects were gradually added to the model. First, four models were fitted with one random effect on *A*, *I*, *T70* and *S*, respectively. Secondly, the random effects from the models with the lowest likelihood function (-2ll) values were combined and a model with two random effects was fitted. Finally, more random effects were gradually added to this model to obtain a model with a rich random effects structure.

In the second phase, attention was paid to the mean structure. To get a first idea about the dose effect on the different parameters (*A*, *S* and *T70*), four models were fitted:

- Model 1: dose effect on *S* in an unstructured way (i.e., one parameter per dose level)
- Model 2: dose effect on *A* in an unstructured way
- Model 3: dose effect on *T70* in an unstructured way
- Model 4: dose effect on *S*, *A* and *T70* in an unstructured way

An intermediate random effects structure (i.e., two random effects) was included in all four models to correct for the correlated nature of the data. The fit statistics (AIC and -2ll value) of Model 1, 2 and 3 were compared to understand the importance of the dose effect on the different parameters.

To get a preliminary idea about the functional relationship between the parameters and dose level, plots were made of the parameter estimates together with the 95% confidence intervals versus dose level of *T70*, *S* and *A*, as estimated by Model 4. The parameter estimates and the 95% confidence intervals were back-transformed to their original scale.

In the final phase of the model building, a model with a rich mean and random effects structure was fitted. Dose effects were included on all three parameters (*T70*, *S* and *A*). Random effects were gradually added to the model. First, likelihood ratio tests based on a mixture of χ^2 distributions were performed to test whether random effects could be excluded from the model. Secondly, a manual backwards selection procedure based on likelihood ratio tests was performed to simplify the mean structure (i.e., the dose effects with the highest insignificant pvalues were gradually removed from the model until no more dose effects could be removed).

To check the fit of the model, several procedures were performed. As a first and most important model fit diagnostic, all 96 individual learning curves were plotted both as observed versus predicted animal specific profiles on the original scale and on the logit scale.

A scatter plot of the observed versus predicted response values on the original and on the logit scale were made to detect any deviations from the 45 degree line through the origin. In addition to the scatter plot, the Pearson correlation coefficient was calculated between the observed and predicted proportions.

Histograms (per day and per dose level) of the errors on the logit scale were obtained to identify any unexpected patterns. The errors were calculated as the observed response value minus the predicted response value on the logit scale.

To check the appropriateness of the distributional assumptions and of the model, histograms of the observed proportions, overlaid with an appropriate mixture of beta distributions were made. The components of this mixture are: (1) the marginalized predicted proportions per dose-day combination, (2) the phi parameter, as estimated by the model and (3) the weights, based on the number of animals per dose-day combination. The marginalized predicted proportions were obtained by taking the average of 10.000 profiles from hypothetical animals with random effects drawn randomly from a multivariate normal distribution of random effects as estimated by the model.

One overall histogram, histograms per day and histograms per dose level of the observed proportions, overlaid with the appropriate mixture of beta distributions were obtained to detect any deviations between the histograms and the mixtures.

A plot of the observed average dose profiles versus the marginalized predicted dose profiles was obtained to check how well the model fitted the data. The observed average dose profiles were based on the beta means instead of the arithmetic means.

To detect model deviations or animals with outlying profiles, scatter plots of the empirical Bayes estimates of the random effects were obtained.

Adaptive Gaussian quadrature integration method was used to obtain the parameter estimates. A minimum of 11 qpoints was used for all models. To assure numerical stability, the qpoints were gradually increased until no changes were detected in -2ll value (if computational time stayed within a reasonable time frame). The starting values were obtained from previous models in the model building procedure. When necessary, a grid search on some starting values was performed. To study animal specific learning curves, the empirical Bayes estimates of the random effects and the best linear unbiased predictions were obtained.

2.3 Time-to-event analysis

Instead of modeling the full profiles like in the non-linear model, the longitudinal profiles can be summarized by the time to reach a certain proportion of correctly executed trials and a time-to-event analysis can be performed. The event is proportion 0.7 reached. As such, the analysis has affinities to *T70* as performed before.

This section explains the dataset, the exploratory data analysis, the Cox proportional hazard model and finally a sensitivity analysis testing the robustness of the analysis on the definition of *T70* chosen.

2.3.1 Data

The variables in the dataset used for the time-to-event analysis are presented in Table 3.

Table 3: A summary of the variables in the dataset used for the time-to-event-analysis.

Variable	
Animal ID	A numerical indicator for the identification of the animal.
Dose	Dose level of compound X in mg: 0, 0.25, 0.5, 0.75, 1.
Time	Time until proportion 0.7 was reached (*T70*).
Censor	Censoring indicator: 0 for censored time-to-event and 1 for true time-to-event.

The longitudinal animal specific profiles were summarized by two variables. The first variable is *Time*, corresponding to *T70* and the second variable, *Censor*, gives the censoring status taken value one (*Censor*= 1) if *T70* is observed and zero (*Censor*= 0) otherwise.

Due to the erratic behavior of the observed profiles (i.e., there is considerable amount of variability present between the observed proportions within animals), defining *T70* is not straightforward. This is illustrated in Figure 5 by the individual profile of animal 114. The dot-dashed line is the observed profile over time, the horizontal line represents proportion 0.7.

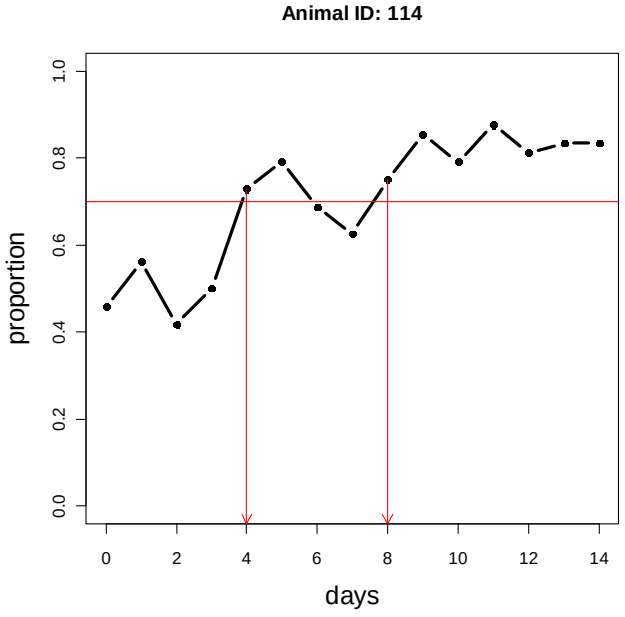

Figure 5: Illustration of the difficulty in defining *T70* for an individual profile. The dot-dashed line is the observed profile for animal 114. The horizontal solid line represents the level at proportion 0.7, the vertical arrows represent the time when the profile exceeds this level.

The observed profile crosses the horizontal line at two different points, corresponding to two different values for *T70*. According to the left arrow, *T70* is 4 days. The decrease of the profile after 4 days under the horizontal line could be due to variability or due to the fact that proportion 0.7 was not reached yet at day 4. The arrow on the right corresponds to *T70* at 8 days. The profile no longer decreases under the horizontal line after day 8, indicating that proportion 0.7 was probably reached after day 8. This illustrates that caution must be taken while defining *T70* for each animal. Therefore four definitions were proposed. For each definition, a dataset was generated with 96 values for the variable *Time* and *Censor*. The definitions are presented in Table 4.

Table 4: Summary of the definitions used to define *T70*.

Definition	Explanation of definition
Definition 1	*T70*: the first day when proportion >= 0.7 was reached and the median of the remaining proportions later in time >= 0.7.
Definition 2	*T70*: the first day when proportion >= 0.7 was reached and at least two consecutive proportions >= 0.7.
Definition 3	*T70*: the first day when proportion >= 0.7 was reached.
Definition 4	*T70*: the median day corresponding to the points where the profile exceeds the 0.7 horizontal line.

According to the first definition, *T70* is reached at the first day when an observed proportion exceeds 0.7 and when the median of the remaining proportions after that day is higher or equal to 0.7. According to the second definition, *T70* is reached at the first day when an observed proportion exceeds 0.7 and when at least two consecutive proportions exceed 0.7. The third definition is a rather naïf approach. *T70* is defined as the first day when an observed proportion exceeds 0.7 despites what happens to the remaining part of the profile after that day. In the fourth definition, *T70* is calculated as the median of the days when the profile exceeds the level of 0.7.

The definitions were evaluated by plotting *T70* according to the four definitions on the observed individual profiles for all 96 animals.

2.3.2 Exploratory data analysis

A summary of the number of censored observations was obtained.

To explore the dose effect on *T70*, the Kaplan-Meier estimator of the survival function was plotted per dose level stratum. The median survival time with 95% confidence interval based on the Kaplan-Meier estimate was obtained per dose level stratum. The 95% confidence intervals were based on the log-log transformation.

2.3.3 Cox proportional hazard model

A Cox proportional hazard model was fitted on the data based on definition 1. *Dose* was included as a continuous covariate:

$$\frac{h_i(t)}{h_0(t)} = \exp(\beta * dose_i) \qquad (14)$$

$dose_i$ refers to the dose level for animal i. $h_0(t)$ refers to the baseline hazard (i.e., the hazard function of animals with dose level 0mg) and $h_i(t)$ refers to the hazard function of animals with dose level i. The parameter estimate β represents the dose effect. It refers to the increase in the log-hazard with a unit increase in dose level. The hazard ratio (HR), $exp(\beta * dose_i)$, corresponds to the ratio of the hazard of an animal with dose level i and the hazard of an animal with dose level 0mg.

The median survival time together with the 95% confidence interval based on the log-log transformation were obtained per dose level as estimated by the model. A likelihood ratio test was performed to test the significance of dose effect on survival. The tied failure times were handled using the Efron approximate likelihood.

To test the proportional hazard (PH) assumption, a time-dependent covariate (i.e., an interaction between *dose* and *time*) was included into the model. A likelihood ratio test was done to test whether the interaction was significant (indicating a violation of the proportionality assumption). The deviance residuals were obtained per dose level to check for outliers, the score residuals were obtained to check for influential observations.

2.3.4 Sensitivity analysis

To check the robustness of the analysis on the choice of the definition, a sensitivity analysis was performed. Previous assessments were repeated using the datasets based on definition 2, 3 and 4 and compared with the analysis based on definition 1. First, the discrepancy between the different *T70* values within each animal was studied by plotting *T70* according to the four definitions on the observed individual profiles for all 96 animals. Secondly, the summary of censored observations and the plot of the Kaplan-Meier estimator per dose level stratum were compared. Finally, the parameter estimate of dose effect as estimated by the Cox proportional hazard model were compared.

2.4 Comparison of a non-linear model and a Cox proportional hazard model

A direct answer to the research question was provided by plotting *T70* versus dose levels ranging between 0mg and 1mg with 95% pointwise confidence interval as estimated by the model. This way, the dose level, inducing the desired biological effect (*T70*), can be read from the graph. First, a plot was made of *T70* versus dose level for an average animal as estimated by the non-linear model. Also a table of *T70* at different dose levels with 95% confidence interval is provided in the appendix (Appendix Table 4). Secondly, a plot of the median *T70* versus dose level as estimated by the Cox proportional hazard model, with 95% pointwise confidence interval (based on the log-log transformation) was overlaid to compare the results of the non-linear model and the time-to-event analysis.

The analyses of the non-linear models were performed in SAS 9.2. The time-to-event analyses, the model fit procedures of the non-linear models and the graphical presentations were performed in R. An alpha level of 0.05 was used throughout the study.

3 Results

In the first two parts of this section, the results of the non-linear model and the time-to-event analysis will be presented. In the final part, the results of both analysis will be compared.

3.1 Non-linear model

3.1.1 Exploratory data analysis

Plots of animal specific profiles per dose level are shown in Figure 6. The horizontal line refers to the level at proportion 0.7.

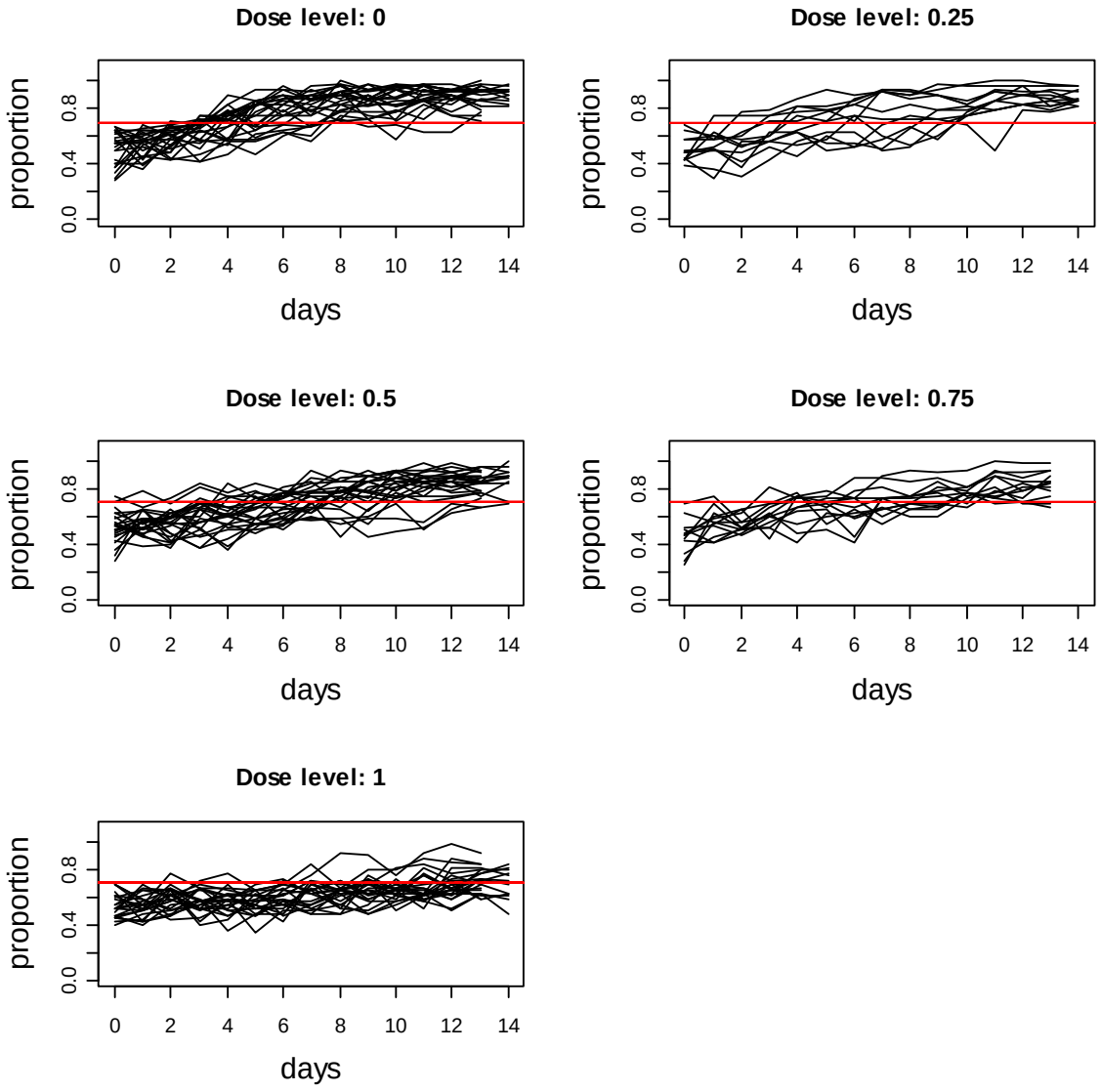

Figure 6: Plots of animal specific profiles per dose level (mg). The horizontal line refers to the level at proportion 0.7.

A substantial amount of variability between and within different animals is observed as seen in Figure 6. Most profiles seem to start at a proportion around 0.5, as expected. There is variability present around the intercept, suggesting the inclusion of a random intercept into the model. All profiles show an increasing behavior, though the increase seems to change with dose level. The increase of the profiles at dose level 1mg is less steep compared to the increase at dose level 0mg. This suggests a dose effect on the profiles. Most profiles seem to cross the level at proportion 0.7 (with some exceptions at dose level 1mg, though this is difficult to assess based on the plots in Figure 6). *T70* can be read from the X-axis, underneath the point where the profiles cross the horizontal line at proportion 0.7. Substantial variability is present around *T70*, suggesting the inclusion of a random effect on *T70* into the model.
At dose level 0mg, most profiles seem to have reached their asymptotic levels after 7-8 days, which are between 0.8 and 0.9. The assessment of the asymptotic level is more difficult at the other dose levels.
To take a closer look at the form of the individual profiles, all 96 profiles were plotted separately (not shown). Most profiles at dose level 0mg show an S-shaped evolution over time. There is substantial variability present in the forms of the profiles between different animals. At dose level 1mg, most profiles show a considerable less steep increase compared to the profiles at lower dose levels.

The average profiles per dose level are shown in Figure 7. The horizontal solid line presents the level at proportion 0.7, the vertical dotted lines indicate the corresponding *T70* at different dose levels. All average profiles seem to start at a proportion around 0.5. The higher the dose level, the less steep the increase of the average profiles. The differences between the average profiles seem to decrease with increasing dose level, with an exception of the average profile at dose level 1mg. (i.e., the average profiles at dose level 0.5mg and 0.75mg lie closer to each other compared to the average profiles of dose level 0mg and 0.25mg.). *T70* seems to increase with increasing dose levels. *T70* at dose level 1mg lies far beyond *T70* at lower dose levels.

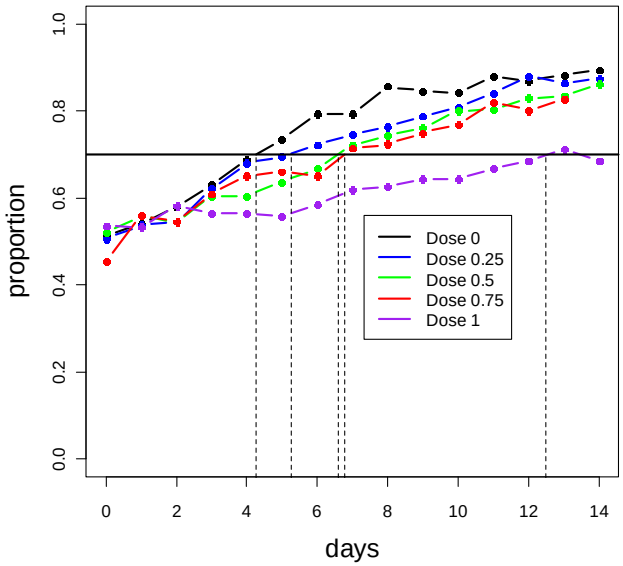

Figure 7: The average observed profiles (dot-dashed lines) per dose level. The horizontal solid line presents the level at proportion 0.7, the vertical dotted lines indicate the corresponding *T70* at different dose levels.

3.1.2 Model building

In the first phase of the model building procedure, a model with 3 random effects (random effect on *T70*, *S* and *I*) was obtained (not shown). Unfortunately, models with a random effect on *A* did not converge. A table of the fitted models is included in the appendix (Appendix Table 1).

The results from the second phase of the model building procedure are presented in Table 5. All models were fitted with the same intermediate random effects structure (a random effect on *T70* and *S*). AIC of the model without dose effect (Model 0: AIC= -3186) decreases substantially after the inclusion of a dose effect on *T70* (Model 3: AIC= -3243). The decrease in AIC of Model 0 is less pronounced after inclusion of a dose effect on *A* (Model 2: AIC= -3184) and AIC slightly increases after the inclusion of a dose effect on *S* (Model 1: AIC= -3188). This suggests an important role of the dose effect on *T70* in the model.

Table 5: Model building: Models including a dose effect on *S*, *A* and *T70* in an unstructured way (i.e., one parameter per dose level). Note: Unstr: unstructured, RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood.

Model	Dose effect:	RE:	#p	AIC	-2ll
Model 0	No dose effect	*T70*+*S*	8	-3186	-3202
Model 1	*S*: Unstr	*T70*+*S*	12	-3188	-3212
Model 2	*A*: Unstr	*T70*+*S*	12	-3184	-3208
Model 3	*T70*: Unstr	*T70*+*S*	12	-3243	-3267
Model 4	(*T70*: Unstr)+(*S*:Unstr)+(*A*:Unstr)	*T70*+*S*	20	-3237	-3277

Figure 8 presents the plots of the parameter estimates together with the 95% confidence intervals versus dose level for *T70*, *S* and *A*, as estimated by Model 4.

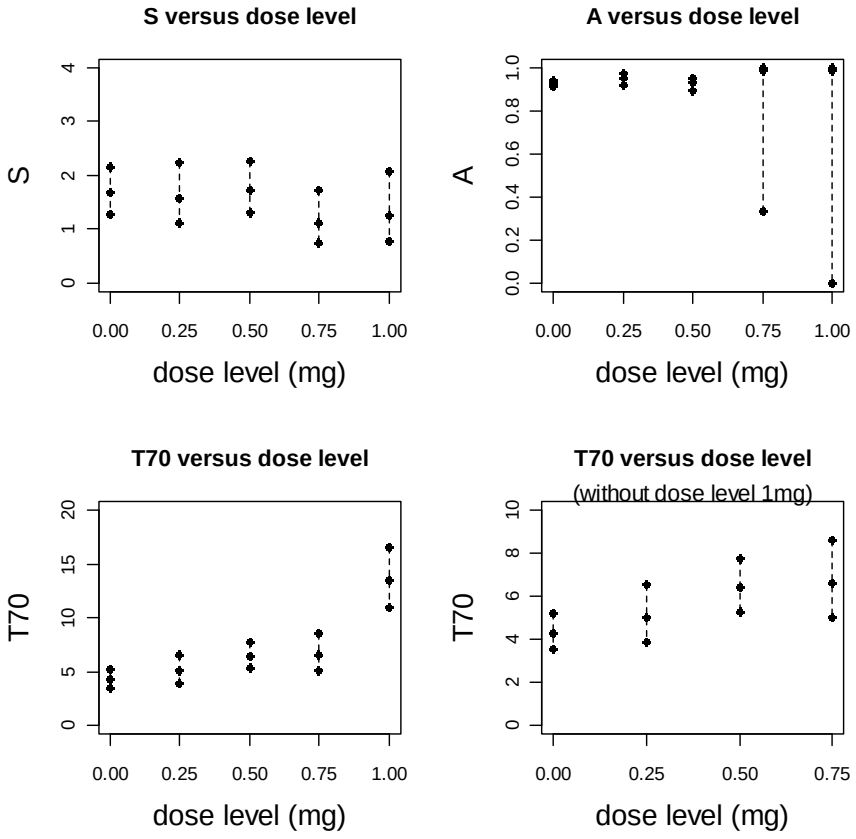

Figure 8: Parameter estimates of dose effect on *S* (upper left panel), *A* (upper right panel) and *T70* (lower left and right panel) with 95% confidence intervals at different dose levels as estimated by Model 4. The lower left panel shows the parameter estimates of *T70* on the complete dose range (0mg-1mg), the lower right panel shows the parameter estimates of *T70* on the lower dose range (0mg-0.75mg). The parameter estimates and the 95% confidence intervals were back-transformed to their original scale.

The upper left panel shows the parameter estimates for *S* versus dose level. The estimates for *S* ranges between 1 and 2. There seems to be a slight decrease of *S* at dose level 0.75mg and 1mg. The upper right panel shows the parameter estimates for *A* versus dose level. The estimates for *A* ranges between 0.92 and 0.98 and seem to be less affected by dose level. Note the wide confidence intervals around the parameter estimates at dose level 0.75mg and 1mg. It reflects the uncertainty around the estimate of the asymptotic level at higher dose levels. The reason might be that most of the profiles at higher dose levels have not yet reached their asymptotic level within the range of the observed data. This can also been seen on Figure 7.

The bottom left panel shows the parameter estimates for *T70* versus dose level. The estimates for *T70* ranges between 4 and 13 days. *T70* increases with increasing dose levels. Dose level 1mg seems to behave different compared to dose levels 0mg to 0.75mg. To take a closer look at the dose effect on *T70* at lower dose levels, the parameter estimates for *T70* versus dose levels 0mg-0.75mg are shown at the lower right panel. Possibilities to describe the dose response relationship between *T70* at the lower dose levels are a hyperbolic Emax curve or the log-linear curve. Both curves will be fitted to the data later.

Based on the data exploration (Figure 7) and the exploratory analysis of dose effect on *T70* as described above, one might raise two hypotheses. In the first hypothesis, dose level 1mg is believed to show outlying behavior. The dose effect of 1mg on *T70* does not follow the same pattern as expected from the lower dose levels (i.e., dose level 0mg to 0.75mg). Under the assumption of the first hypothesis, two models were fitted. In Model 3.1 the dose effect on *T70* at the lower dose levels was modeled with an Emax curve. A separate parameter was used to estimate the dose effect on *T70* at dose level 1mg. In the second model (Model 3.2), the dose effect on *T70* at the lower dose levels was modeled with a log-linear curve and a separate parameter was used to estimate the dose effect on *T70* at dose level 1mg.

According to the second hypothesis, dose level 1mg is not believed to show outlying behavior. The discrepancy found is considered mere as a coincidence due to random behavior. Under this assumption, the dose effect on *T70* at the whole dose range (i.e., dose level 0mg to 1mg) was modeled with a log-linear curve in Model 3.3.

All three models included 2 random effects (a random effect on *T70* and *S*). No dose effects on other parameters were included. The fit statistics for the three models are presented in Table 6 together with the fit statistics for Model 3.

Table 6: Model building: Models with dose effect on *T70* in different functional forms. Note: Unstr: unstructured, Logl: log-linear, D1: dose level 1mg, RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood.

Model	Dose effect:	RE:	#p	AIC	-2ll
Model 3	*T70*: Unstr	*T70+S*	12	-3243	-3267
Model 3.1	*T70*: Emax+Unstr on D1	*T70+S*	11	-3244	-3266
Model 3.2	*T70*: Logl+Unstr on D1	*T70+S*	10	-3246	-3266
Model 3.3	*T70*: Logl	*T70+S*	9	-3241	-3259

Model 3.1 (AIC= -3244) and Model 3.2 (AIC= -3246) show lower AIC values compared to Model 3.3 (AIC= -3241). Adding an extra parameter to capture the dose effect at dose level 1mg seems to improve the fit of the model. Because the difference in AIC values is rather subtle (AIC value should be used as an exploratory tool only) and because of the different biological assumptions made, we will continue with the functional forms on *T70* as used in Model 3.2 and Model 3.3. Model 3.1 with an Emax structure on *T70* at the lower dose levels is left behind due to its increase in complexity (four parameters were used for five dose levels) and the fact that it does not show an improved fit based on the AIC value compared to Model 3.2. No further effort was done in finding the correct functional form between the parameters and dose levels to avoid a model that will over fit the data. There were only five dose levels available for the analysis.

The final model building phase was done for two models. In the first model, dose effect on *T70* was modeled with a log-linear curve at the lower dose levels and a separate parameter was used for dose effect at dose level 1mg. We will refer to these models as Model_LU. In the second model, dose effect on *T70* was modeled with a log-linear curve over the complete dose level range. We will refer to these models as Model_L.

The most important steps for the model building procedure for Model_LU are presented in Table 8. A complete overview of the model building procedure can be found in the appendix (Appendix Table 2). The full model (Model_LU_3) included a dose effect on *T70*, *S* and *A* and a random effect on *T70, S* and *I* with an unstructured 3x3 variance-covariance matrix. Unfortunately, models with a random effect on *A* did not converge. Likelihood ratio tests based on a mixture of χ^2 distributions revealed that no random effects could be removed from the model. The dose effect on *S* did not decreased -2ll value and was removed from the model (LR test Model_LU_3 versus Model_LU_2: G^2= 0, DF= 1, pvalue= 1). The dose effect on *A* was borderline significant (LR test Model_LU_2 versus Model_LU_1: G^2= 4, DF= 1, pvalue= 0.046) and could not be removed from the model.

The most important steps for the model building procedure for Model_L are presented in Table 9. (for a complete overview, see Appendix Table 3). The full model (Model_L_3) included a dose effect on *T70, S* and *A*, and a random effect on *T70, S* and *I* with an unstructured 3x3 variance-covariance matrix. Models with a random effect on *A* did not converge, like before. Likelihood ratio tests based on a mixture of χ^2 distributions revealed that no random effects could be removed from the model. The dose effect on *S* did not decreased -2ll value and was removed from the model (LR test Model_L_3 versus Model_L_2: G^2= 0, DF= 1, pvalue= 1). The dose effect on *A* was again borderline significant (LR test Model_L_2 versus Model_L_1: G^2= 5, DF= 1, pvalue= 0.025) and could not be removed from the model.

3.1.3 Non-linear model: Model_LU_2

The final model (Model_LU_2) includes a dose effect on *T70* and *A*. The parameter estimates and 95% confidence intervals for the fixed effects of Model_LU_2 are presented in Table 7. The parameter estimate and confidence interval of the dose effect on *A* (*A_slope*) and *T70* (*T70_slope*) are reported on the logit scale and log scale, respectively. The remaining parameter estimates and confidence intervals were back-transformed to the original scale, for the ease of the interpretation.

Table 7: Parameter estimates and 95% confidence intervals (CI) for the fixed effects of Model_LU_2. The parameter estimate and 95% confidence interval of *A_slope* and *T70_slope* are reported on the logit scale and log scale, respectively. The remaining parameter estimates and 95% confidence intervals were back-transformed to the original scale.

Parameter	Estimate	95% CI
I_int	0.52	(0.50, 0.54)
S_int	1.64	(1.40, 1.91)
A_int	0.93	(0.92, 0.94)
A_slope	0.71	(-0.16, 1.58)
T70_int	4.2	(3.6, 5.0)
T70_slope	0.74	(0.38, 1.10)
T70_dose 1	13.9	(11.4, 16.9)

The estimate of the intercept (*I_int*= 0.52) is close to the proportion of 0.5, as expected. The abruptness (*S_int*) is estimated to be 1.64. The asymptotic level at dose level 0mg (*A_int*) is estimated to be 0.93. The positive estimate for the dose effect on *A* (*A_slope*= 0.71) indicates an increase of the asymptotic level with an increasing dose level. This result is somewhat unexpected. The increase of the asymptotic level is estimated to be from 0.93 (at dose level 0mg) up to 0.96 (at dose level 1mg).

Table 8: Model building procedure Model_LU: functional form on *T70*: Log-linear + Unstructured at dose level 1mg. Note: RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood, G^2: likelihood ratio statistic, DF: degrees of freedom, LR test: likelihood ratio test.

Model	Dose effect:	RE:	# p	AIC	-2ll	G^2	DF	pvalue	LR test
Model_LU_3	*T70+S+A*	*T70+S+I*	15	-3288	-3318				
Model_LU_2	*T70+A*	*T70+S+I*	14	-3290	-3318	0	1	1	remove dose effect on *S*
Model_LU_1	*T70*	*T70+S+I*	13	-3288	-3314	4	1	0.046	remove dose effect on *A*

Table 9: Model building procedure Model_L: functional form on *T70*: Log-linear. Note: RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood, G^2: likelihood ratio statistic, DF: degrees of freedom, LR test: likelihood ratio test.

Model	Dose effect:	RE:	# p	AIC	-2ll	G^2	DF	pvalue	LR test
Model_L_3	*T70+S+A*	*T70+S+I*	14	-3283	-3311				
Model_L_2	*T70+A*	*T70+S+I*	13	-3285	-3311	0	1	1	remove dose effect on *S*
Model_L_1	*T70*	*T70+S+I*	12	-3282	-3306	5	1	0.025	remove dose effect on *A*

A plot of the five dose profiles for an average animal (i.e., an animal with random effects equal to zero) as estimated by the model with dose effect on *A* (Model_LU_2) and the model without the dose effect on *A* (Model_LU_1) revealed almost no differences in the estimated profiles within the observed time frame of 15 days. The plot is provided in the appendix (Appendix Figure 1). The small differences are not considered to be biologically relevant.

T70 at dose level 0mg (*T70_int*), is estimated to be 4.2 days. The positive parameter estimate for the dose effect on *T70* (*T70_slope*= 0.74) indicates an increase of *T70* with increasing dose level between the range 0mg to 0.75mg. *T70* at dose level 0mg increases exponentially by factor 1.74 (= $\exp(0.74)^{0.75}$) with a 0.75 unit increase in dose level (i.e., going from dose level 0mg to dose level 0.75m). *T70* at dose level 1mg (*T70_dose 1*) is estimated to be 13.9 days. A plot of the five dose profiles for an average animal as estimated by Model_LU_2 is presented in Figure 10, left panel.

The estimates and 95% confidence intervals of the variance components of Model_LU_2 are presented in Table 10. The correlation coefficient between the random effects was estimated to be 0.19 (random effect *T70* and *S*), -0.30 (random effect *T70* and *I*) and 0.31 (random effect *S* and *I*). The negative correlation between random effects *T70* and *I* indicated that animals with a high intercept tend to have a lower *T70* compared to animals with a low intercept. The correlation between random effects *S* and *I* and between random effects *S* and *T70* are more difficult to interpret since the biological meaning of *S* depends on the value of *T70*.

Table 10: Parameter estimates and 95% confidence intervals (CI) for the variance components of Model_LU_2.

Parameter	Estimate	95% CI
var (t_i)	0.186	(0.131, 0.264)
rho (t_i, s_i)	0.19	(-0.12, 0.46)
var (s_i)	0.254	(0.151, 0.425)
rho (t_i, i_i)	-0.30	(-0.56, 0.01)
rho(s_i, i_i)	0.31	(0.01, 0.57)
var (i_i)	0.061	(0.039, 0.094)
phi	44.81	(41.21, 48.73)

During the model fit procedures, no indication of severe model misspecifications were found. The plots of the predicted versus the observed individual profiles showed a good fit on both the original and logit scale (the plots on the original scale are included in the appendix, Appendix Figure 3-7).

The correlation between the observed and predicted proportions was 0.92. The scatter plot of the observed and predicted values on the logit scale (see Appendix Figure 8) showed a few points deviating from the 45 degree line through the origin at higher logit values. These values corresponded to the few observations with an observed proportion equal to 1. These deviations were expected since the model cannot predict values above its estimated asymptotic value(s).

The histograms of the errors on the logit scale showed no unexpected patterns. The histograms showed some skewness to the right. This can be explained as a consequence of the use of the logit link function. Small deviations between proportions will create large deviations at the logit scale.

The histograms of the observed response values overlaid with the appropriate mixture of beta distributions showed no extreme deviations between the histograms and the mixtures (see Appendix Figure 9-11). The plot of the observed average dose profiles versus the marginalized predicted dose profiles showed a good fit of the data (see Figure 9, left panel).

The scatter pots of the empirical Bayes estimates of the random effects did not revealed extreme outlying estimations or severe model deviations (see Appendix Figure 12).

3.1.4 Non-linear model: Model_L_2

The final model (Model_L_2) includes again a dose effect on *T70* and *A*. The parameter estimates and 95% confidence intervals of the fixed effects of Model_L_2 are presented in Table 11. The parameter estimates and 95% confidence intervals are reported in the same way as before.

Table 11: Parameter estimates and 95% confidence intervals (CI) for the fixed effects of Model_L_2. The parameter estimate and 95% confidence interval of *A_slope* and *T70_slope* are reported on the logit scale and log scale, respectively. The remaining parameter estimates and 95% confidence intervals were back-transformed to the original scale.

Parameter	Estimate	95% CI
I_int	0.52	(0.50, 0.54)
S_int	1.66	(1.42, 1.94)
A_int	0.93	(0.92, 0.94)
A_slope	0.81	(-0.13, 1.74)
T70_int	3.9	(3.4, 4.6)
T70_slope	1.11	(0.86, 1.36)

The results from Model_L_2 were very similar compared to Model_LU_2, except for the estimation of *T70*. The intercept (*I_int*) was estimated to be 0.52, as before. The abruption (*S_int*) was estimated to be 1.66. The asymptotic level at dose level 0mg was estimated to be 0.93, as before. The parameter estimate of the dose effect on *A* (*A_slope*) was again positive, indicating an increase in asymptotic level

with increasing dose levels. The asymptotic level was estimated to increase from 0.93 (at dose level 0mg) up to 0.97 (at dose level 1mg). A plot of the dose profiles of an average animal as estimated by the model with a dose effect on *A* (Model_L_2) compared to the profiles as estimated by the model without a dose effect on *A* (Model _L_1) indicated again only minor changes between the two models in the observed time frame. The plot is provided in the appendix (Appendix Figure 2). *T70* at dose level 0mg (*T70_int*) was estimated to be 3.9 days. The positive estimate for the dose effect on *T70* (*T70_slope*= 1.11) revealed an increase of *T70* with increasing dose levels. *T70* at dose level 0mg increases exponentially by factor 3.03 (= exp(1.11)) with a unit increase in dose level (i.e., going from dose level 0mg to 1mg). A plot of the five dose profiles for an average animal as estimated by Model_L_2 is presented in Figure 10, right panel.

The estimates and 95% confidence intervals of the variance components of Model_L_2 are presented in Table 12. The estimates are similar to the estimates from Model_LU_2. The correlation between random effects *T70* and *S*, *T70* and *I*, *S* and *I* are estimated to be 0.24, -0.24 and 0.35, respectively.

Table 12: Parameter estimates and 95% confidence intervals (CI) for the variance components of Model_L_2.

Parameter	Estimate	95% CI
var (t_i)	0.197	(0.138, 0.279)
rho (t_i, s_i)	0.24	(-0.07, 0.50)
var (s_i)	0.260	(0.154, 0.438)
rho (t_i, i_i)	-0.24	(-0.51, 0.07)
rho(s_i, i_i)	0.35	(0.02, 0.61)
var (i_i)	0.061	(0.038, 0.099)
phi	44.84	(41.23, 48.77)

The plots of the predicted versus the observed individual profiles showed a good fit on both the original and logit scale (the plots on the original scale are included in the appendix, Appendix Figure 13-17).
The correlation between the observed and predicted proportions was again 0.92. The scatter plot of the observed and predicted values on the original and logit scale showed similar pattern as Model_LU_2 (see Appendix Figure 18).
The histograms of the errors on the logit scale showed no unexpected patterns. The histograms of the observed response values overlaid with the appropriate mixture of beta distributions showed more deviation between the histograms and the mixtures as compared to Model_LU_2 (see Appendix Figure 19-21).

To study the difference in the model fit between the two models more in detail, the plot of the observed average dose profiles versus the marginalized predicted dose profiles is shown in Figure 9 for Model_LU_2 (left panel) and Model_L_2 (right panel). The observed average profile at dose level 0.75 is not captured well by Model_L_2.

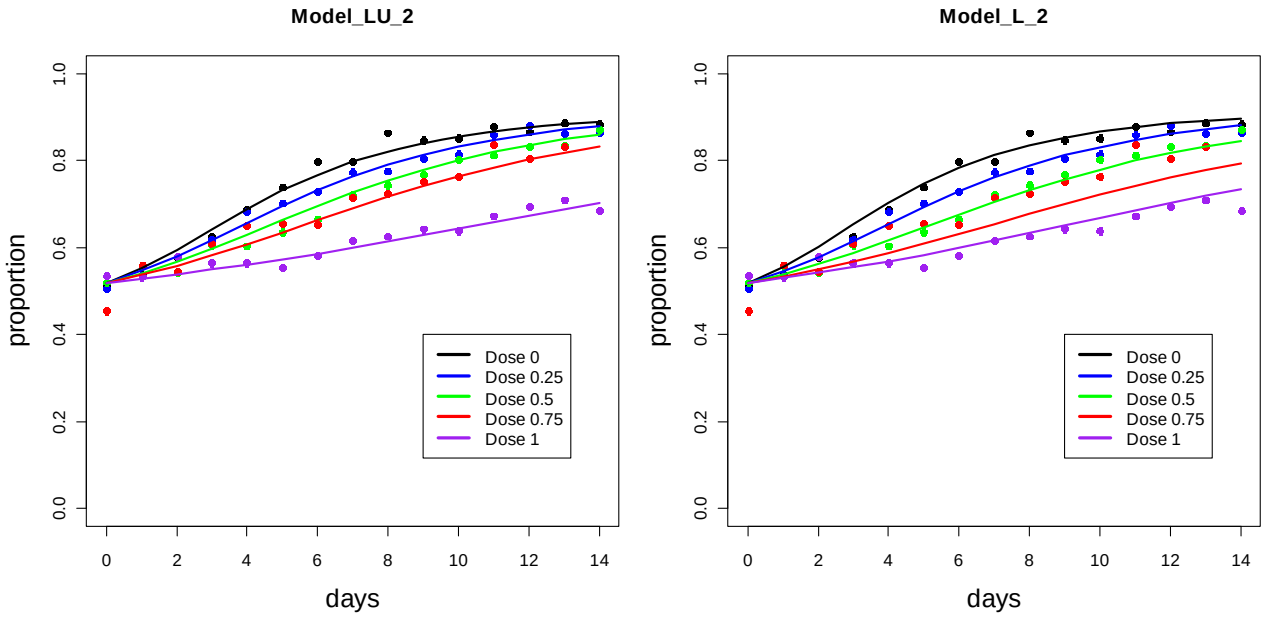

Figure 9: Observed average dose profiles (dots) versus the marginalized predicted dose profiles (solid lines) as predicted by Model_LU_2 (left panel) and Model_L_2 (right panel). The observed average proportions were based on the means of the beta distribution. The marginalized predicted dose profiles were obtained by averaging over the random effects based on sampling.

The scatter pots of the empirical Bayes estimates of the random effects did not revealed extreme outlying estimations, though the random effects for *T70* for profiles at dose level 0.75 showed systematically more negative values, indicating some model deviation for Model_L_2 (see Appendix Figure 22).

3.1.5 Comparison of two non-linear models

In Figure 10, the two models are compared by plots of the predicted dose profiles of an average animal, as estimated by Model_LU_2 (left panel) and Model_L_2 (right panel). The horizontal solid line represents the level at proportion 0.7. The vertical dotted lines represents the time when proportion 0.7 was reached (*T70*) as estimated by the model. In Model_LU_2, *T70* increases exponentially by factor 2.10

(= exp(0.74)) with a unit increase in dose level in the range of dose level 0mg to 0.75mg. The separate parameter for the estimation of dose effect at dose level 1mg allows *T70* to move freely. The estimation (= 13.9 days) lies far beyond of what would be expected from the estimation at the lower dose levels, which is 8.8 days (= 4.2* exp(0.74)).

In Model_L_2, *T70* increases exponentially by factor 3.03 with a unit increase of dose level. The estimation of dose effect at dose level 0.75 (= 9 days) is higher compared to the estimation based on Model_LU_2 (= 7.4 days), the estimation of dose effect at dose level 1mg (= 11.9 days) is lower compared to the estimation based on Model_LU_2 (= 13.9 days).

Both plots also reveal that according to both models, the asymptotic levels are not reached yet at the higher dose profiles. The estimated profile at dose level 0mg (black line) seems to reach its asymptote at day 12-14 in both plots, whereas the higher dose profiles, like dose level 1mg (purple line) is still increasing at day 14.

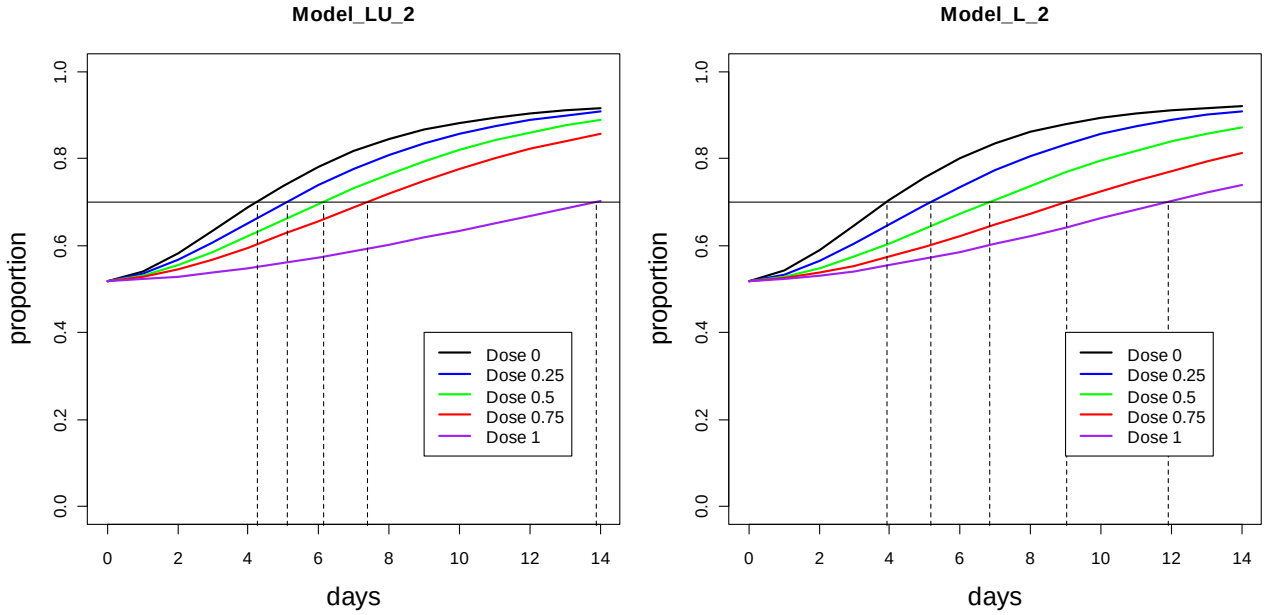

Figure 10: Predicted dose profiles of an average animal as estimated by Model_LU_2 (left) and Model_L_2 (right). The predictions were based on the hierarchical model.

3.2 Time-to-event analysis

3.2.1 Exploratory data analysis

The dataset based on definition 1 contained 12.50% censored observations. The censored observations were mainly in the stratum of dose level 1mg (see Table 14). The Kaplan-Meier estimate per dose level stratum is presented in Figure 11, upper left panel. The estimated survival curves for dose level strata

0mg up to 0.75mg lie close to each other whereas the estimated curve for dose level stratum 1mg lies considerably more to the right compared to lower dose levels. The median survival time based on the Kaplan-Meier estimate per dose level stratum shows similar results as presented in Table 13. The median survival times estimated for the lower dose levels ranges between 4 and 6 days whereas the median survival time for dose level 1mg is estimated to be 13 days. Note that the confidence interval for dose level 0.25mg and 0.75mg are wider since these strata contain less events.

3.2.2 Cox proportional hazard model

The parameter estimate for dose effect (β) and the corresponding hazard ratio (HR, *exp(β)*) as estimated by the Cox proportional hazard model are presented in Table 15.

The dose effect (β= -1.843, SE= 0.306) was significant according to the likelihood ratio test (G^2= 36.47, DF= 1, pvalue <0.0001). A negative dose effect indicates a decrease in the log-hazard by 1.843 with a unit increase in dose level (i.e., going from dose level 0mg to 1mg). It corresponds to a hazard ratio equal to 0.153 (95% CI: (0.087, 0.288)). A decreasing hazard with increasing dose level corresponds to a prolongation of *T70* with increasing dose level. The median survival time per dose level as estimated by the model are compared to the median survival times estimated by the Kaplan-Meier estimate in Table 13. The median survival times are comparable except for dose level 0.75mg, were the median survival time is estimated higher by the model (median= 8, 95% CI: (7, 11)) compared to the Kaplan-Meier estimate (median= 4, 95% CI: (4, 10)).

The time-dependent covariate was not significant according to the likelihood ration test (G^2= 0.82, DF= 1, pvalue = 0.36), indicating no violation of the proportionality assumption. More positive deviance residuals were found at dose level 0.75mg (11 out of 12 deviance residuals were positive), indicating some lack of fit at dose level 0.75mg. Positive deviance residuals correspond to observations with a shorter than expected observed survival time. No observations were found with deviance residuals outside the interval [-3, 3], indicating no extreme outliers. One observation was found with a deviating score residual. The observation corresponds to animal 16 from dose level 0mg group who has a *T70* value equal to 13 days.

Table 13: Median *T70* with 95% confidence interval (CI) as estimated by the Kaplan-Meier estimator per dose level stratum and by the Cox proportional hazard model based on definition 1. The confidence intervals were based on the log-log transformation. Note: NA indicates that the median value was not reached.

	Kaplan-Meier estimate		Cox PH Model Definition 1	
Dose level	**Median**	**95% CI**	**Median**	**95% CI**
0mg	4	(3, 5)	4	(3, 4)
0.25mg	4.5	(2, 9)	5	(4, 6)
0.5mg	6	(4, 7)	6	(5, 8)
0.75mg	4	(4, 10)	8	(7, 11)
1mg	13	(12, NA)	12	(9, 13)

3.2.3 Sensitivity analysis

Assessing the discrepancy of *T70* based on the four definitions within each animal (not shown) revealed considerable different *T70* values proposed by the four definitions. Definition 2 tends to propose more conservative values for *T70* (i.e., higher *T70* values) Definition 3, on the other hand, seems to propose more early *T70* values as it is a more liberal approach. Definition 4 provides *T70* values in between the ones proposed by definition 2 and 3.

A summary of the censored observations were compared between the four definitions in Table 14. The percentage of censored observations is quite similar between lower dose levels (0mg to 0.75mg). Substantial differences are present at dose level 1mg with a percentage of censored observations of 37.50%, 75.00%, 8.33% and 8.33% for definition 1, 2, 3 and 4, respectively.

Table 14: Summary of the censored observations based on the dataset by definition 1, 2, 3 and 4. N: number of observations.

Dose level	Total N	N (%) Censored Definition 1	N (%) Censored Definition 2	N (%) Censored Definition 3	N (%) Censored Definition 4
0mg	24	0 (0.00%)	1 (4.17%)	0 (0.00%)	0 (0.00%)
0.25mg	12	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
0.5mg	24	2 (8.33%)	3 (12.50%)	2 (8.33%)	2 (8.33%)
0.75mg	12	1 (8.33%)	1 (8.33%)	0 (0.00%)	0 (0.00%)
1mg	24	9 (37.50%)	18 (75.00%)	2 (8.33%)	2 (8.33%)
Total	96	12 (12.50%)	23 (23.96%)	4 (4.17%)	4 (4.17%)

The plots of the Kaplan-Meier estimates per dose level stratum are shown in Figure 11.

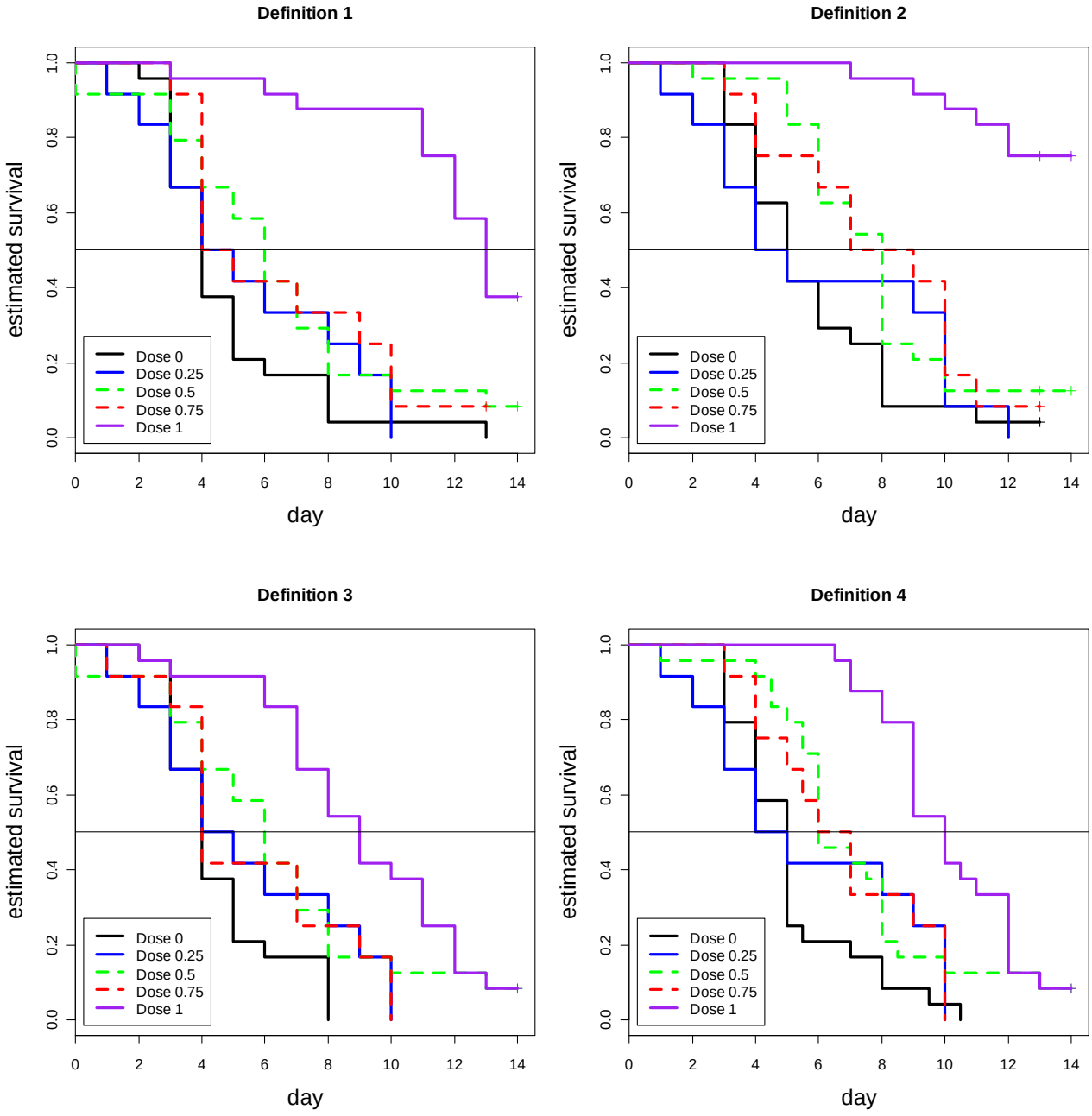

Figure 11: Kaplan-Meier estimator for the survival function per dose level stratum applied to the datasets based on definition 1, 2, 3 and 4. The horizontal line corresponds to an estimated survival equal to 0.5.

The plots based on definition 2, 3 and 4 deviated from the plots based on definition 1. The estimated curve of the dose level 1mg stratum lies considerable lower for definition 3 and 4, and higher for definition 2.

A comparison of the parameter estimates based on the Cox proportional hazard model is presented in Table 15.

Table 15: Parameter estimate of dose effect (β), standard error (SE), corresponding hazard ratio (HR) and 95% confidence interval (CI) of the hazard ratio (HR) for on the Cox proportional hazard model based on definition 1, 2, 3 and 4. The pvalues were based on the likelihood ratio tests.

Definition	β	SE(β)	pvalue	Hazard ratio (HR)	95% CI HR
Definition 1	-1.843	0.306	<0.0001	0.158	(0.087, 0.288)
Definition 2	-2.148	0.330	<0.0001	0.117	(0.061, 0.223)
Definition 3	-1.318	0.302	<0.0001	0.268	(0.148, 0.483)
Definition 4	-1.478	0.294	<0.0001	0.228	(0.128, 0.406)

The likelihood ratio tests revealed significant negative dose effect (β) in all four analyses (pvalue <0.0001 in all four analyses) but the parameter estimates for β differ between the analyses. The parameter estimates from the analyses based on definition 3 and 4 show a less negative dose effect (β_{def3}= -1.318, SE= 0.302 and β_{def4}= -1.478, SE= 0.294) and the parameter estimated from the analysis based on definition 2 shows a more negative dose effect (β_{def2}= -2.148, SE= 0.330) compared to the parameter estimate from the analysis based on definition 1 (β_{def1}= -1.843, SE= 0.306).

Note also that β_{def2} is estimated with slightly more uncertainty. The standard error for β_{def2} (= 0.330) is slightly higher compared to the standard errors of β_{def1}(= 0.306), β_{def3} (= 0.302) and β_{def4}(= 0.294). It is probably due to the fact that the data according to definition 2 contains the most censored values (% censored values definition 2= 23.96%).

Likelihood ratio tests revealed no violation of the proportionality assumption in the analysis based on definition 2 (G^2= 0.74, DF= 1, pvalue= 0.39) and definition 3 (G^2= 1.17, DF= 1, pvalue= 0.28). The proportionality assumption was violated in the analysis based on definition 4 (G^2= 8.28, DF= 1, pvalue= 0.004).

3.3 Comparison of a non-linear model and a Cox proportional hazard model

In this section, a direct answer to the research question is given based on the non-linear model (Model_L_2) and compared with the results from the time-to-event analysis. The non-linear model Model_L_2 is chosen instead of Model_LU_2, since it provides an estimate of *T70* over the whole dose range [0mg, 1mg]. Model_L_2 showed a less good fit compared to Model_LU_2, especially at dose level 0.75mg but we have to keep in mind that only 12 animals were available at dose level 0.75mg. The discrepancy between the model and the observed data at dose level 0.75mg might be due to random behavior. Only additional data at intermediate dose levels between 0.75mg and 1mg can elucidate this issue (see discussion).

In Figure 12, upper left panel, the non-linear model is compared to the time-to-event analysis based on definition 1. The red solid curve represents *T70* values for an average animal versus dose level with a 95% pointwise confidence intervals as estimated by the non-linear model. *T70* increases exponentially with increasing dose levels from 3.9 days at dose level 0mg up to 11.9 days at dose level 1mg. The median *T70* versus dose level with a 95% pointwise confidence intervals based on the Cox proportional hazard model is plotted by the black stepwise curve. The median *T70* increases with increasing dose levels in a stepwise manner from 4 days at dose level 0mg up to 12 days at dose level 1mg. The red solid line based on the non-linear model overlaps with the black stepwise curve based on the Cox model, indicating similar estimations for *T70* by the two analyses. However, the estimations for *T70* based on the non-linear model were estimated with more precision, especially at higher dose levels. The 95% pointwise confidence intervals based on the non-linear model are completely enclosed by the 95% pointwise confidence intervals based on the Cox model at dose levels above 0.5mg.

One of the reasons might be that summarizing the full profiles into one single value for *T70* increases imprecision. i.e., estimations of *T70* based on the full profiles like in the non-linear model are more accurate compared to estimations of *T70* based on one single value per animal like in the time-to-event analysis. A second reason might be that the estimations from the non-linear model were based on a subject specific level, removing the inter-animal variability, whereas the estimations from the time-to-event analysis were based on the population level. Additionally, the time-to-event analysis is subjected to censored data. The effective sample size in a time-to-event analysis is based on the number of events and not on the complete sample size. At higher dose levels, more censored observations were found, making the estimations of *T70* at higher dose levels less efficient. This is not the case in the non-linear model, where the complete sample size contributes to the estimation of *T70*, making the estimation more efficient.

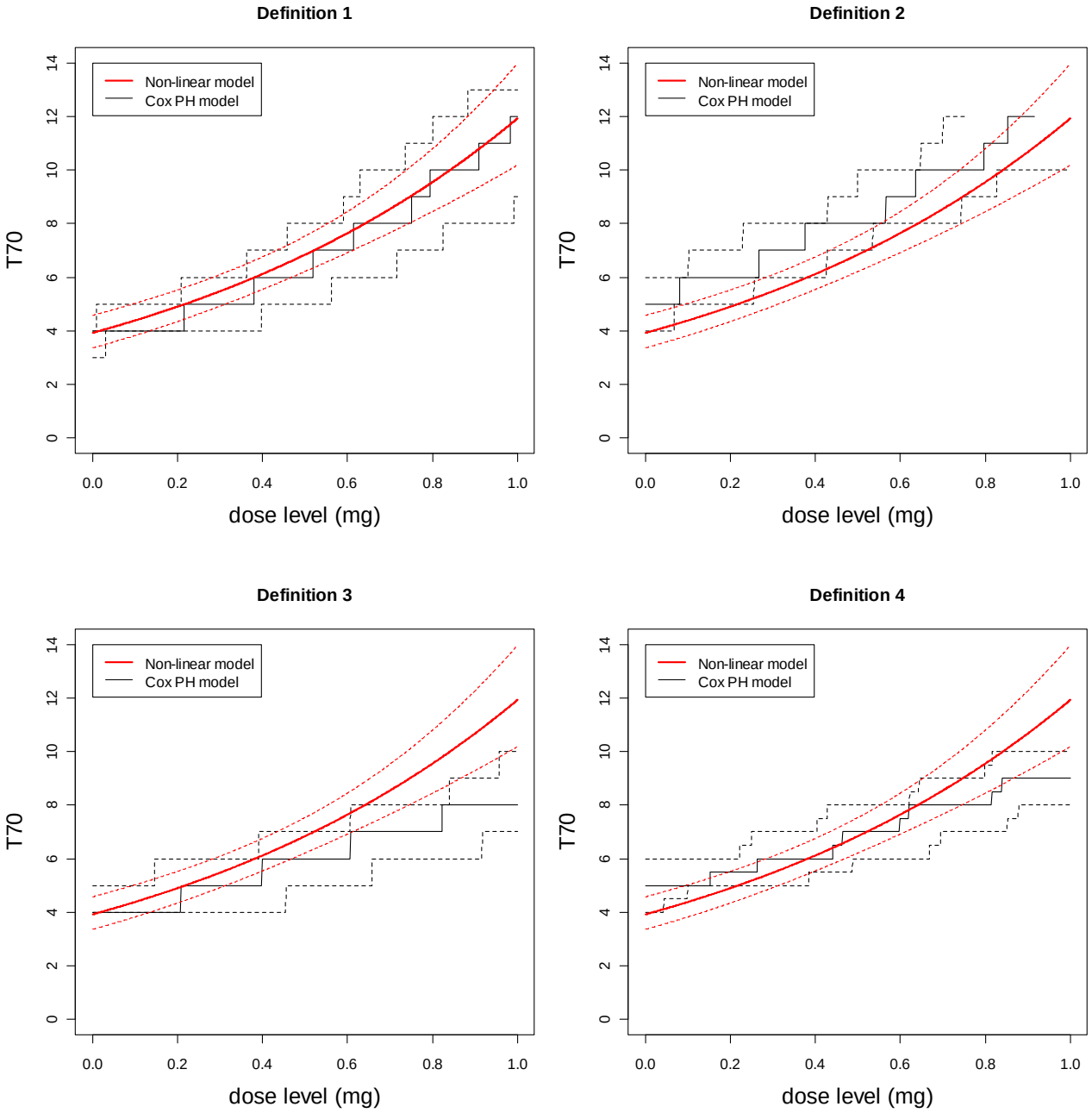

Figure 12: Comparison of the estimated *T70* values with 95% pointwise confidence intervals as estimated by the non-linear model and the Cox proportional hazard model. The red solid line represents the *T70* values for an average animal as estimated by the non-linear model (Model_L_2). The red dotted curves are the upper and lower 95% pointwise confidence limits. The black solid stepwise curve represents the median *T70* values as estimated by the Cox proportional hazard model and the black dotted stepwise curves are the upper and lower 95% pointwise confidence limits based on the log-log transformation. In the upper left, upper right, lower left and lower right panel the comparisons were made with a Cox proportional hazard model based on definition 1, 2, 3 and 4, respectively.

Another source of uncertainty addressed to the time-to-event analysis is the sensitivity of the analysis to the definition of *T70*. The upper right, lower left and lower right panel in Figure 12 compare the results of the non-linear model and the Cox model based on definition 2, 3 and 4, respectively. The estimations of *T70* based on the two models were no longer similar. The Cox model based on definition 2 estimates *T70* systematically higher compared to the estimations from the non-linear model (see Figure 12, upper right panel). This is a consequence of the strong conditions imposed by the definition. Note that definition 2 defines *T70* as the first day when proportion 0.7 is reached and at least two consecutive proportions are higher or equal to 0.7. A profile reaching proportion 0.7 who shows a consecutive lower proportion due to random fluctuation will be assigned a *T70* value later in time compared to the true *T70* value. Note that the median *T70* and the upper 95% confidence limits based on the Cox model were no longer available at dose levels near 1mg (see Figure 12, upper right panel). This might be due to the high percentage of censored observations at higher dose levels for definition 2 (see Table 14).

Likewise, the Cox model based on definition 3 estimates *T70* values systematically lower compared to the non-linear model, especially at higher dose levels (see Figure 12, lower left panel). Definition 3 defines *T70* as the first day the profile reaches proportion 0.7. A profile not yet reaching proportion 0.7 who shows a high proportion by random fluctuation will be assigned a *T70* value more earlier in time compared to the true *T70* value. The estimations of *T70* as estimated by the Cox model based on definition 4 (see Figure 12, lower right panel) deviate strongly from the estimation of *T70* based on the non-linear model. At lower dose levels, the Cox model estimates *T70* values systematically higher and at higher dose levels, the Cox model estimates *T70* values systematically lower compared to the non-linear model.

4 Discussion and conclusion

In this report, the effect of compound X on learning behavior was studied in 96 male wistar rats. Longitudinal data on the proportion of correctly executed trails per training session from an animal behavior experiment through reward/punishment mechanisms was available on daily basis during a period of 14 days. A Weibull learning curve was used to describe learning behavior by four characteristics: an intercept (*I*), the asymptotic level (*A*), the abruptness (*S*) and the time until proportion 0.7 was reached (*T70*). A non-linear model revealed that the most important characteristic to study the effect of compound X on learning behavior was *T70*. *T70* prolongs with increasing dose level.

Two hypotheses can be raised about how the dose level of compound X changes *T70*. In the first hypothesis, dose level 1mg is believed to show outlying behavior. Animals receiving a dose level of 1mg tend to learn more slower as expected from animals receiving lower dose levels. A possible explanation could be that after reaching a certain dose level, different biological effects come into play beyond the expected effect of compound X on learning behavior (e.g., adverse events that have a negative influence on the performance of the test). Extra experiments with animals at intermediate dose levels between 0.75mg and 1mg could elucidate whether this is the case and at which dose level the biological effect starts to change. Another possibility to get more insight in this phenomenon is to look at different response values like the percentage of completed trials, correction trial accuracy, response latency, food collection latency,…

According to the second hypothesis, dose level 0.75mg is believed to show outlying behavior and not dose level 1mg. Animals receiving dose level 0.75mg tend to learn more faster as would be expected from animals receiving other dose levels. The discrepancy found could be considered mere as a coincidence due to random behavior. We have to keep in mind that only 12 or 24 animals were used per dose level, making the second hypothesis reasonable. Therefore the second hypothesis was used to provide a direct answer to the research question i.e., to quantify the dose effect of compound X on *T70*. A graph (Figure 12, red curves) with corresponding table (Appendix Table 4) are provided with estimated *T70* values at dose levels ranging between 0mg and 1mg for an average animal with 95% pointwise confidence intervals.

A borderline significant dose effect on the asymptotic level was found. The biological relevance of this effect was questionable since most profiles have not yet reached their asymptotic level within the observed time frame. If interest lies in studying the dose effect of compound X on the asymptotic level with sufficient precision, the profiles should be studied longer in time.

One might wonder whether modeling the full profiles by a non-linear model is necessary to quantify the dose effect of compound X on *T70*. Fitting a non-linear model while taking into account the heterogeneity between animals by including random effects can be a time consuming exercise. To circumvent this problem, the longitudinal profiles were summarized by the time to reach proportion 0.7 and a time-to-event analysis was performed by fitting a Cox proportional hazard model. A first difficulty was defining *T70*. Reaching a success proportion of 0.7 is not a unambiguous endpoint like death in oncology studies, normally used in time-to-event analysis. Also, exploratory data analysis revealed substantial variability within animals. A sensitivity analysis revealed that the time-to-event analysis is quite sensitive to the definition chosen.

The dose effect of compound X on *T70* was quantified by the median *T70* value induced by dose levels ranging between 0mg and 1mg as estimated by the Cox proportional hazard model.

The estimated *T70* values based on the Cox proportional hazard model were similar compared to the estimated *T70* values based the non-linear model, though the estimation based on the Cox proportional hazard model came with less precision making it less suitable for an estimation problem like the research question of this report.

Additionally, the time-to-event analysis cannot be used to study the effect of compound X on other characteristics of learning behavior beyond *T70* like the non-linear model does.

5 References

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6 Appendix

Appendix Table 1: Model building procedure base model: model without dose effect and with random effects. Note: * indicates no convergence of the model, RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood, G^2: likelihood ratio statistic, DF: degrees of freedom, LR test: likelihood ratio test.

Model	Dose effect:	RE:	# p	AIC	-2ll	G^2	DF	pvalue	LR test
	no dose effect	none	5	-2079	-2089				
	no dose effect	*T70*	6	-3061	-3073				
	no dose effect	*S*	6	-2378	-2390				
	no dose effect	*A*	6	*	*				
	no dose effect	*I*	6	*	*				
	no dose effect	*T70+S*	8	-3186	-3202				
	no dose effect	*T70+I*	8	-3165	-3181				
	no dose effect	*T70+A*	8	*	*				
	no dose effect	*T70+S+I*	11	-3212	-3234				

Appendix Table 2: Model building procedure Model_LU: functional form on *T70*: Log-linear + Unstructured at dose level 1mg. Note: * indicates no convergence of the model, RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood, G^2: likelihood ratio statistic, DF: degrees of freedom, LR test: likelihood ratio test.

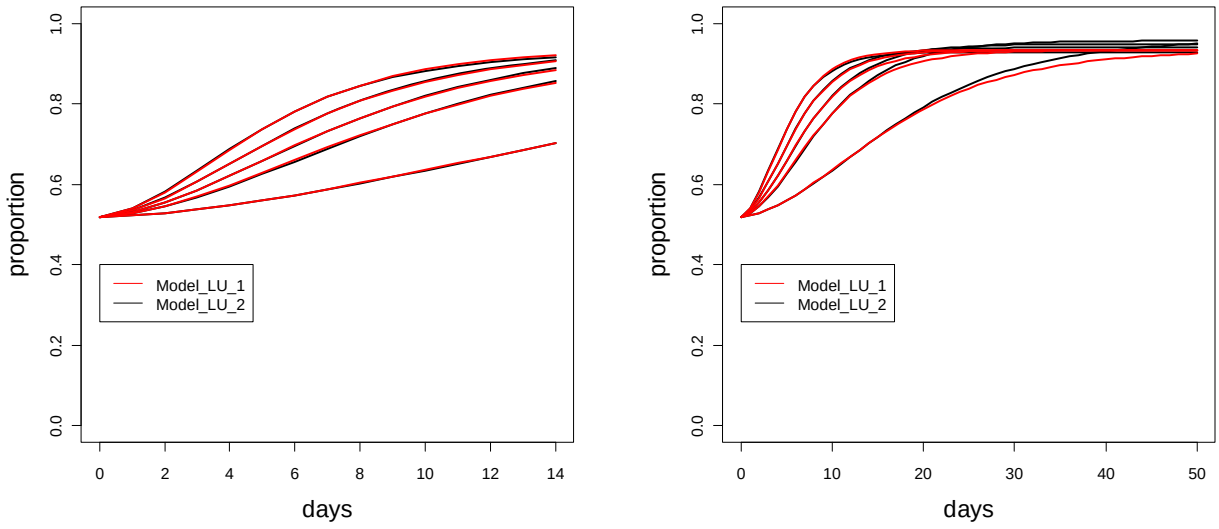
Model	Dose effect:	RE:	# p	AIC	-2ll	G^2	DF	pvalue	LR test
Model_LU_3	*T70+S+A*	*T70+S+I*	15	-3288	-3318				
	T70+S+A	*T70+S*	12	-3248	-3272	46	3/2	<0.0001	remove RE on *I*
	T70+S+A	*T70+I*	12	-3231	-3255	63	3/2	<0.0001	remove RE on *S*
	T70+S+A	*S+I*	12	*	*				remove RE on *T70*
	T70+S	*T70+S+I*	14	-3286	-3314	4	1	0.046	remove dose effect on *A*
Model_LU_2	*T70+A*	*T70+S+I*	14	-3290	-3318	0	1	1	remove dose effect on *S*
	S+A	*T70+S+I*	13	*	*				remove dose effect on *T70*
Model_LU_1	*T70*	*T70+S+I*	13	-3288	-3314	4	1	0.046	remove dose effect on *A*
	A	*T70+S+I*	12	-3225	-3249	69	2	<0.0001	remove dose effect on *T70*
	no dose effect	*T70+S+I*	11	-3224	-3246	68	2	<0.0001	remove dose effect on *T70*

Appendix Table 3: Model building procedure Model_L: functional form on *T70*: Log-linear. Note: * indicates no convergence of the model, RE: random effects, #p: number of parameters, AIC: Akaike information criterion, -2ll: -2 log-likelihood, G^2: likelihood ratio statistic, DF: degrees of freedom, LR test: likelihood ratio test.

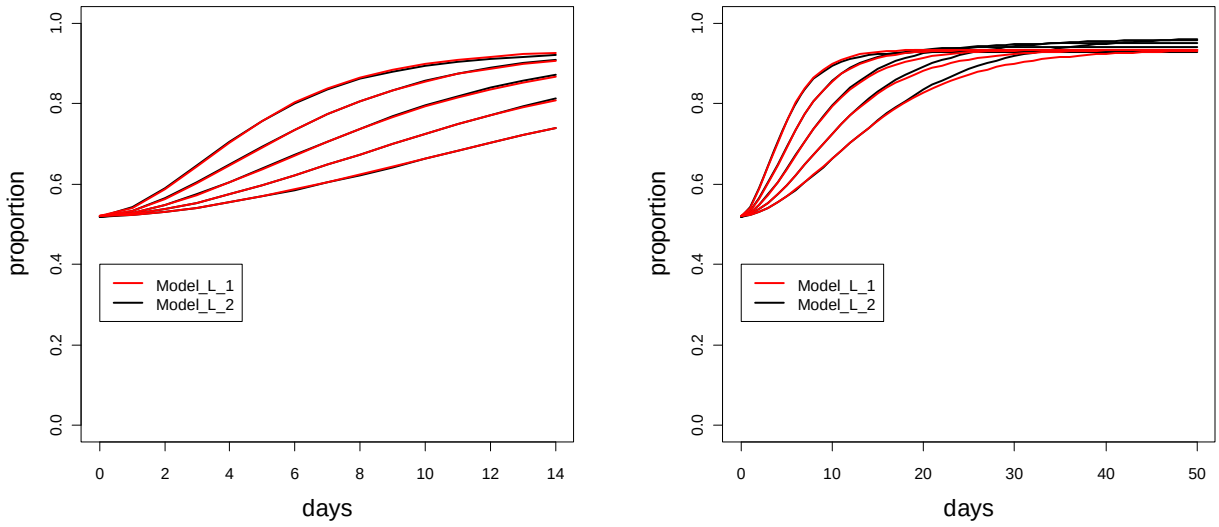
Model	Dose effect:	RE:	# p	AIC	-2ll	G^2	DF	pvalue	LR test
Model_L_3	*T70+S+A*	*T70+S+I*	14	-3283	-3311				
	T70+S+A	*T70+S*	11	-3243	-3265	46	3/2	<0.0001	remove RE on *I*
	T70+S+A	*T70+I*	11	-3226	-3248	63	3/2	<0.0001	remove RE on *S*
	T70+S+A	*S+I*	11	*	*				remove RE on *T70*
	T70+S	*T70+S+I*	13	-3280	-3306	5	1	0.025	remove dose effect on *A*
Model_L_2	*T70+A*	*T70+S+I*	13	-3285	-3311	0	1	1	remove dose effect on *S*
	S+A	*T70+S+I*	13	*	*				remove dose effects on *T70*
Model_L_1	*T70*	*T70+S+I*	12	-3282	-3306	5	1	0.025	remove dose effect on *A*
	A	*T70+S+I*	12	-3225	-3249	62	1	<0.0001	remove dose effect on *T70*
	No dose effect	*T70+S+I*	11	-3224	-3246	60	1	<0.0001	remove dose effect on *T70*

Appendix Table 4: *T70* values and 95% confidence interval (CI) at different dose levels for an average animal as estimated by the non-linear model (Model_L_2).

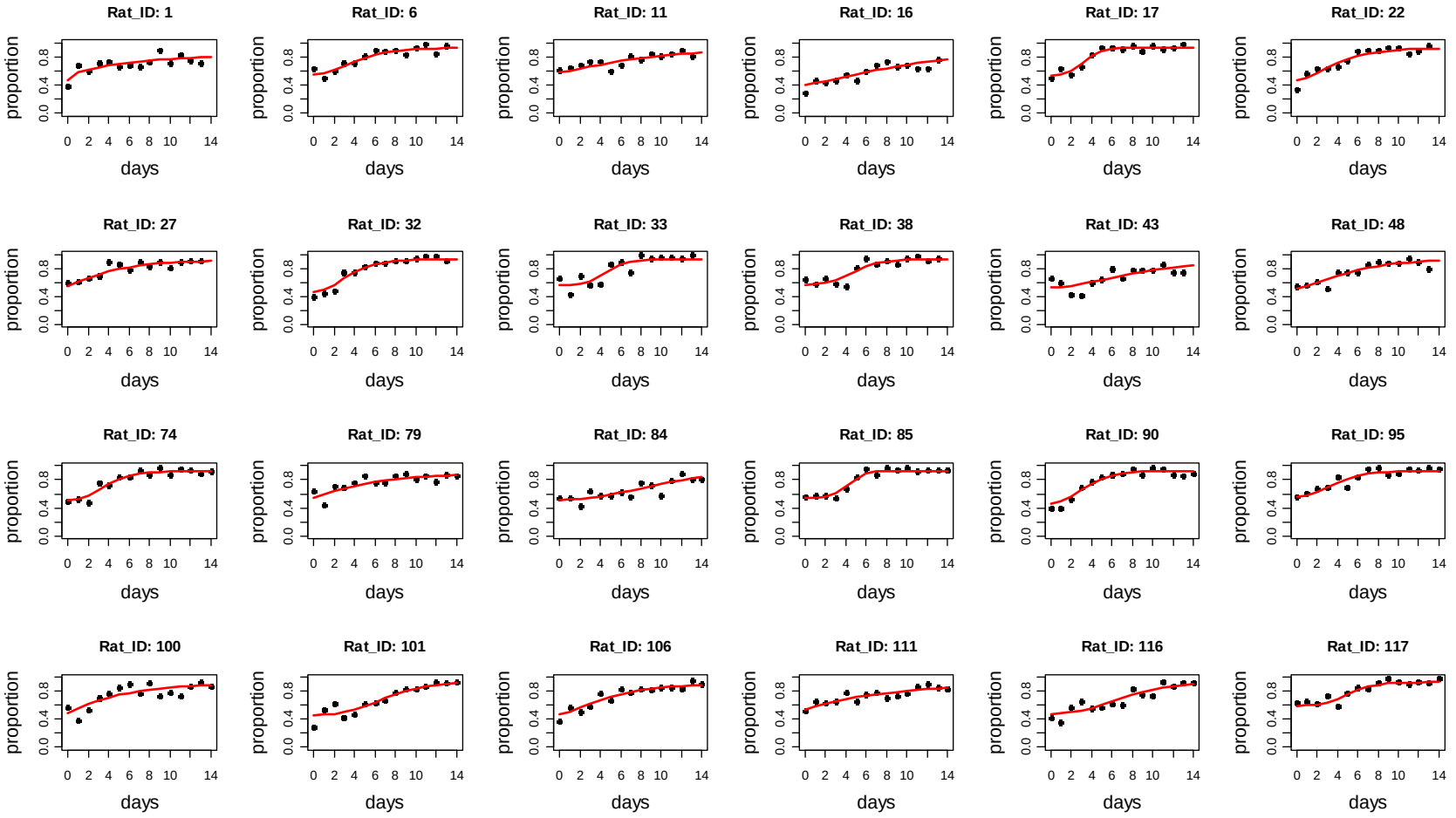
Dose level (mg)	*T70* (days)	95% CI
0	3.9	(3.4, 4.6)
0.05	4.1	(3.6, 4.8)
0.1	4.4	(3.8, 5.0)
0.15	4.6	(4.1, 5.3)
0.2	4.9	(4.3, 5.5)
0.25	5.2	(4.6, 5.8)
0.3	5.5	(4.9, 6.1)
0.35	5.8	(5.2, 6.4)
0.4	6.1	(5.5, 6.8)
0.45	6.5	(5.9, 7.1)
0.5	6.8	(6.2, 7.5)
0.55	7.2	(6.6, 8.0)
0.6	7.6	(6.9, 8.4)
0.65	8.1	(7.3, 9.0)
0.7	8.5	(7.7, 9.5)
0.75	9.0	(8.0, 10.1)
0.8	9.5	(8.4, 10.8)
0.85	10.1	(8.9, 11.5)
0.9	10.7	(9.3, 12.3)
0.95	11.3	(9.7, 13.1)
1	11.9	(10.2, 14.0)



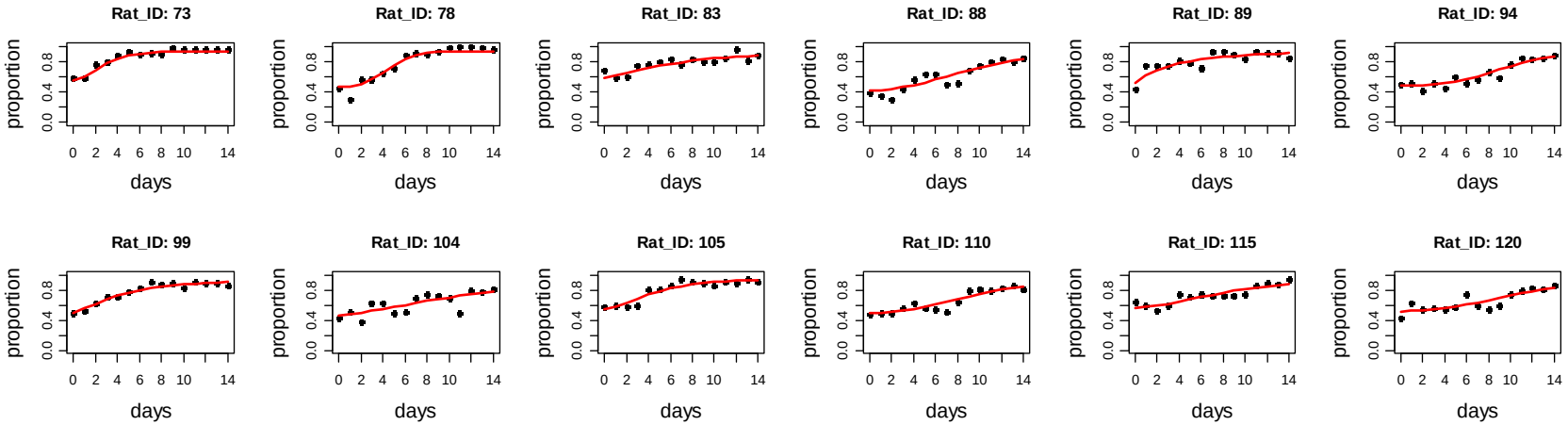
Appendix Figure 1: Model_LU: comparison of predicted dose profiles for an average animal as predicted by the model with dose effect on *A* (Model_LU_2) and the model without dose effect on *A* (Model_LU_1). The predicted profiles for Model_LU_2 and Model_LU_1 are shown by the black and red curves, respectively. The left panel and right panel shows the predicted profiles in the observed time frame (days within the range [0, 14]) and days within the range [0, 50], respectively.



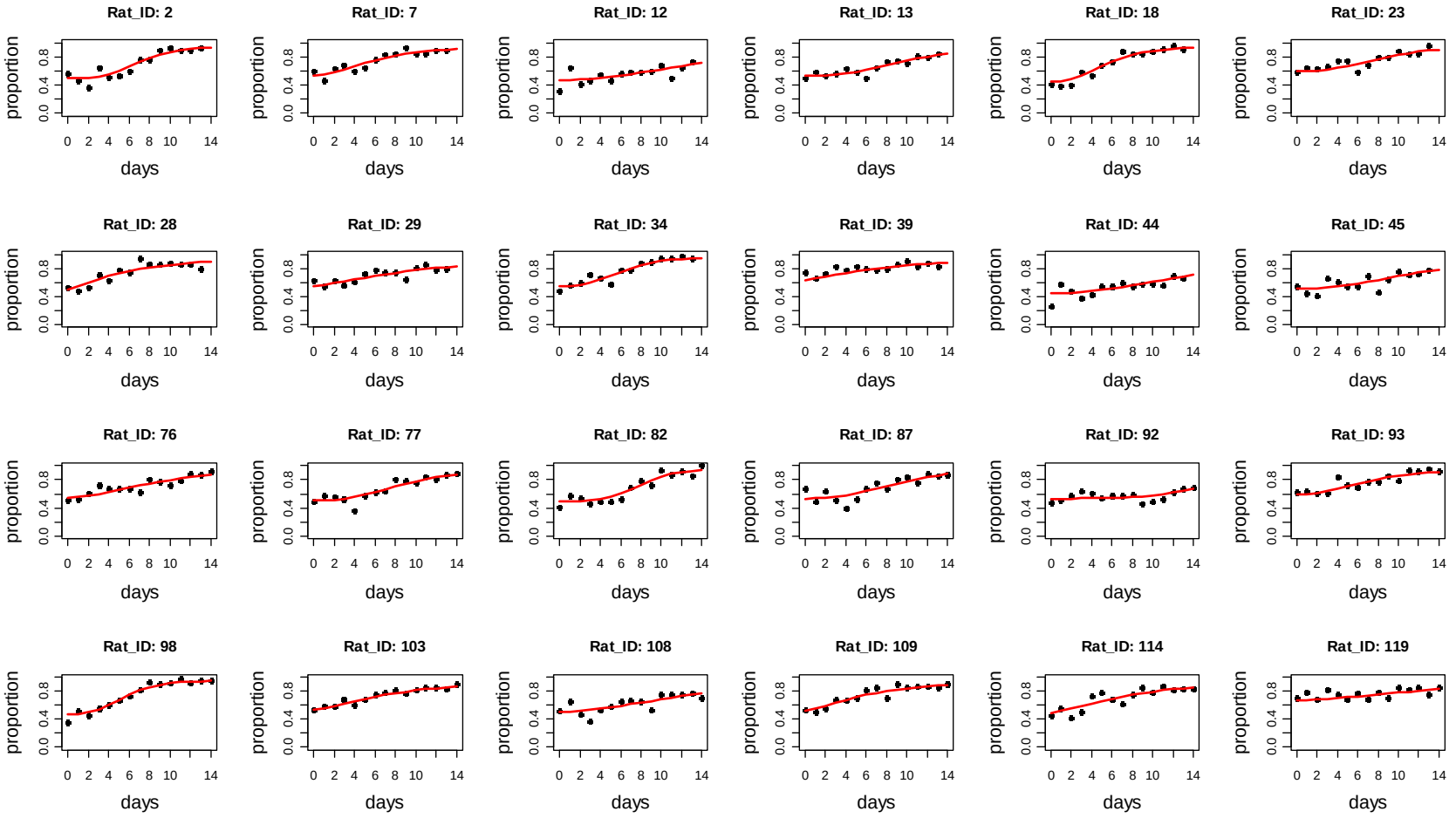
Appendix Figure 2: Model_L: comparison of predicted dose profiles for an average animal as predicted by the model with dose effect on *A* (Model_L_2) and the model without dose effect on *A* (Model_L_1). The predicted profiles for Model_L_2 and Model_L_1 are shown by the black and red curves, respectively. The left panel and right panel shows the predicted profiles in the observed time frame (days within the range [0, 14]) and days within the range [0, 50], respectively.



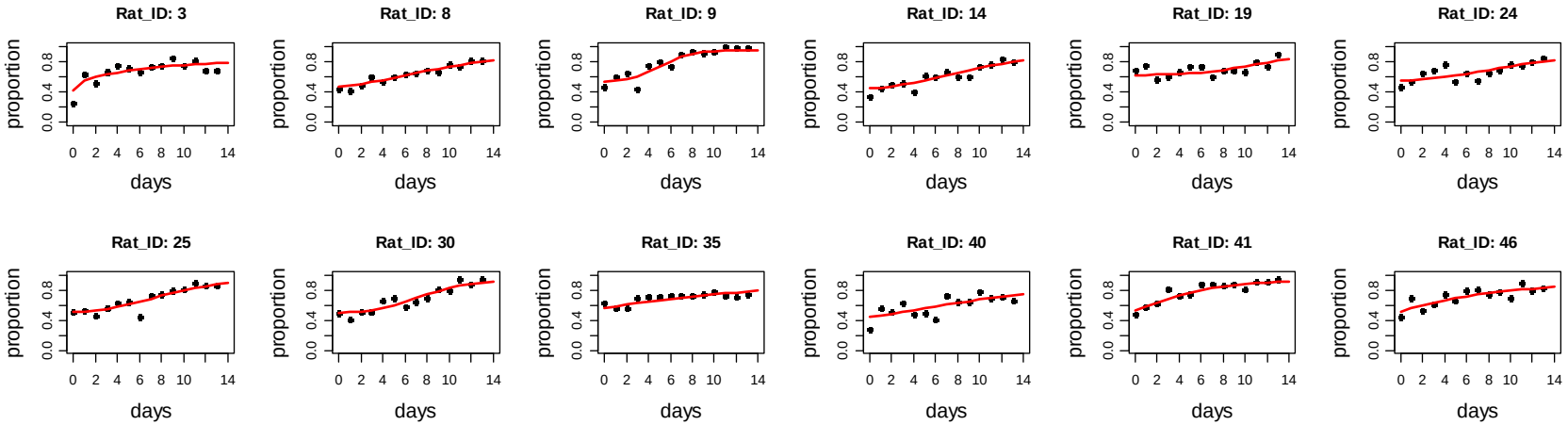
Appendix Figure 3: Model fit Model_LU_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0mg.



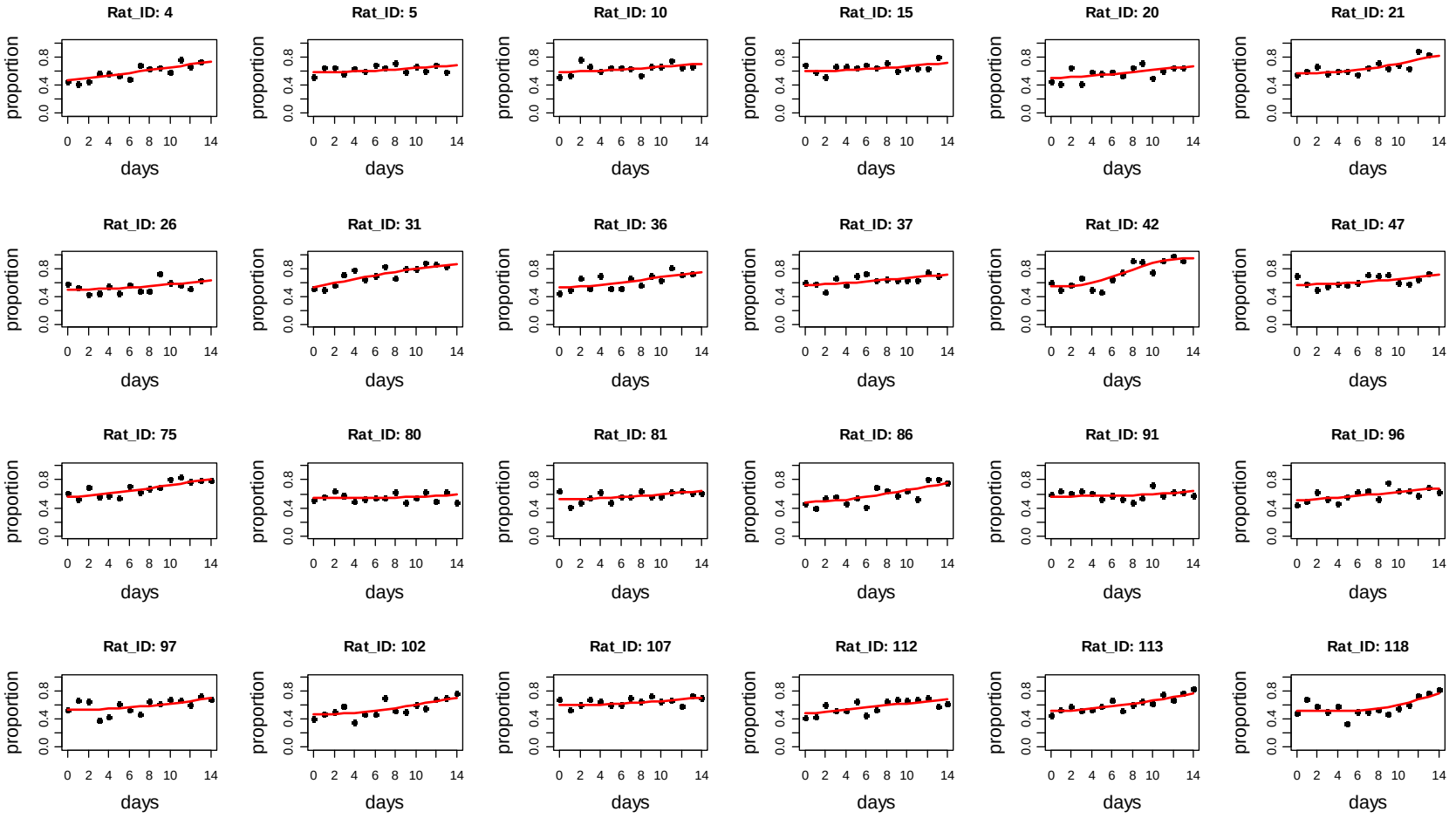
Appendix Figure 4: Model fit Model_LU_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.25mg.



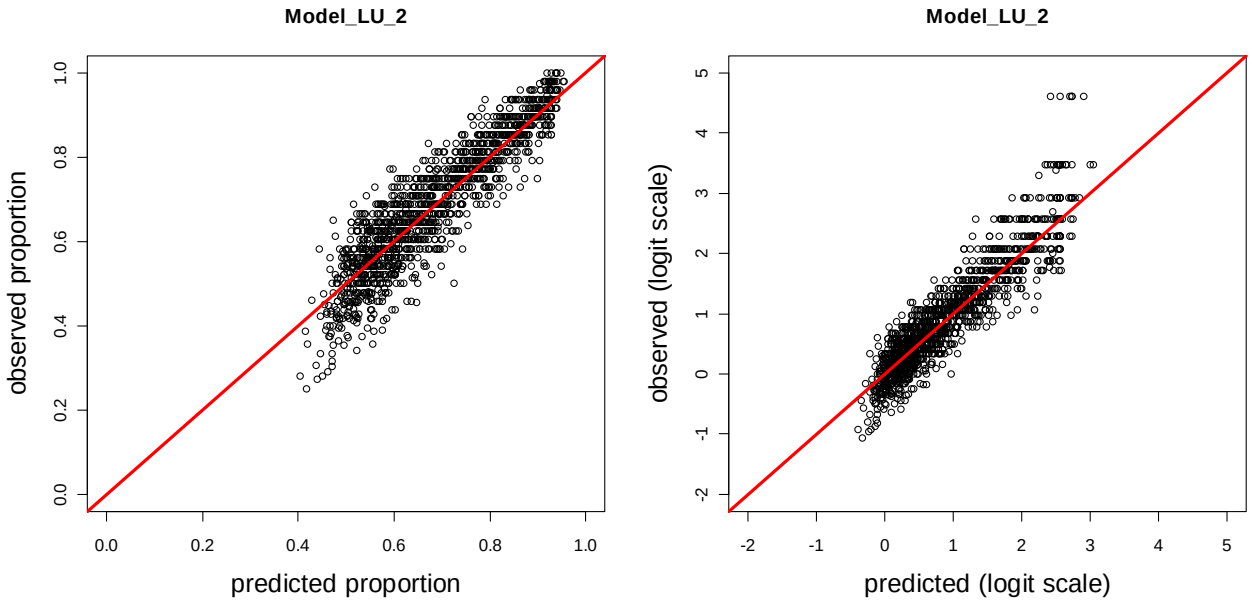
Appendix Figure 5: Model fit Model_LU_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.50mg.



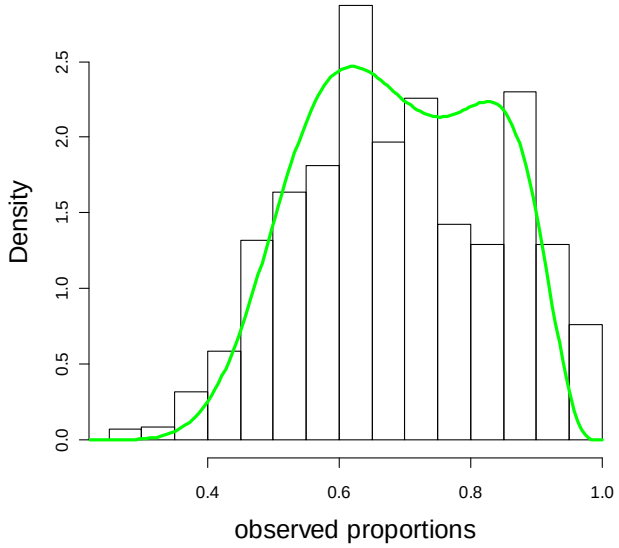
Appendix Figure 6: Model fit Model_LU_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.75mg.



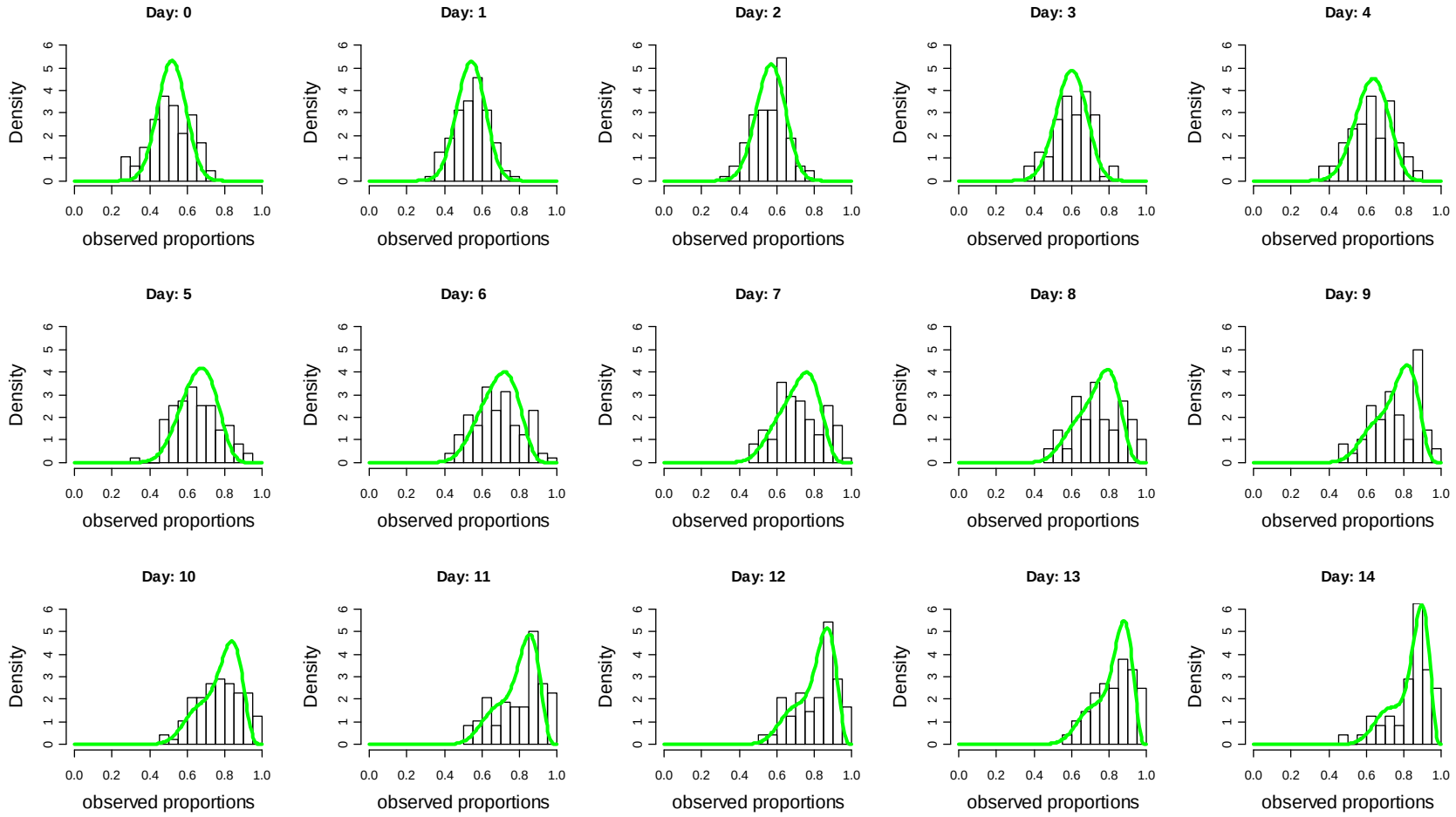
Appendix Figure 7: Model fit Model_LU_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 1mg.



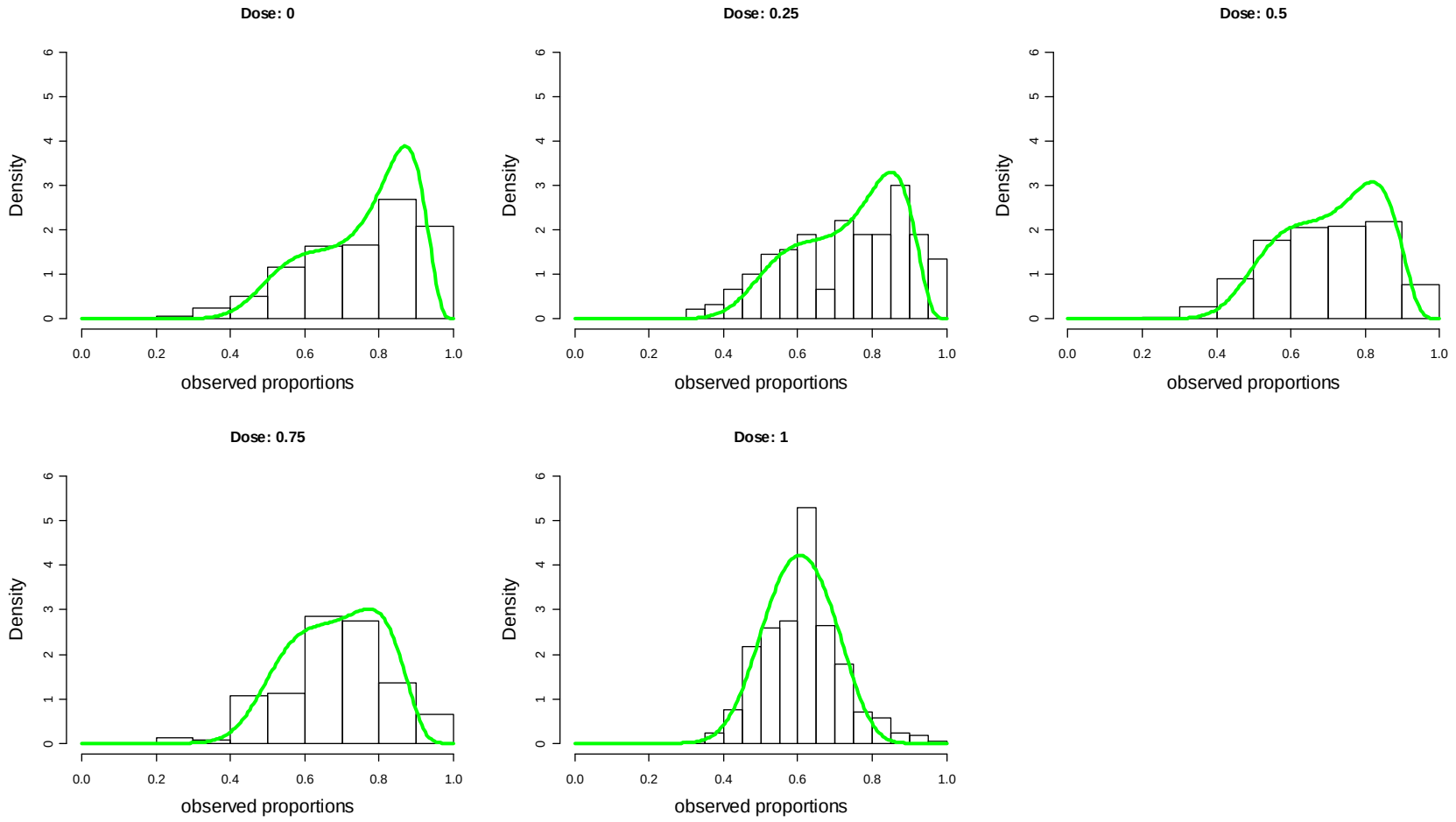
Appendix Figure 8: Model fit Model_LU_2: observed proportions versus predicted proportions (left panel) and observed values on the logit scale versus predicted values on logit scale (right panel). The red curve represents the 45 degree line through the origin.



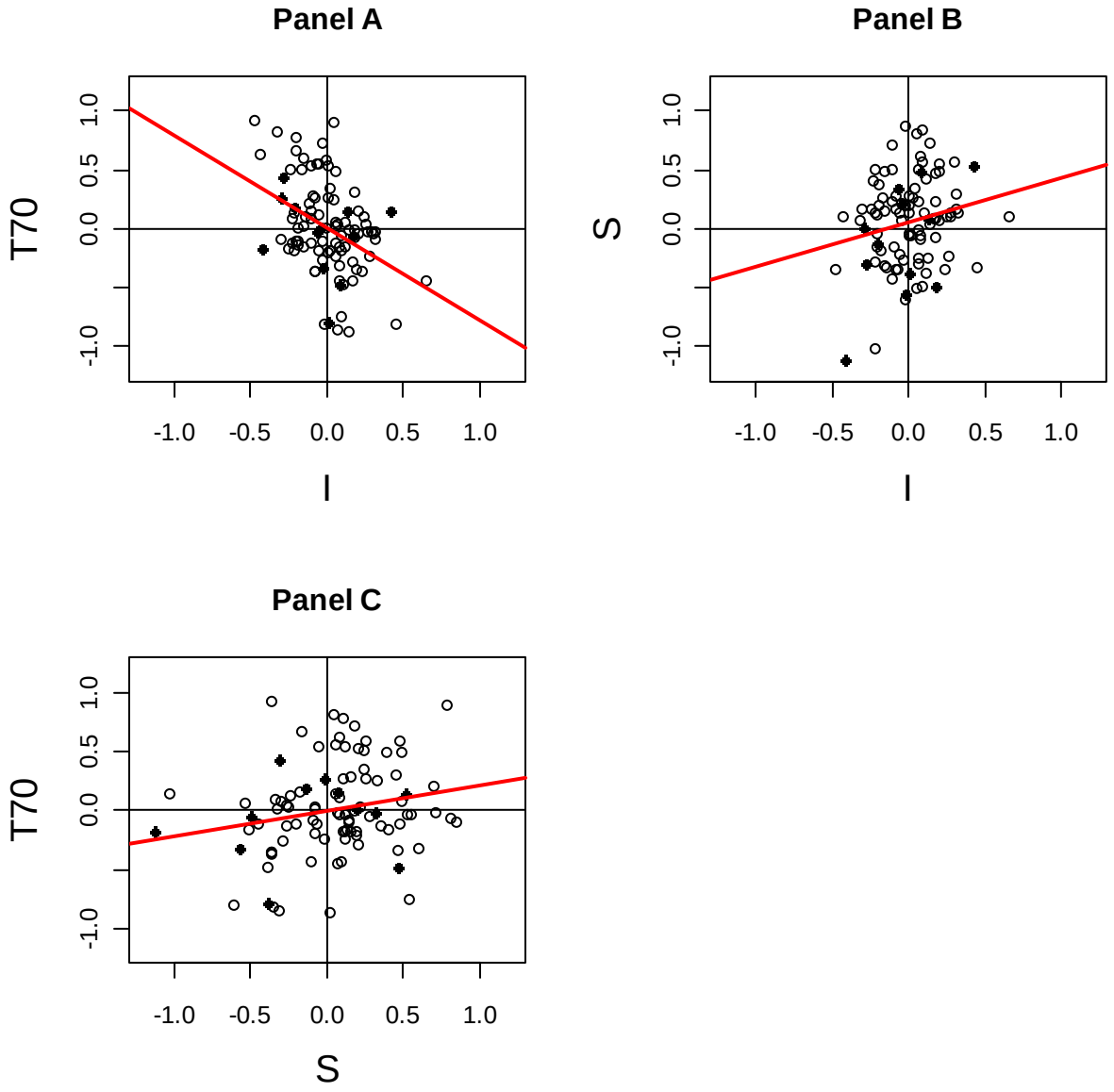
Appendix Figure 9: Model fit Model_LU_2 : mixture of beta distributions, overall histogram. The histogram of the observed proportions overlaid with a mixture of 75 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



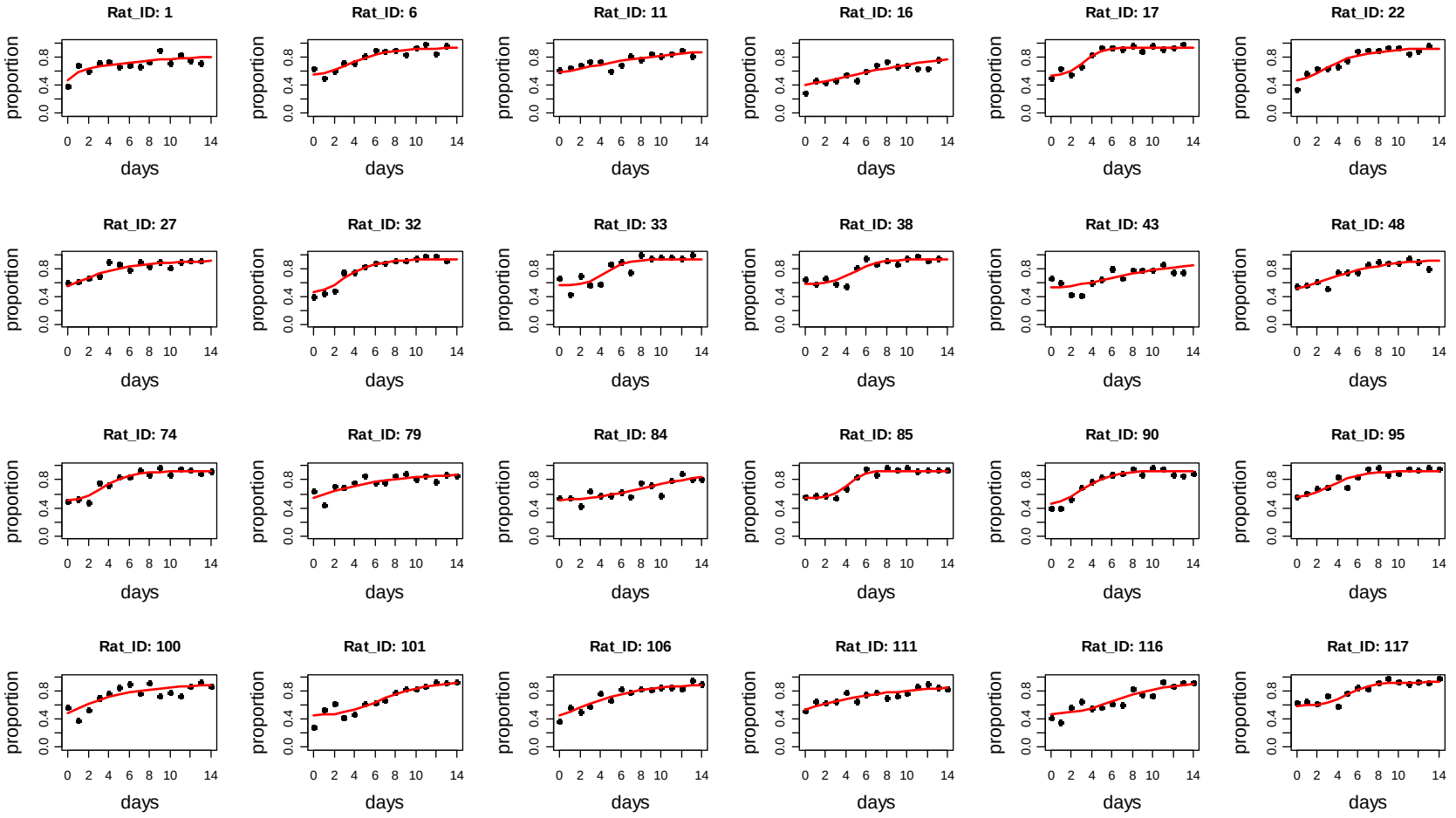
Appendix Figure 10: Model fit Model_LU_2 : mixture of beta distributions, histograms per day. The histograms of the observed proportions per day overlaid with a mixture of 5 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



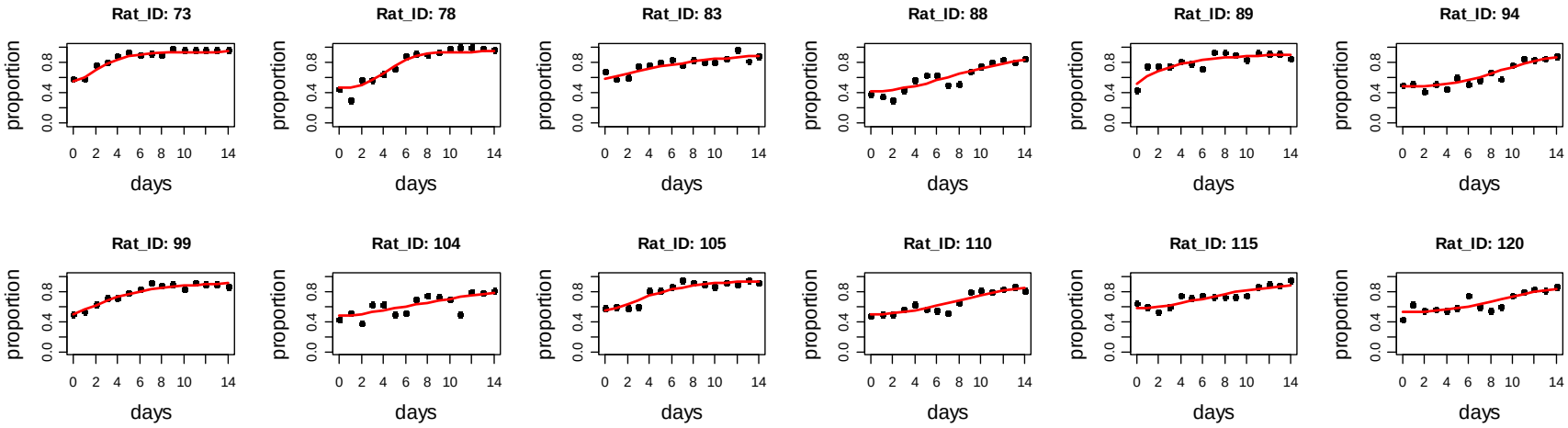
Appendix Figure 11: Model fit Model_LU_2 : mixture of beta distributions, histograms per dose level. The histograms of the observed proportions per dose level overlaid with a mixture of 15 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



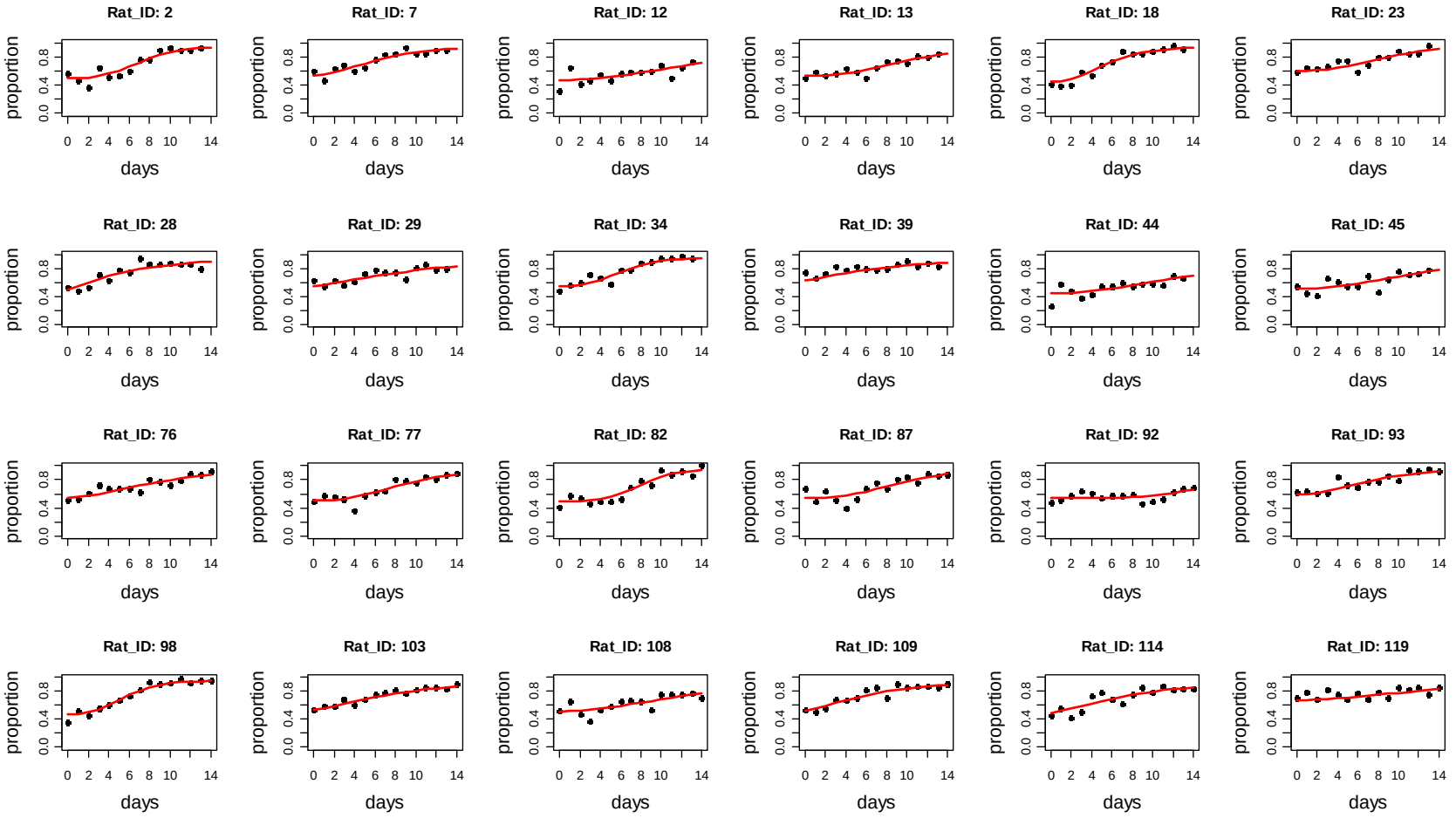
Appendix Figure 12: Model fit Model_LU_2 : scatter plot of empirical Bayes estimates of the random effects. Panel A: random *T70* versus random *I*, panel B: random *S* versus random *I* and panel C: random *T70* versus random *S*. The red line represents the estimated regression line. The horizontal and vertical black lines represents the average random effect, which is zero. The black filled dots represents the random effects for animals at dose level 0.75mg.



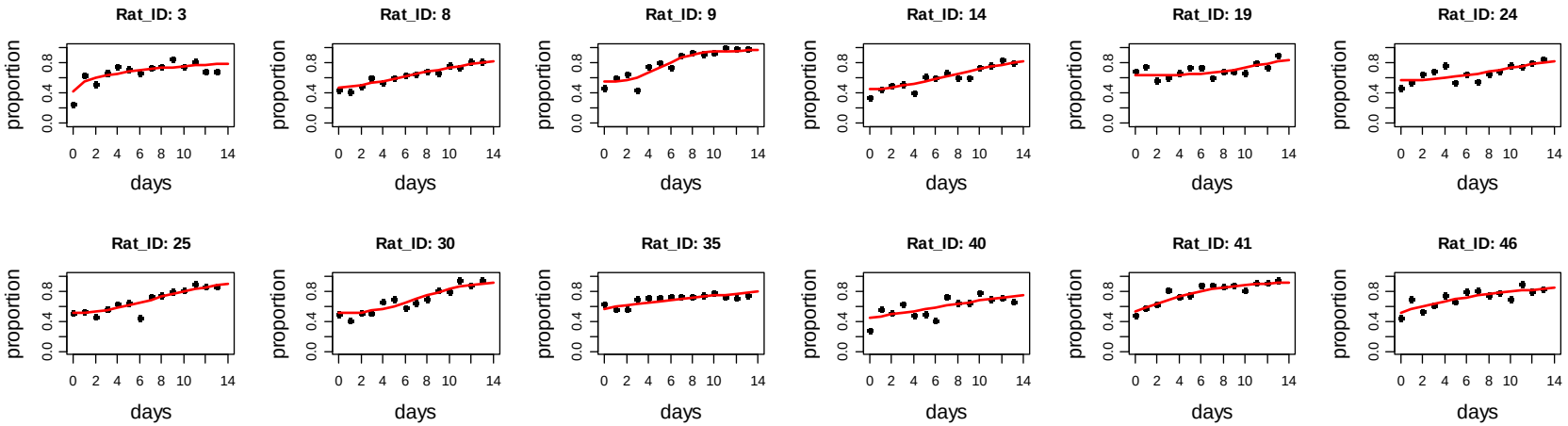
Appendix Figure 13: Model fit Model_L_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0mg.



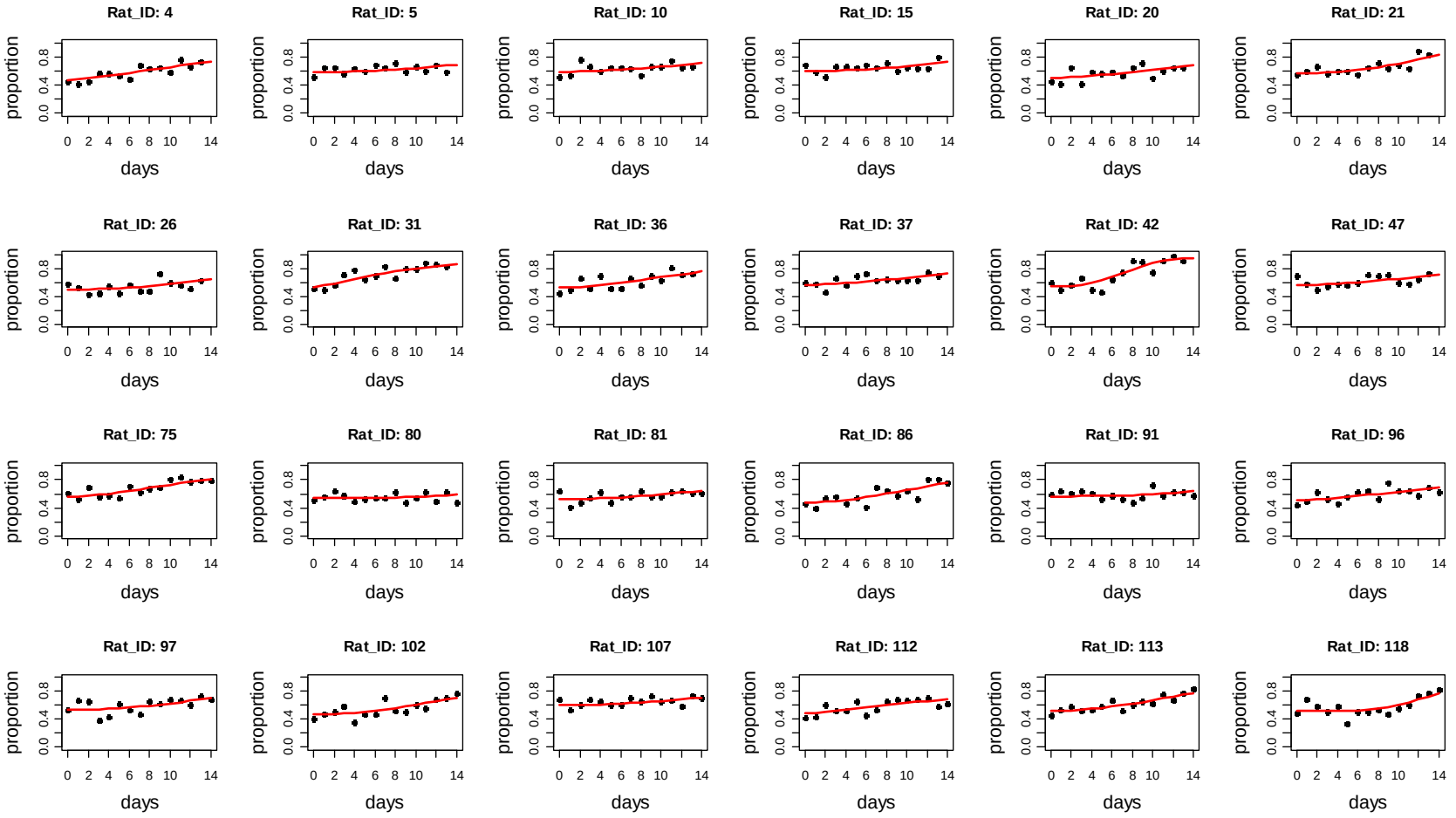
Appendix Figure 14: Model fit Model_L_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.25mg.



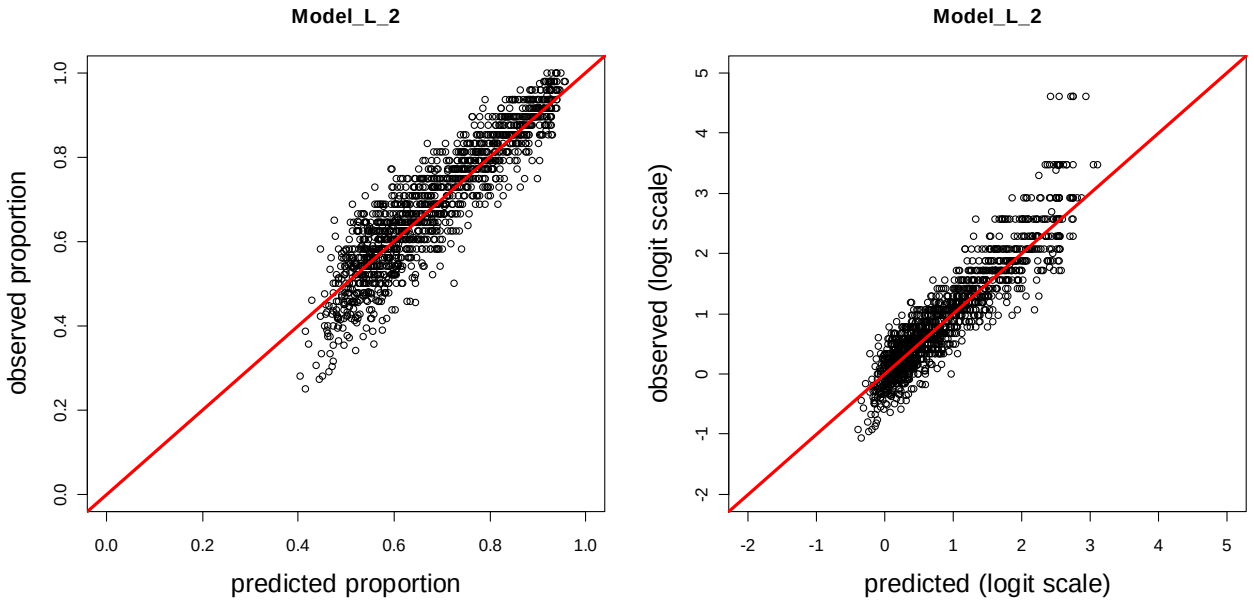
Appendix Figure 15: Model fit Model_L_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.5mg.



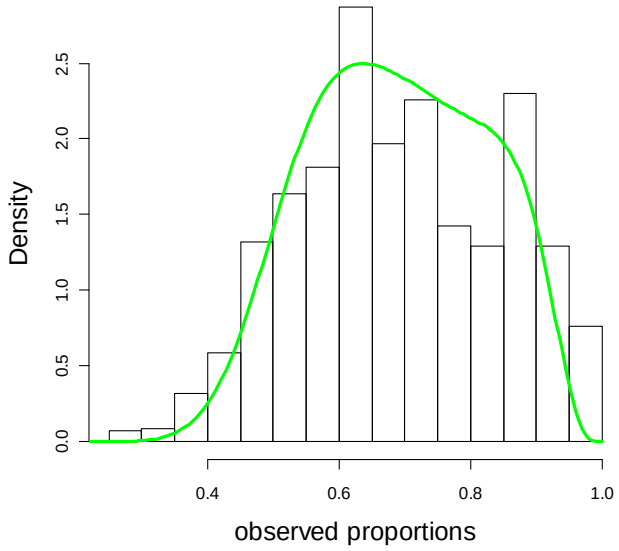
Appendix Figure 16: Model fit Model_L_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 0.75mg.



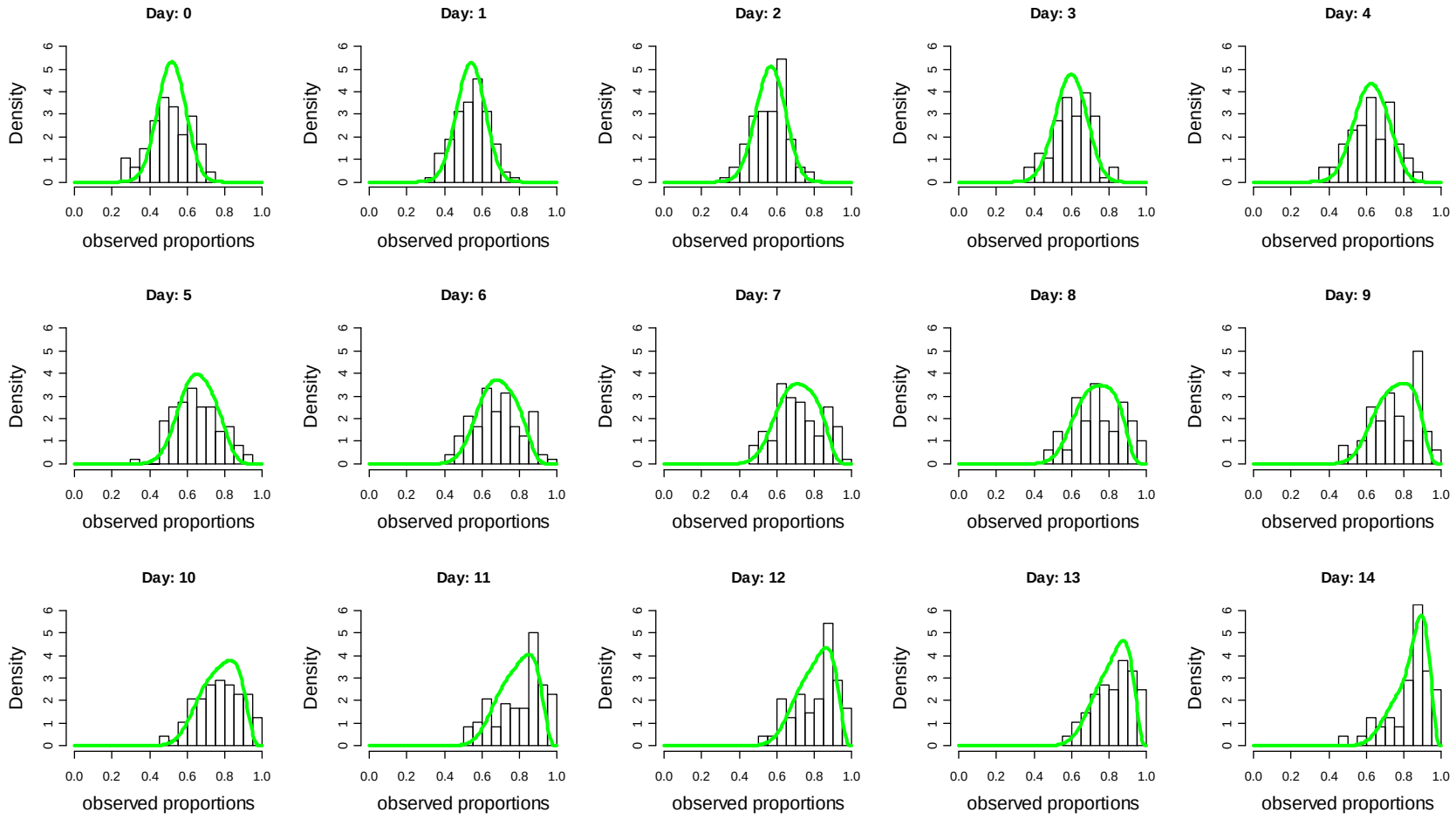
Appendix Figure 17: Model fit Model_L_2 : observed (black dots) versus predicted animal specific profiles (red line) for animals at dose level 1mg.



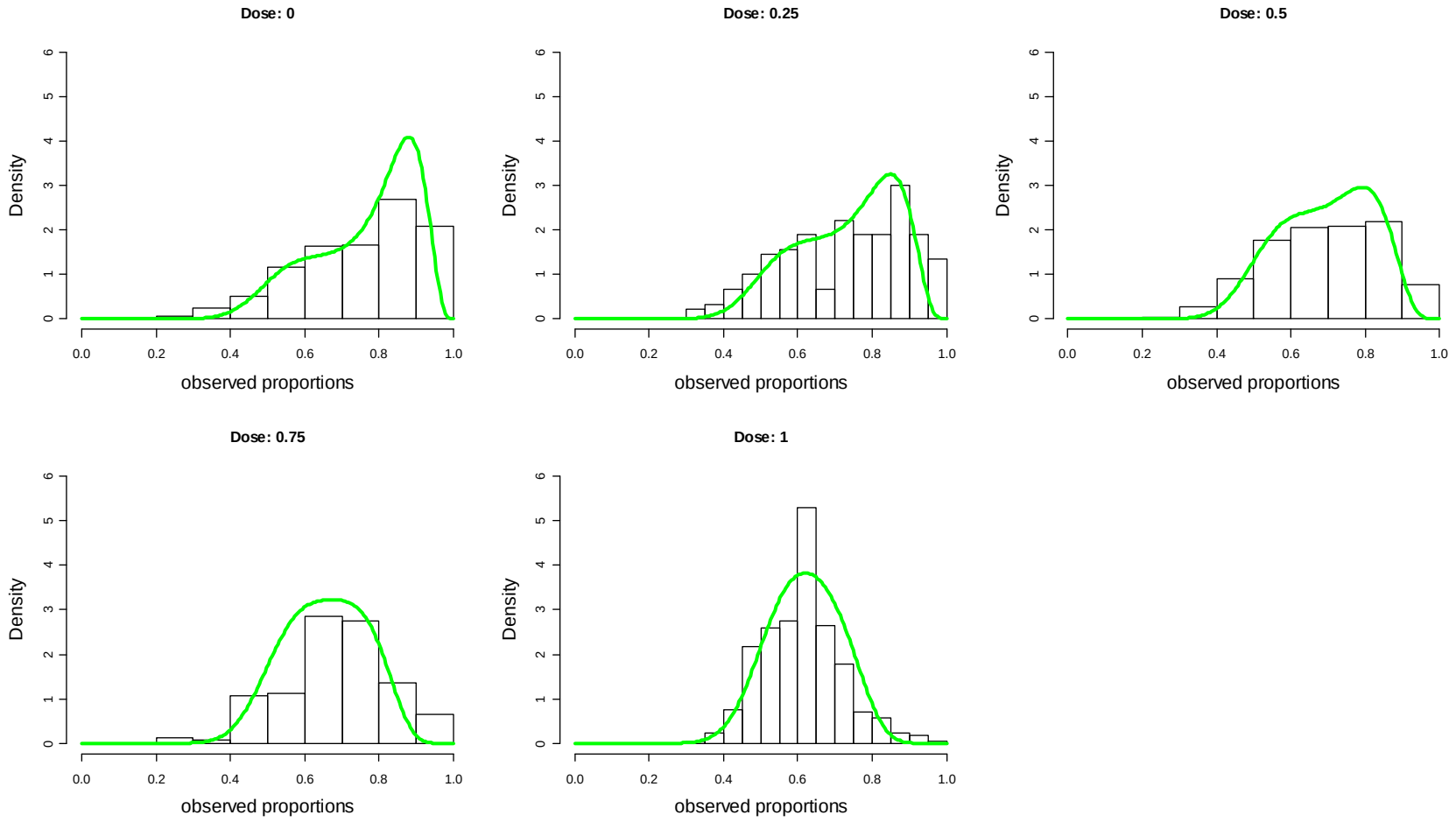
Appendix Figure 18: Model fit Model_L_2: observed proportions versus predicted proportions (left panel) and observed values on the logit scale versus predicted values on logit scale (right panel). The red curve represents the 45 degree line through the origin.



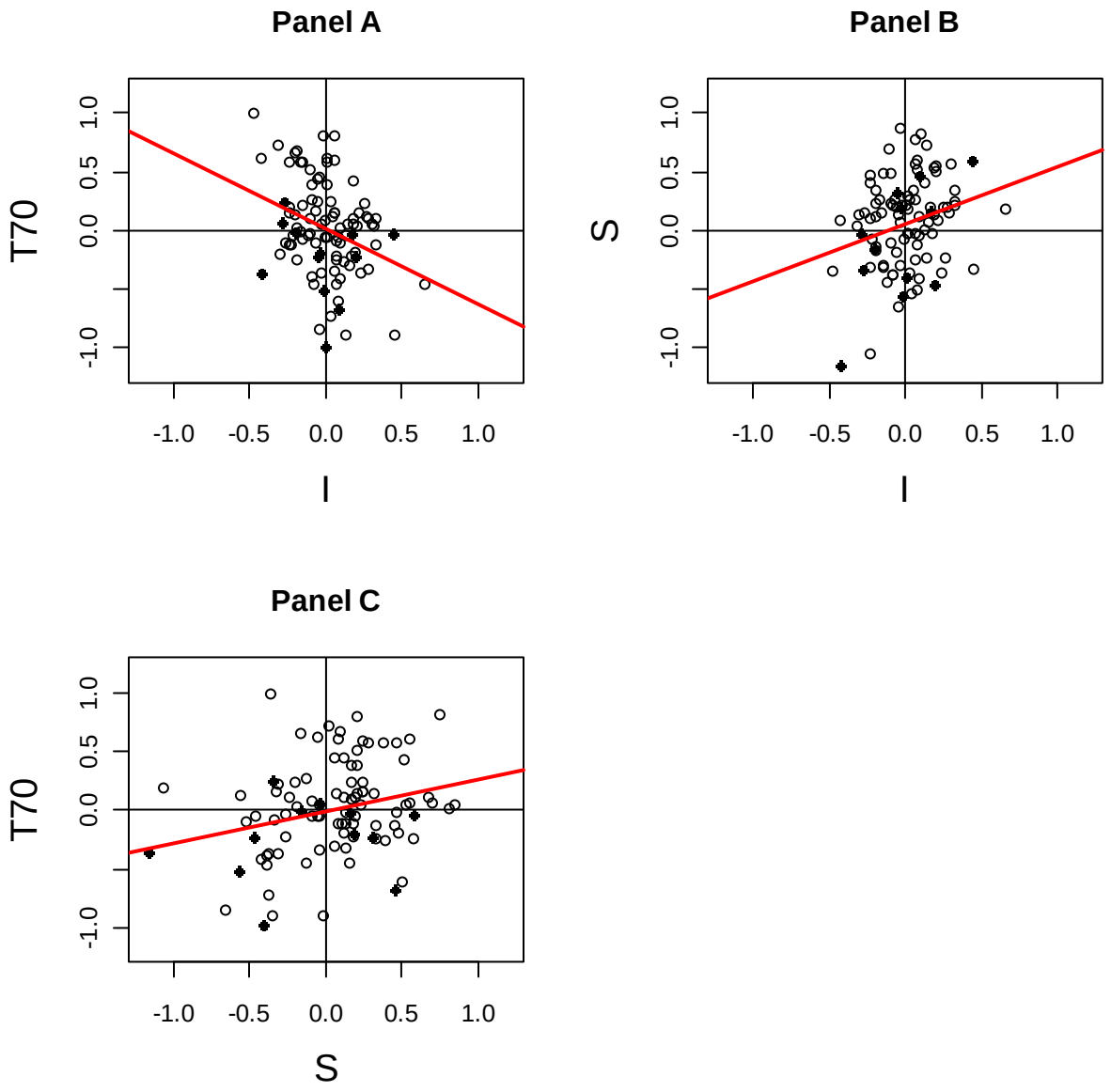
Appendix Figure 19: Model fit Model_L_2 : mixture of beta distributions, overall histogram. The histogram of the observed proportions overlaid with a mixture of 75 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



Appendix Figure 20: Model fit Model_L_2 : mixture of beta distributions, histograms per day. The histograms of the observed proportions per day overlaid with a mixture of 5 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



Appendix Figure 21: Model fit Model_L_2 : mixture of beta distributions, histograms per dose level. The histograms of the observed proportions per dose level overlaid with a mixture of 15 beta distributions (green curve) based the marginalized average proportions per dose-day combination.



Appendix Figure 22: Model fit Model_L_2 : scatter plot of empirical Bayes estimates of the random effects. Panel A: random *T70* versus random *I*, panel B: random *S* versus random *I* and panel C: random *T70* versus random *S*. The red line represents the estimated regression line. The horizontal and vertical black lines represents the average random effect, which is zero. The black filled dots represents the random effects for animals at dose level 0.75mg.

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Janssen, Sarah

Datum: **13/09/2012**