

Analyzing Rat Bioassays

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September 13, 2017

Introduction

In this report, we analyze data from an international validation study to measure the effectiveness of the rat uterotrophic bioassay. The bioassay being studied attempts to measure the estrogenic effect of certain chemicals. The two chemicals used in this study have well known effects, so we would like to verify that the bioassay produces consistent results in rats that have been administered various dosages of the chemicals across two possible protocols. If the uterotrophic bioassay is an effective procedure for measuring the effects of these chemicals, then we expect to see consistent responses to various dosages across all labs and groupings.

Methods

To measure the consistency of the responses, we fit iterations of a linear mixed effects model on the provided dataset. The model is conditioned on the labs to account for lab-to-lab variability. Each iteration of the model differs slightly in transformations and conditioning of the predictors/responses until we arrive at a model that we deem most appropriate for the dataset. We then evaluate the final model to determine whether it predicts large variation in responses to the dosages between labs, or if it predicts a consistent measured response to the chemicals.

Model-Fitting

$$y_{ij} \sim \beta_{0,i} + \beta_{1,i}x_{ij,d_1} + \beta_{2,i}x_{ij,d_2} + \beta_{3,i}x_{ij,p_B} + \beta_{4,i}x_{ij,p_C} + \beta_{5,i}x_{ij,p_D} + \beta_{6,i}x_{ij,\log(w)} + \epsilon$$

$$\beta_{0:5,i} \sim N(\mu_{0:5,i}, \sigma_{0:5,i}^2)$$

$$\epsilon \sim N(0, \sigma^2)$$

To fit the data, we used a mixed model, with fixed and random effects. Let y_{ij} be the observed log(blotted uterus weight) for subject x_{ij} , the j th individual in lab i . x_{ij,d_1} and x_{ij,d_2} are the values of dose1 and dose2 for subject x_{ij} . x_{ij,p_B} , x_{ij,p_C} , and x_{ij,p_D} are dummy variables indicating which protocol x_{ij} was subjected to. $x_{ij,\log(w)}$ is the log(body weight) for x_{ij} . Body weight and uterus weight are log-transformed to account for the right skew in the data (Fig. 1). We make the Gaussian assumption that the coefficients, β , are normally distributed according to some μ_i and σ_i . We add a random effect on all $\beta_{0:5,i}$ to account for the lab-to-lab variability in the intercepts and slopes of the blotted weight (Fig. 2). The summary statistics of the model described above can be found on Table 1.

We start at a reduced form of this model and augment it to its full form after initial analyses.

Table 1: Summary Statistics of Initial Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	4.9844	0.1421
protoB	0.0397	0.0007
protoC	1.2966	0.0193
protoD	1.2949	0.0204

	Mean Fixed Effects	Variance Fixed Effects
log(body)	-0.0264	0.0092

	Basf	Bayer	Berlin	...	WIL	TNO	Sumitomo
(Intercept)	-1.6101	-1.2217	-1.25	...	-1.314	-1.1664	-1.1322
dose1	0.1838	0.1394	0.1427	...	0.15	0.1331	0.1292
dose2	-0.6198	-0.4303	-0.2757	...	-0.4934	-0.3356	-0.4921

	Variance Random Effects
(Intercept)	1.6181
dose1	0.0211
dose2	0.2691

	Variance Residuals
residual	0.1922

Among the problems in these plots is the nonlinearity in the Residuals vs. Predictor plot for dose1. To counter this, we transformed dose 1 by the reciprocal of $(\text{dose1} + 1/2)$ (we add a small number to dose because we cannot take the reciprocal of zero). We evaluate this transformed model using summary statistics, diagnostic plots, and out-of-sample predictive accuracy, which we measure using Mean Absolute Error ($\text{MAE} = E[|y - \hat{y}|]$) and Root Mean Squared Error ($\text{RMSE} = \sqrt{E[(y - \hat{y})^2]}$). Root Mean Squared Error penalizes more for extreme errors, while Mean Absolute Error simply averages all of the errors. The results are in the following tables and figures:

Table 5: Summary Statistics of Transformed Dose 1

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	2.6379	0.0728
protoB	0.0457	0.0003
protoC	0.8553	0.0094
protoD	0.8524	0.0100
log(body)	0.2946	0.0046

	Basf	Bayer	Berlin	...	WIL	TNO	Sumitomo
(Intercept)	0.6012	0.8169	1.3398	...	0.9083	1.3319	1.1546
$I(1/(\text{dose1} + 1/2))$	-0.7142	-0.5655	-0.9166	...	-0.7126	-0.9437	-0.8103
dose2	-1.0239	-0.6824	-0.794	...	-0.9702	-0.9614	-1.1834

	Variance Random Effects
(Intercept)	0.9593
$I(1/(\text{dose1} + 1/2))$	0.5371
dose2	1.0481

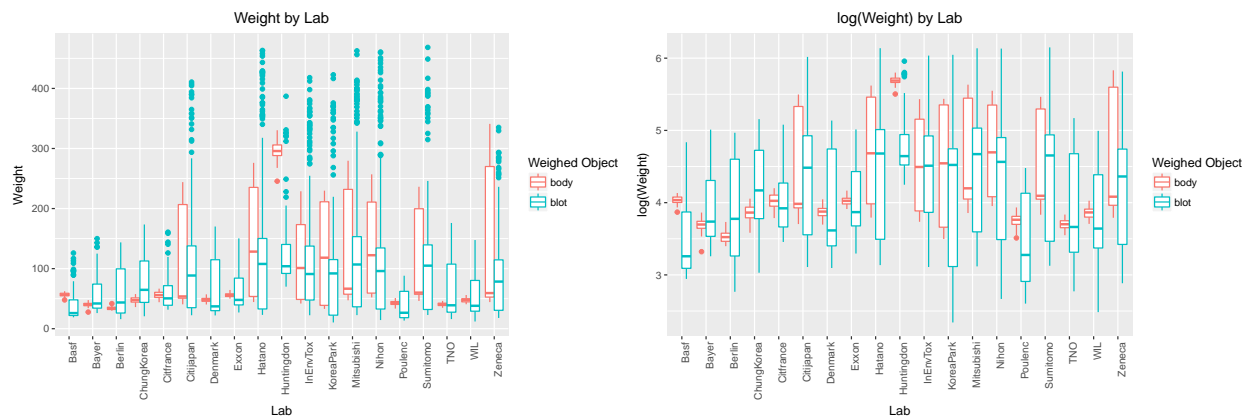


Figure 1: Log Transformations of Weights

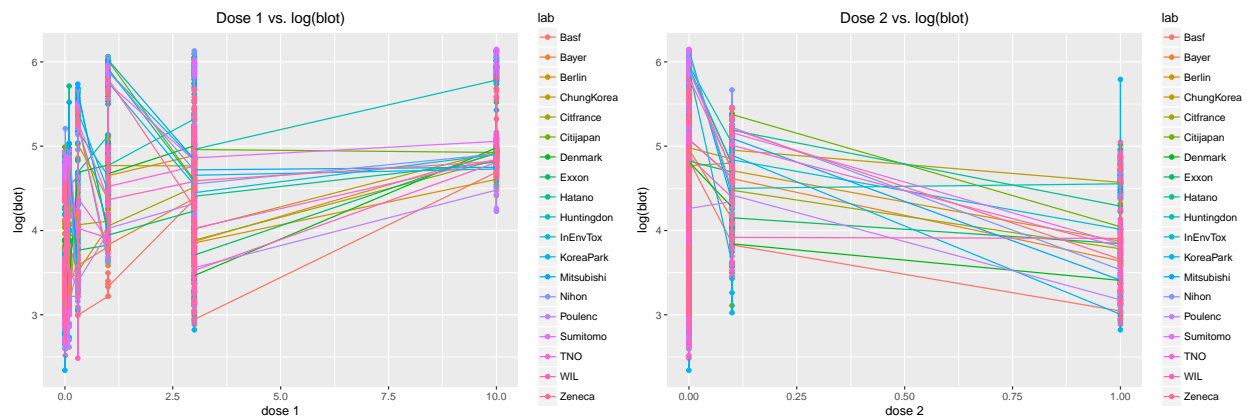


Figure 2: Lab-to-Lab Variability in Intercepts and Slopes

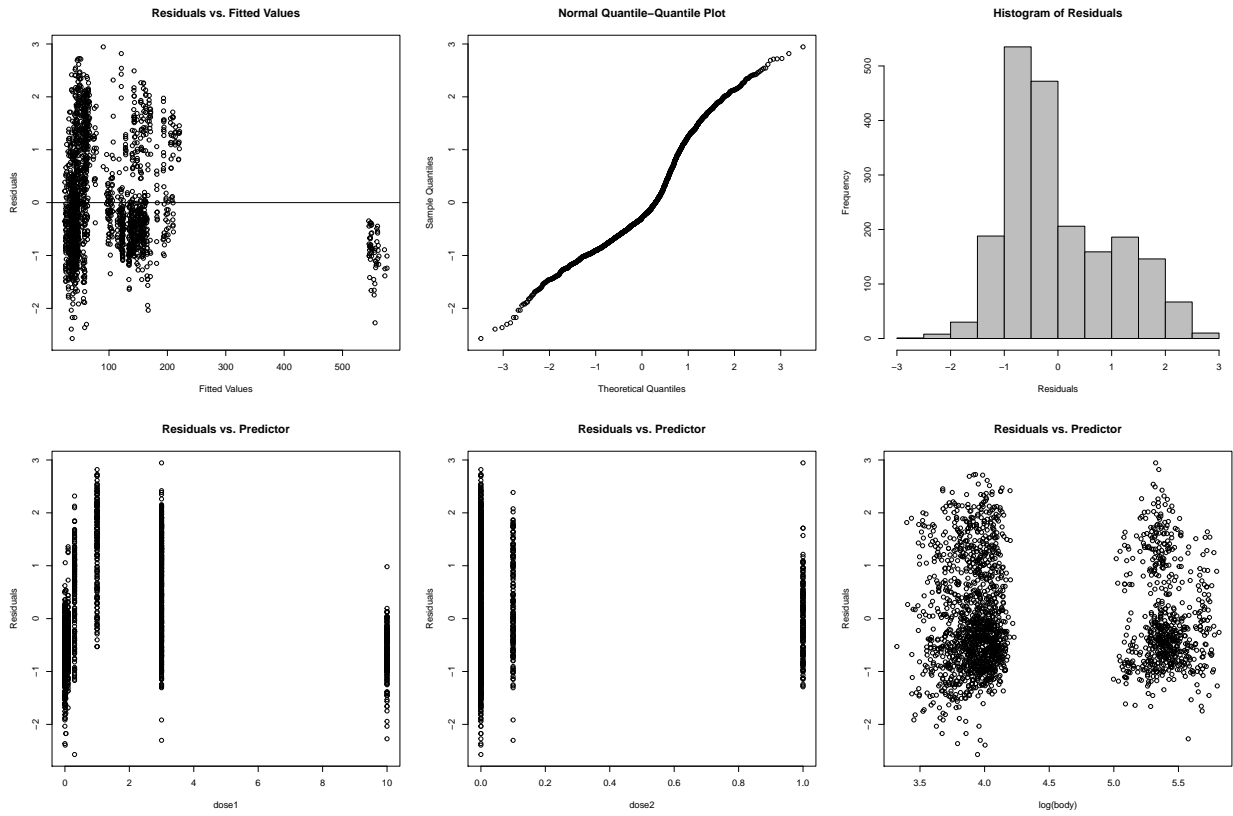


Figure 3: Diagnostic Plots of Initial Model

Variance Residuals	
residual	0.0797

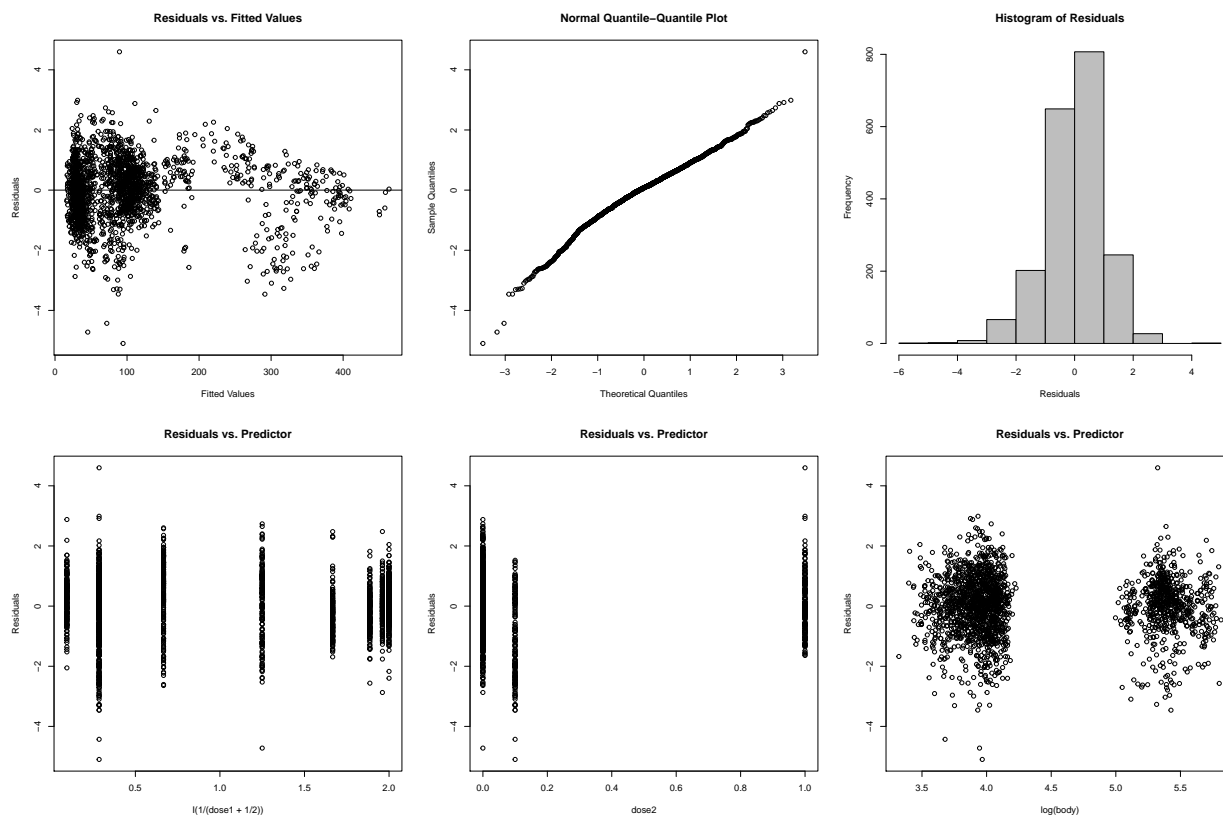


Figure 4: Diagnostic Plots of Model with Transformed Dose 1

Table 9: Predictive Error of First Two Models

	MAE	RMSE
Initial	36.4387	61.0759
Reciprocal Dose 1	21.7968	37.7587

comment comment comment. Another problem with these residual plots is the slight arching in the Residuals vs. Fitted Values. To counteract this, we added a square root transformation to the response. **well then again we might not*** The results are below:

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	1.6255	0.0045
protoB	0.0095	0.0000
protoC	0.1815	0.0006
protoD	0.1792	0.0006
log(body)	0.0871	0.0003

	Basf	Bayer	Berlin	...	WIL	TNO	Sumitomo
(Intercept)	0.1276	0.184	0.3134	...	0.2051	0.3095	0.2511
$I(1/(\text{dose1} + 1/2))$	-0.1846	-0.1407	-0.2306	...	-0.1797	-0.2361	-0.1943
dose2	-0.2628	-0.1661	-0.1881	...	-0.2407	-0.2305	-0.2805

Variance Random Effects	
(Intercept)	0.0471
$I(1/(\text{dose1} + 1/2))$	0.0325
dose2	0.0613

Variance Residuals	
residual	0.0049

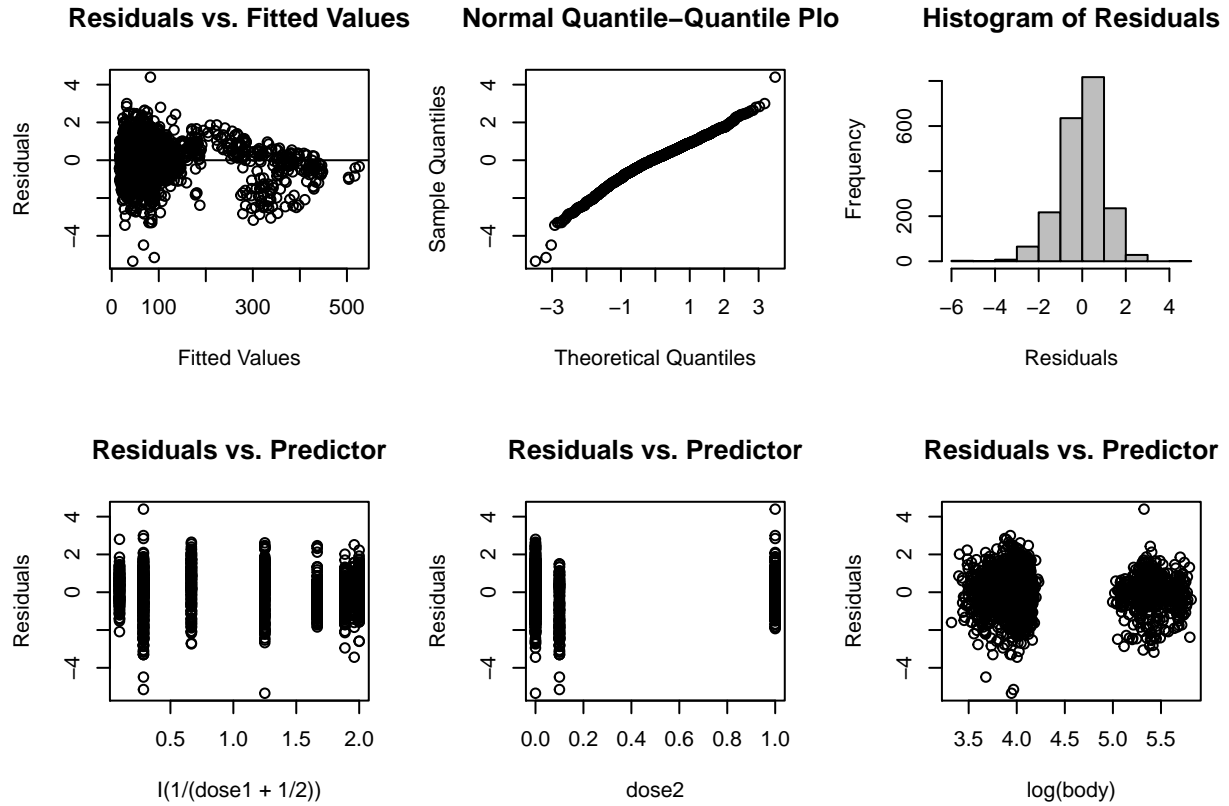


Figure 5: Diagnostic Plots of Model with Transformed Dose 1 and Blotted Uterus Weight

Table 14: Predictive Error of All Models

	MAE	RMSE
Initial	36.4387	61.0759
Reciprocal Dose 1	21.7968	37.7587

	MAE	RMSE
Transformed Dose 1 and Uterus Weight	22.8674	40.5363

This is our best-fitting mixed model we could fit. **maybe. it looks like sqrt log blot improves residual plot but worsens prediction**

Results

INCOMPLETE

The plot below samples random dose1 effects (slopes) and lab effects (intercepts) from our final mixed model, holding all other predictors constant, and plots the resulting dose effect curve.

Discussion

The variance in the dose1 effects by lab is noticeably high, so we say that the bioassay does depend on lab and thus this study fails miserably.

This explodes around 10 and it's only applicable for dose less than or equal to 10.

Contributions

Nathaniel Brown made the visualizations for this report. He also organized the relevant files in a Github repository for the group to access and edit. Annie Tang compiled the group work done on EDA into a .rmd and wrote the accompanying explanations for the EDA and approaches to analysis. William Yang helped pair on EDA analysis and identify approaches to handle the data. Approaches to analysis were a joint effort by all members of the group. Nathaniel implemented analysis for the univariate normal and multivariate normal approaches. Implementation and analysis of the mixed effects model was a joint effort by all members of the group.

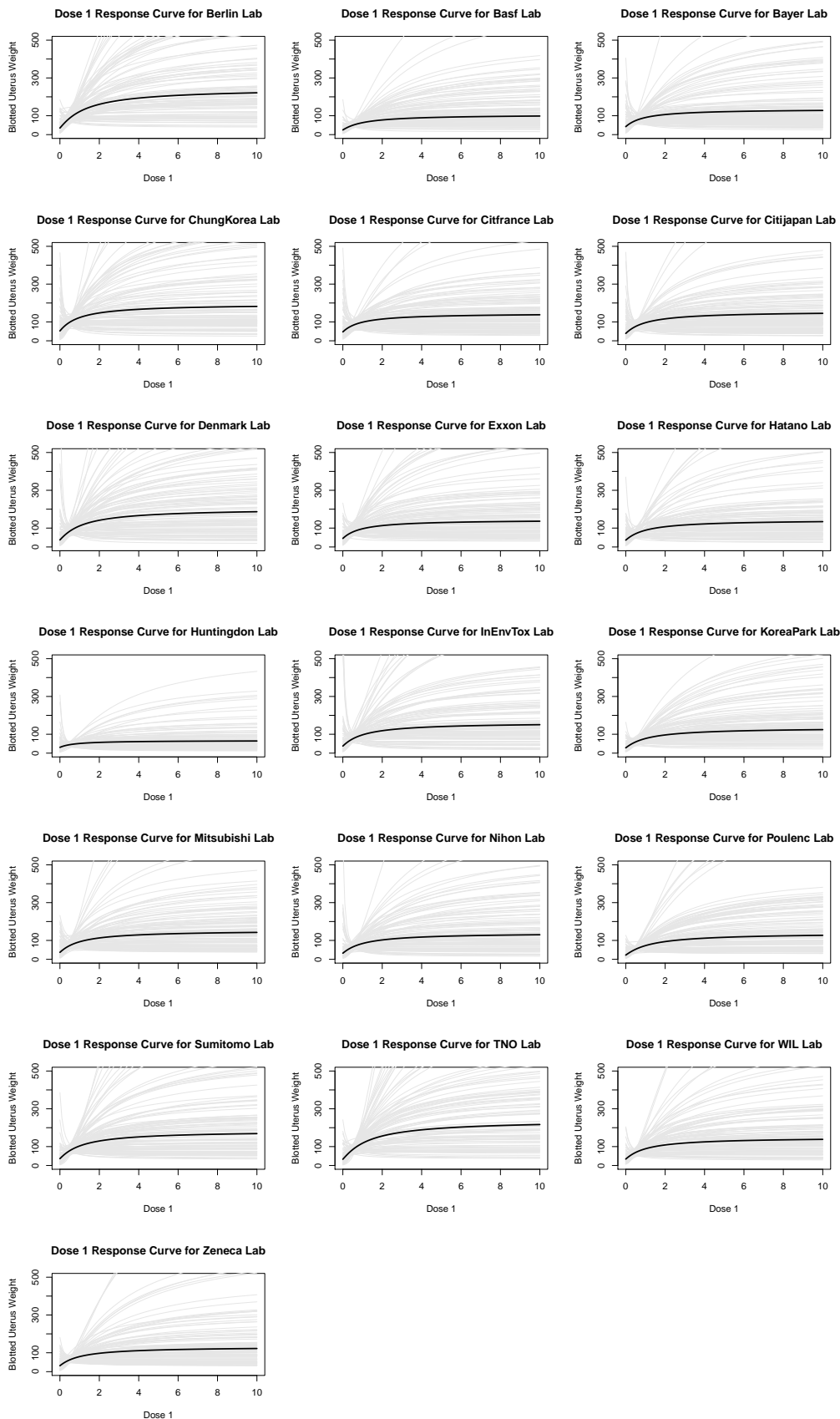


Figure 6: Dose 1 Response Curves for Each Lab