Analyzing Rat Bioassays

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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. We do this by drawing samples of the random effects from the final model and determining if the dose-response curves across labs display homogeneous trends.

Model-Fitting

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

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

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

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 jth 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)	4.8875	0.1421
protoB	0.0429	0.0007
protoC	1.2723	0.0193

	Mean Fixed Effects	Variance Fixed Effects
protoD	1.2716	0.0204
log(body)	-0.0065	0.0092

	Basf	Bayer	Berlin	...	WIL	TNO	Sumitomo
(Intercept)	-1.5851	-1.1978	-1.2306	...	-1.2962	-1.1556	-1.1252
dose1	0.1838	0.1411	0.1513	...	0.1519	0.1406	0.1312
dose2	-0.6631	-0.442	-0.2795	...	-0.4994	-0.3045	-0.4503

	Variance Random Effects
(Intercept)	1.5746
dose1	0.0214
dose2	0.2730

	Variance Residuals
residual	0.192

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 (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 (MAE $= E[|y - \hat{y}|]$) and Root Mean Squared Error (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)	2.6378	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	0.817	1.3398	...	0.9083	1.3319	1.1546
I(1/(dose1 + 1/2))	-0.7142	-0.5655	-0.9166	...	-0.7126	-0.9437	-0.8103
dose2	-1.0239	-0.6824	-0.7941	...	-0.9702	-0.9614	-1.1834

	Variance Random Effects
(Intercept)	0.9593
I(1/(dose1 + 1/2))	0.5371
dose2	1.0481

	Variance Random Effects

	Variance Residuals
residual	0.0797

Table 9: Predictive Error of First Two Models

	MAE	RMSE
Initial	36.4126	60.8907
Reciprocal Dose 1	21.7968	37.7587

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. The model summary statistics, diagnostic plots, and predictive accuracy 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/(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/(dose1 + 1/2))	0.0325
dose2	0.0613

	Variance Residuals
residual	0.0049

Table 14: Predictive Error of All Models

	MAE	RMSE
Initial	36.4126	60.8907
Reciprocal Dose 1	21.7968	37.7587
Transformed Dose 1 and Uterus Weight	22.8674	40.5363

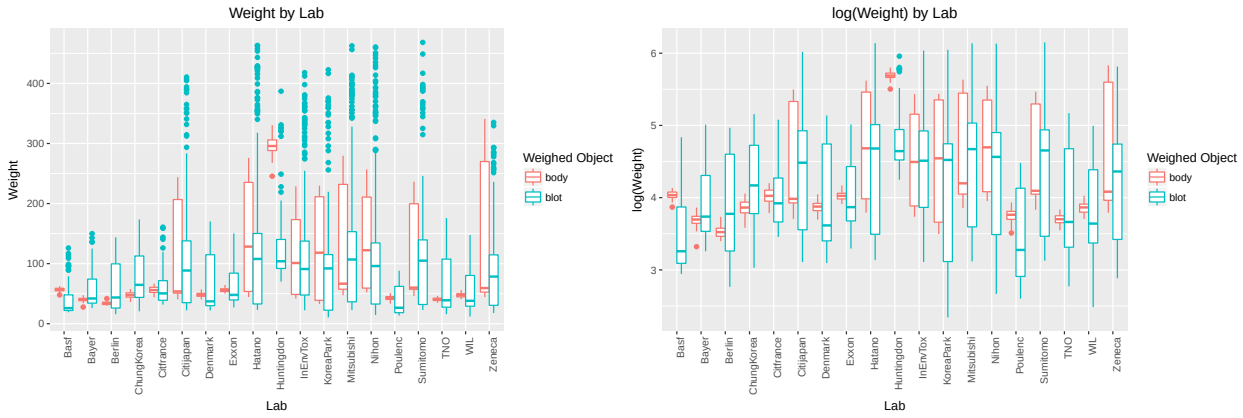

Figure 1: Log Transformations of Weights

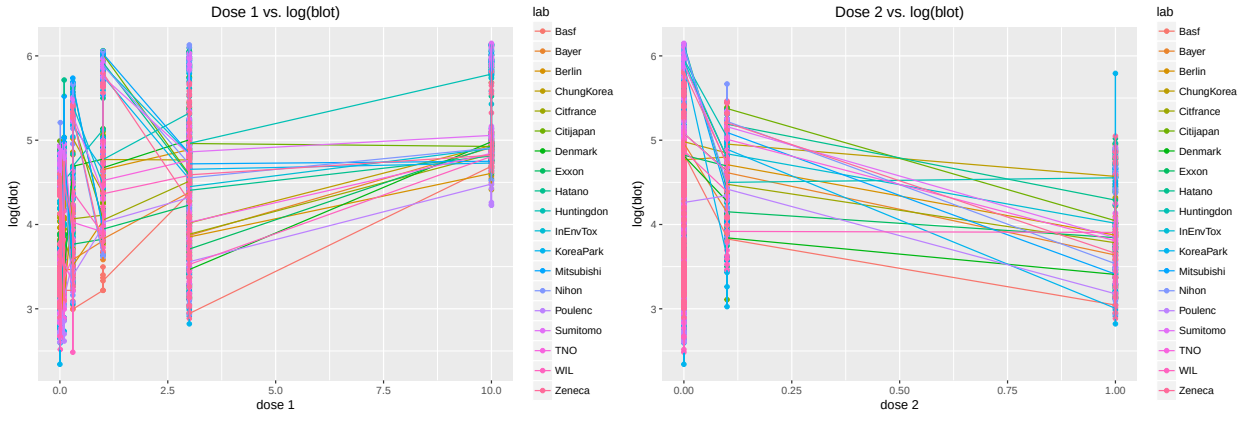

Figure 2: Lab-to-Lab Variability in Intercepts and Slopes

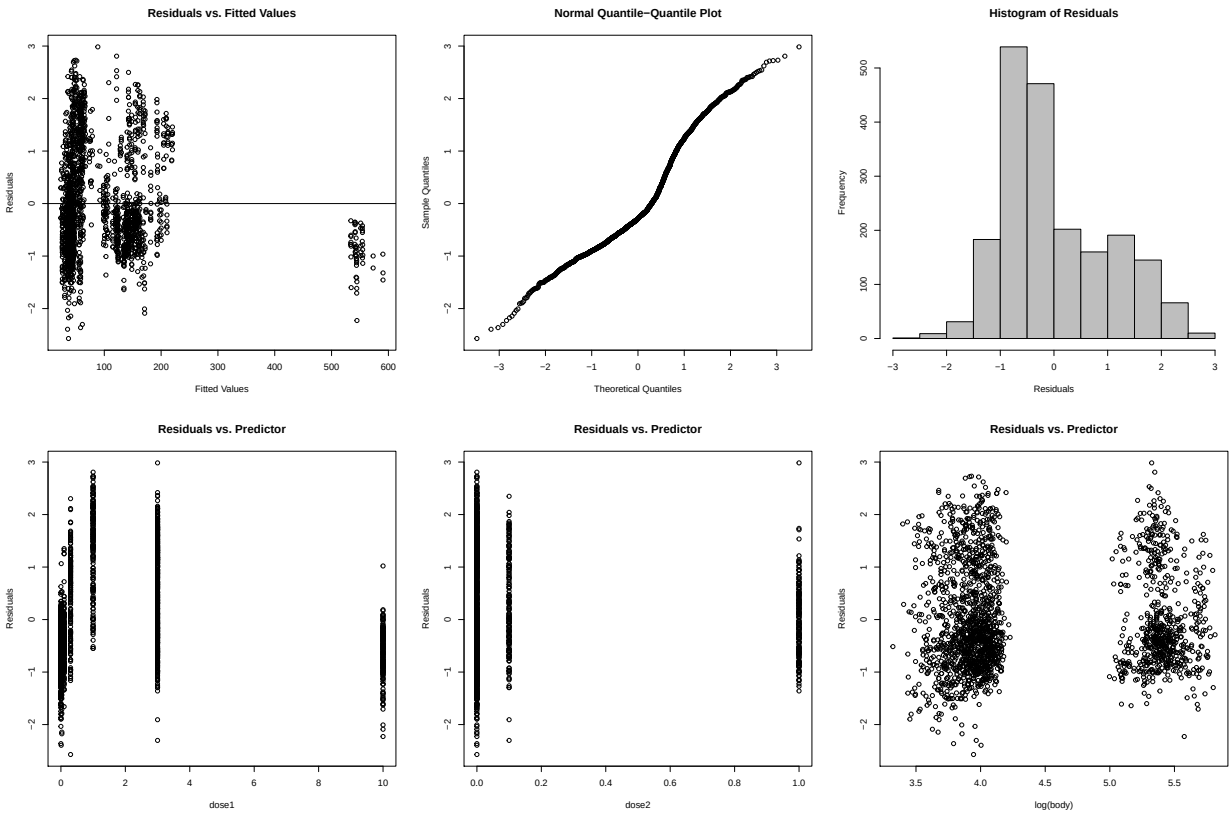

Figure 3: Diagnostic Plots of Initial Model

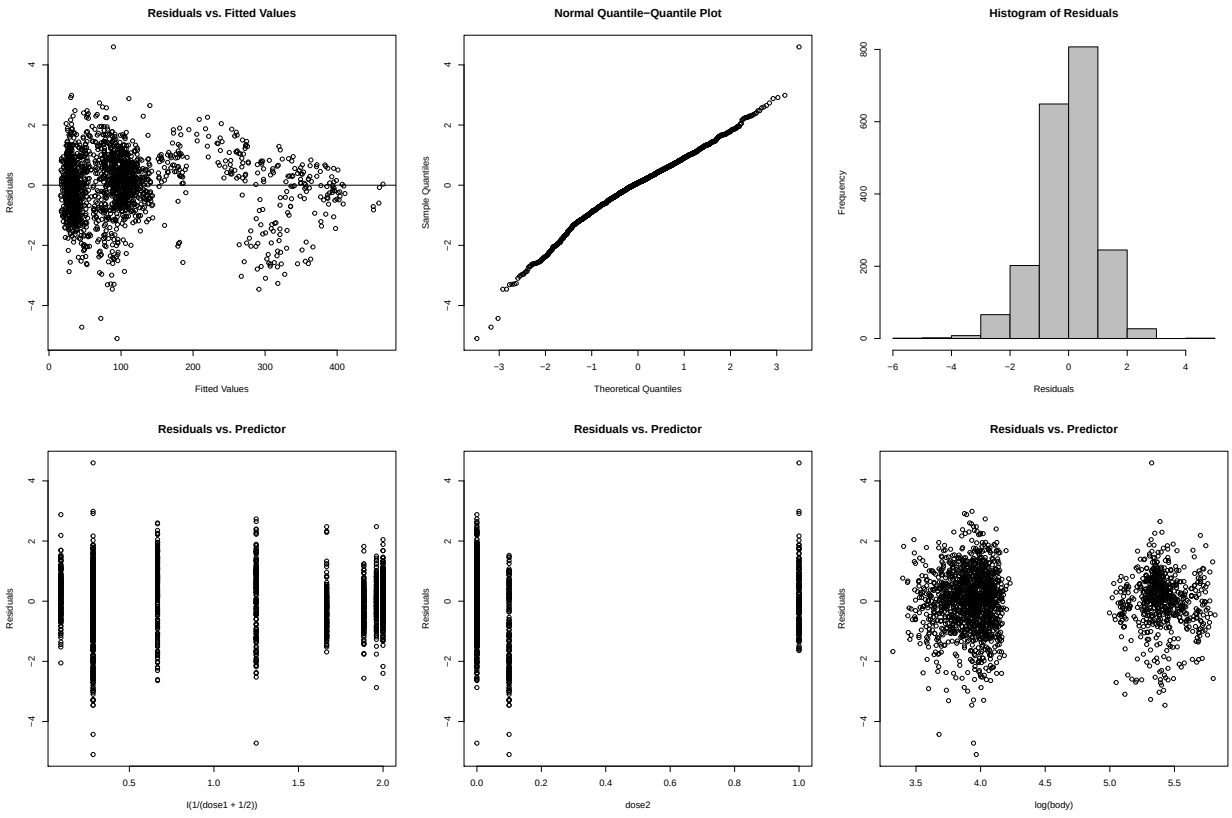

Figure 4: Diagnostic Plots of Model with Transformed Dose 1

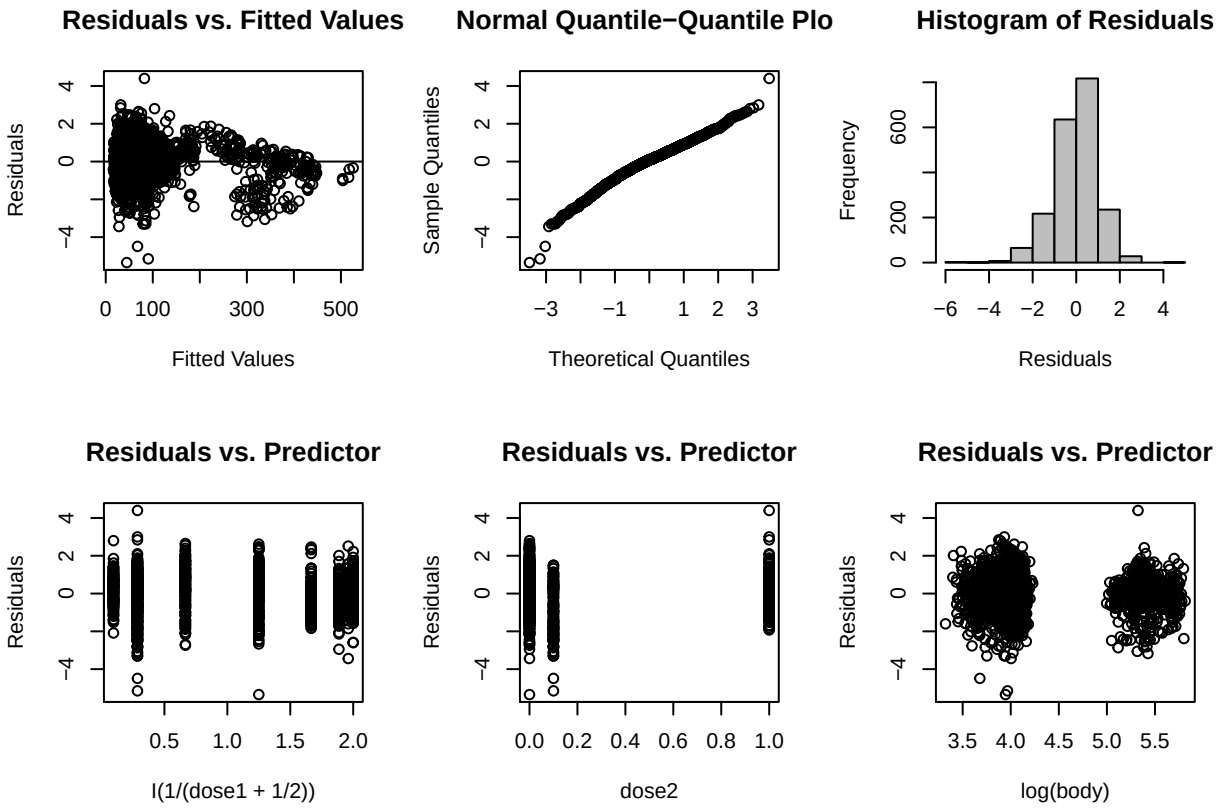

Figure 5: Diagnostic Plots of Model with Transformed Dose 1 and Blotted Uterus Weight

Based on the residual plots, this mixed model best satisfies the assumptions of linear regression. However, we note that taking the square root of log(blot) improves the residual plot at the cost of slightly lower predictive accuracy.

Results

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. The black curves plot the means of the random effects from the model output.

Although the dose effect curves of each lab mostly trend positive, the variance in the dose1 effects by lab is higher than we would like. The distance between the lowest range and highest range of dose effect curves is large enough that we would be skeptical of how close any observed dose effect curve would be to the true effect of a chemical. Therefore, we do not recommend this bioassay procedure as a reliable method for consistently measuring the estrogenic effects of chemicals in rats.

Discussion

While our model seems to mostly satisfy the assumptions for regression, the central range of the residuals do show a slight curve, which may indicate that the response may better fit a different distribution, such as a mixture of gaussians. Some areas of improvement for the model include trying to identify clusters on the initial dose effect curves, or applying a Bayesian approach to mixed effects.

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. Nathanial 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.

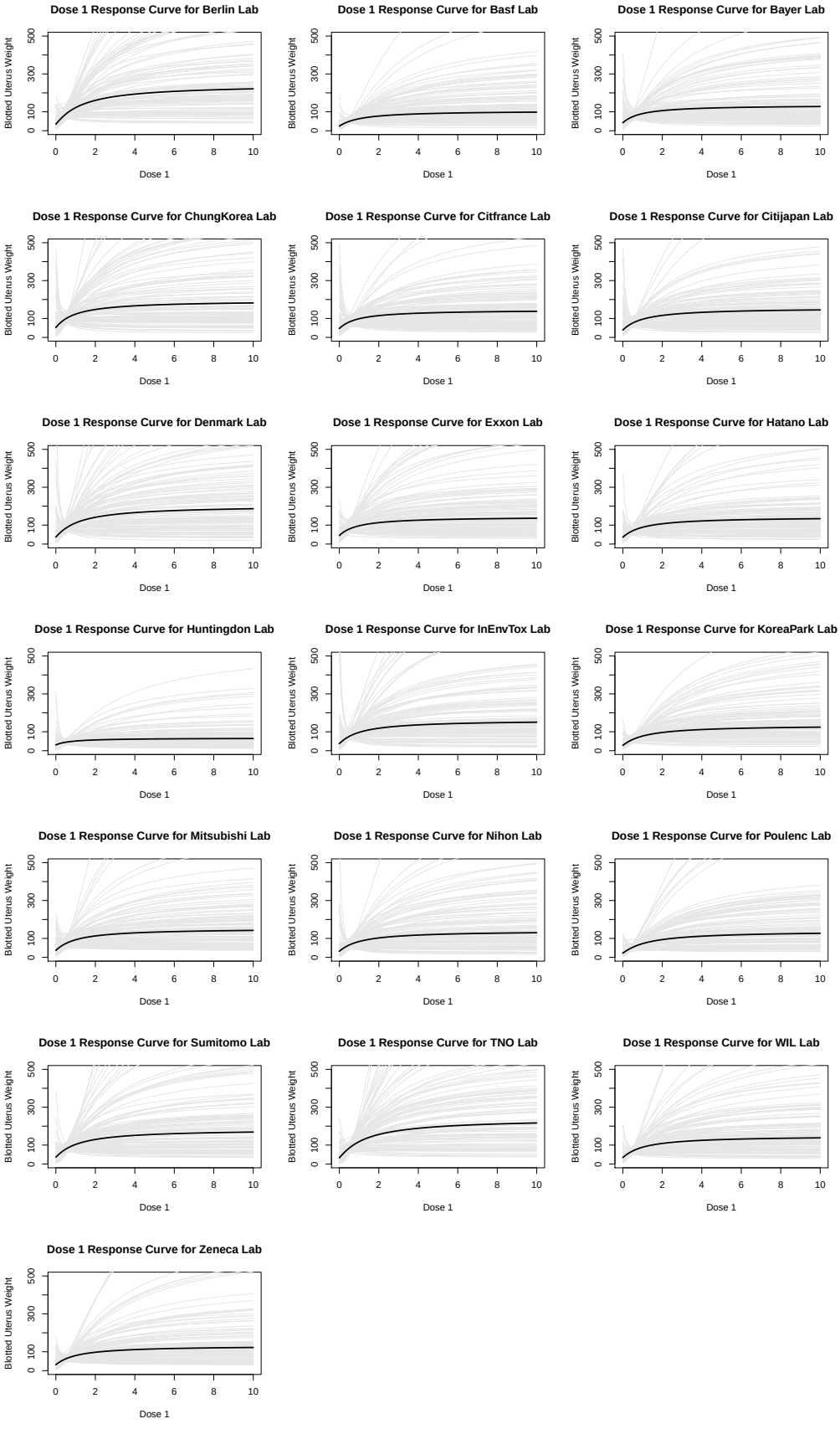

Figure 6: Dose 1 Response Curves for Each Lab