Analyzing Rat Bioassays

Nathaniel Brown, Annie Tang, William Yang

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. 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 in Tables 1-4, and diagnostic plots using the residuals can be found in Figure 3. The results of the random effects that were cut off from Table 2 can be found in the Appendix.

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

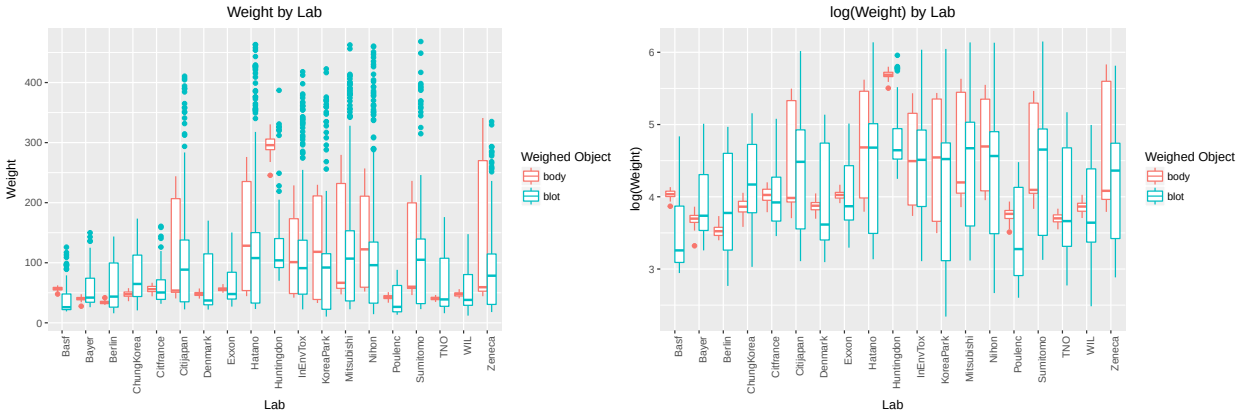

Figure 1: Log Transformations of Weights

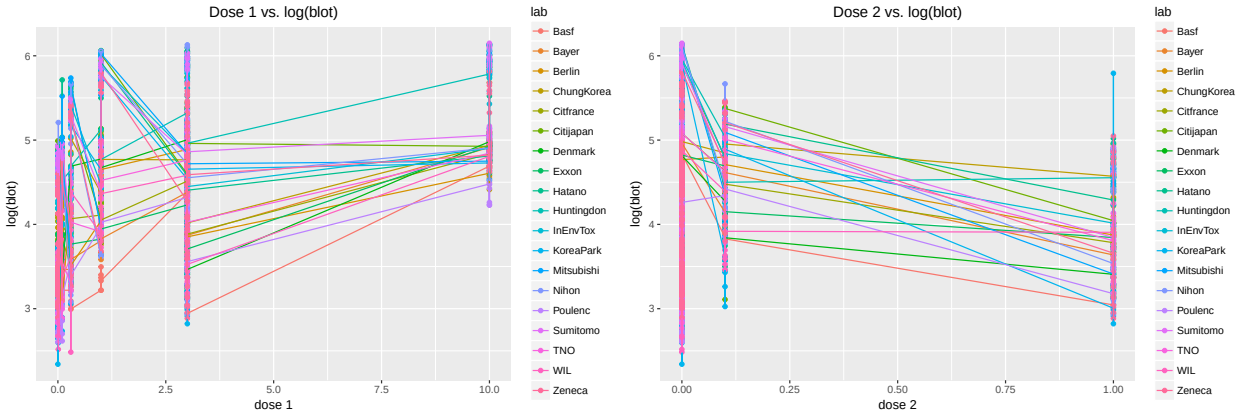

Figure 2: Lab-to-Lab Variability in Intercepts and Slopes

Tables 1-4: 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
log(body)	-0.0264	0.0092

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	-1.6101	-1.2217	-1.25	...	-1.1664	-1.314	-1.3182
dose1	0.1838	0.1394	0.1427	...	0.1331	0.15	0.1505
dose2	-0.6198	-0.4303	-0.2757	...	-0.3356	-0.4934	-0.6048

	Variance Random Effects
(Intercept)	1.6181
dose1	0.0211
dose2	0.2691

	Variance Residuals
residual	0.1922

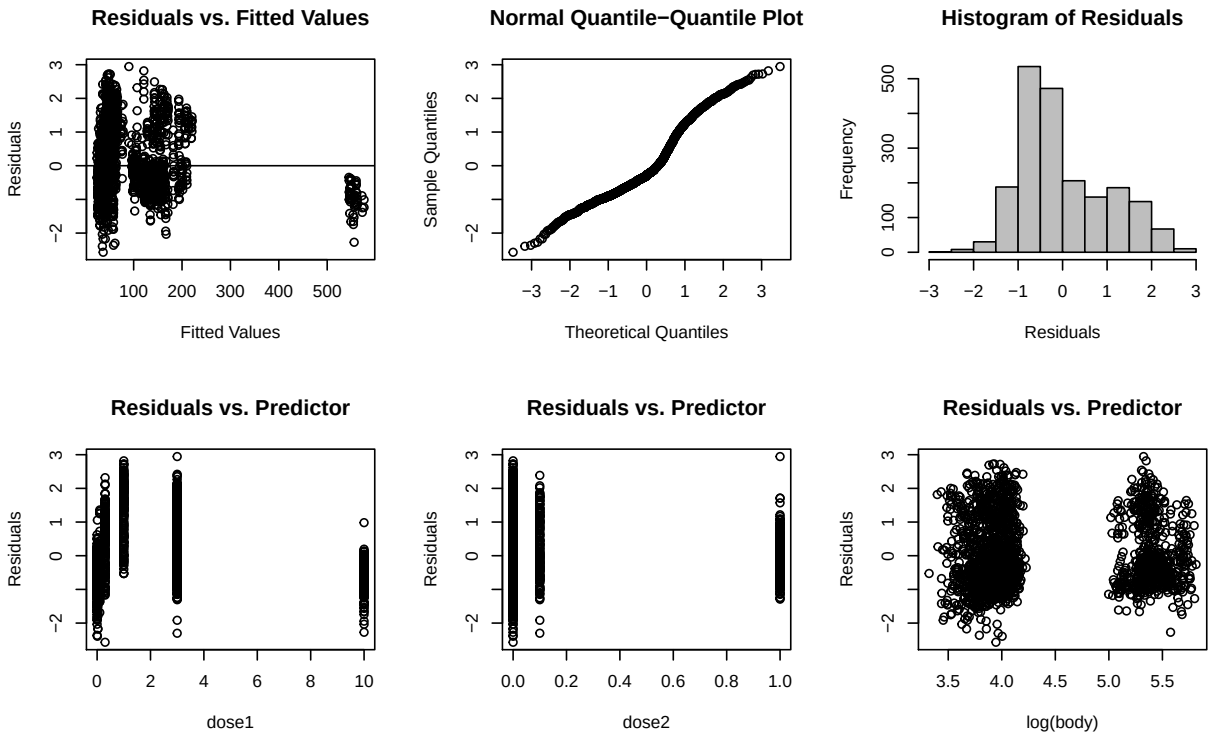

Figure 3: Diagnostic Plots of Initial Model

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 on a single hold-out sample, 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:

Tables 5-8: Summary Statistics of Transformed Dose Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	2.6379	0.0728
protoB	0.0457	0.0003

	Mean Fixed Effects	Variance Fixed Effects
protoC	0.8553	0.0094
protoD	0.8524	0.0100
log(body)	0.2946	0.0046

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	0.6012	0.8169	1.3398	...	1.3319	0.9083	0.8367
I(1/(dose1 + 1/2))	-0.7142	-0.5655	-0.9166	...	-0.9437	-0.7126	-0.722
dose2	-1.0239	-0.6824	-0.794	...	-0.9614	-0.9702	-1.1518

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

	Variance Residuals
residual	0.0797

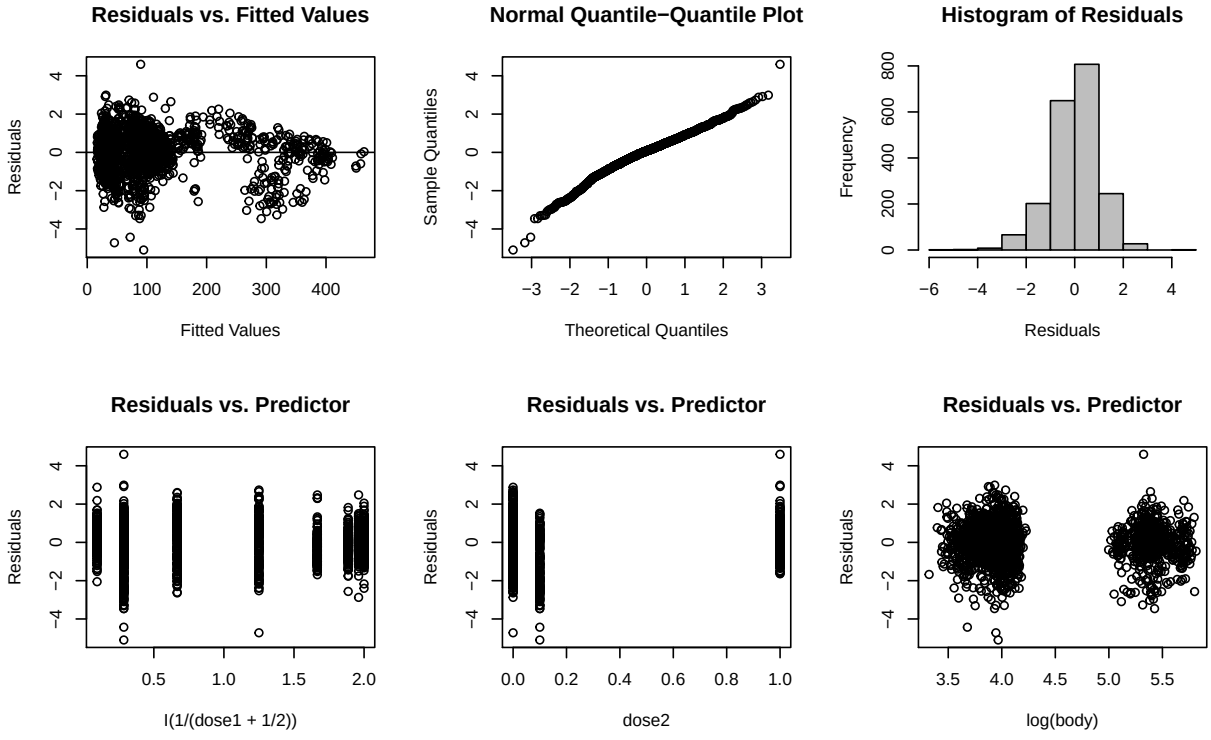

Figure 4: Diagnostic Plots of Model with Transformed Dose 1

Table 9: Predictive Error of First Two Models

	MAE	RMSE
Initial	36.4387	61.0759
Reciprocal Dose 1	21.7968	37.7587

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:

Tables 10-13: Summary Statistics of Transformed Dose and Transformed Uterus Weight Model

	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		TNO	WIL	Zeneca
(Intercept)	0.1276	0.184	0.3134	...	0.3095	0.2051	0.1774
I(1/(dose1 + 1/2))	-0.1846	-0.1407	-0.2306	...	-0.2361	-0.1797	-0.1758
dose2	-0.2628	-0.1661	-0.1881	...	-0.2305	-0.2407	-0.2838

	Variance Random Effects
(Intercept)	0.0471
I(1/(dose1 + 1/2))	0.0325
dose2	0.0613

	Variance Residuals
residual	0.0049

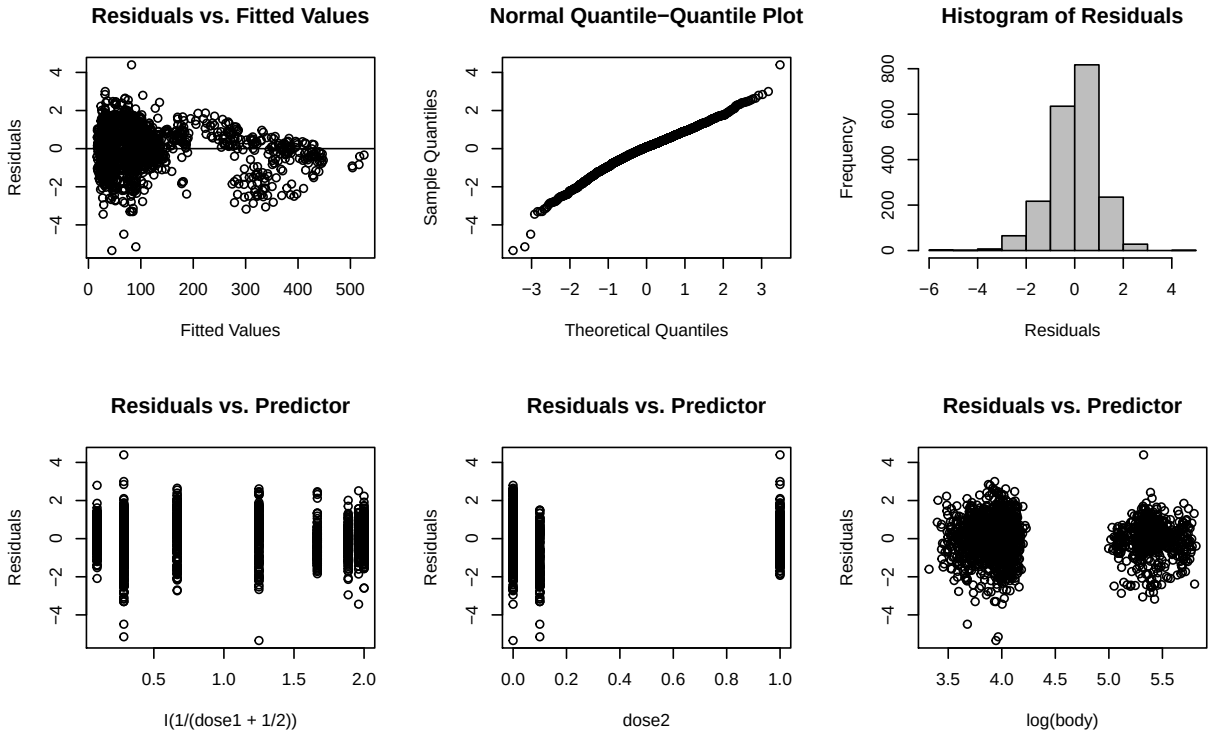

Figure 5: Diagnostic Plots of Model with Transformed Dose 1 and Blotted Uterus Weight

Table 14: Predictive Error of All Models

	MAE	RMSE
Initial	36.4387	61.0759
Reciprocal Dose 1	21.7968	37.7587
Transformed Dose 1 and Uterus Weight	22.8674	40.5363

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 on the hold-out sample dataset.

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.

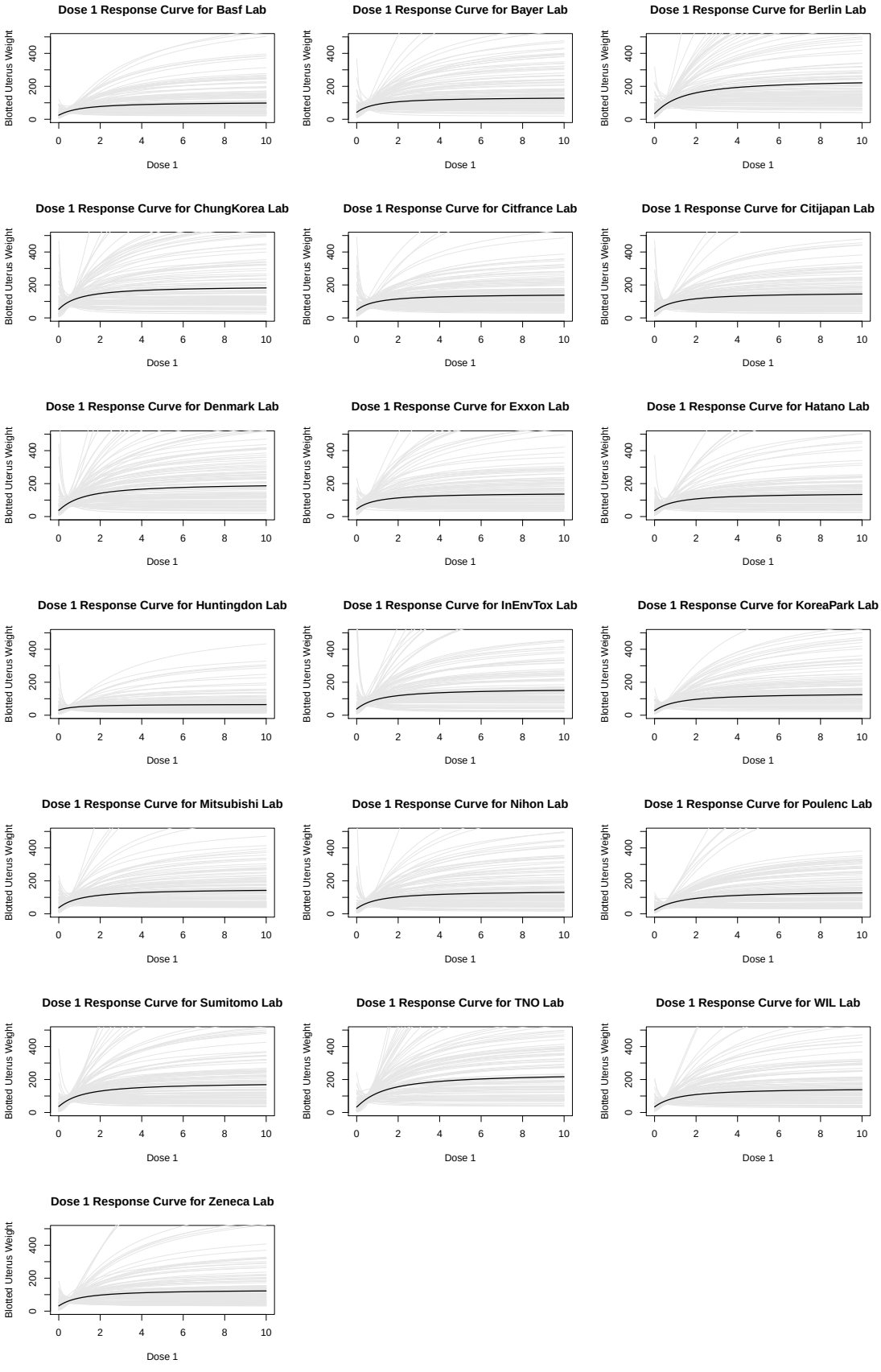
Dose 1 Response Curve for Basf Lab
Dose 1 Response Curve for Bayer Lab
Dose 1 Response Curve for Berlin Lab
Dose 1 Response Curve for ChungKorea Lab
Dose 1 Response Curve for Citfrance Lab
Dose 1 Response Curve for Citijapan Lab
Dose 1 Response Curve for Denmark Lab
Dose 1 Response Curve for Exxon Lab
Dose 1 Response Curve for Hatano Lab
Dose 1 Response Curve for Huntingdon Lab
Dose 1 Response Curve for InEnvTox Lab
Dose 1 Response Curve for KoreaPark Lab
Dose 1 Response Curve for Mitsubishi Lab
Dose 1 Response Curve for Nihon Lab
Dose 1 Response Curve for Poulenc Lab
Dose 1 Response Curve for Sumitomo Lab
Dose 1 Response Curve for TNO Lab
Dose 1 Response Curve for WIL Lab
Dose 1 Response Curve for Zeneca Lab
Blotted Uterus Weight
Dose 1
0
200
400
0
2
4
6
8
10

Figure 6: Dose 1 Response Curves for Each Lab

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. Implementation and analysis of the mixed effects model was a joint effort by all members of the group.

Appendix

Complete Results of Random Means for Each Model

Table 2

	(Intercept)	dose1	dose2
Basf	-1.6101	0.1838	-0.6198
Bayer	-1.2217	0.1394	-0.4303
Berlin	-1.2500	0.1427	-0.2757
ChungKorea	-0.8580	0.0979	-0.2141
Citfrance	-1.0338	0.1180	-0.3777
Citijapan	-1.1414	0.1303	-0.5094
Denmark	-1.0971	0.1252	-0.5356
Exxon	-1.0611	0.1211	-0.4524
Hatano	-1.2059	0.1376	-0.5480
Huntingdon	-1.5436	0.1762	-0.5510
InEnvTox	-1.2422	0.1418	-0.5843
KoreaPark	-1.5166	0.1731	-0.6414
Mitsubishi	-1.1775	0.1344	-0.5862
Nihon	-1.3292	0.1517	-0.6054
Poulenc	-1.6289	0.1859	-0.5460
Sumitomo	-1.1322	0.1292	-0.4921
TNO	-1.1664	0.1331	-0.3356
WIL	-1.3140	0.1500	-0.4934
Zeneca	-1.3182	0.1505	-0.6048

Table 6

	(Intercept)	I(1/(dose1 + 1/2))	dose2
Basf	0.6012	-0.7142	-1.0239
Bayer	0.8169	-0.5655	-0.6824
Berlin	1.3398	-0.9166	-0.7940
ChungKorea	1.1589	-0.6261	-0.6001
Citfrance	0.9091	-0.5523	-0.7315
Citijapan	1.0012	-0.6977	-1.0559
Denmark	1.2001	-0.8188	-1.3949
Exxon	0.9061	-0.5728	-0.8858
Hatano	0.9468	-0.7121	-1.0947
Huntingdon	0.2101	-0.4428	-0.6028
InEnvTox	1.0385	-0.7501	-1.1956
KoreaPark	0.8550	-0.7948	-1.1955
Mitsubishi	1.0052	-0.7298	-1.1966
Nihon	0.9216	-0.7573	-1.1965
Poulenc	0.8069	-0.8571	-0.9633
Sumitomo	1.1546	-0.8103	-1.1834
TNO	1.3319	-0.9437	-0.9614
WIL	0.9083	-0.7126	-0.9702
Zeneca	0.8367	-0.7220	-1.1518

Table 11

	(Intercept)	I(1/(dose1 + 1/2))	dose2
Basf	0.1276	-0.1846	-0.2628
Bayer	0.1840	-0.1407	-0.1661
Berlin	0.3134	-0.2306	-0.1881
ChungKorea	0.2636	-0.1533	-0.1414
Citfrance	0.2000	-0.1347	-0.1759
Citijapan	0.2148	-0.1664	-0.2502
Denmark	0.2739	-0.2024	-0.3463
Exxon	0.1979	-0.1389	-0.2159
Hatano	0.1968	-0.1673	-0.2551
Huntingdon	0.0179	-0.1001	-0.1367
InEnvTox	0.2239	-0.1763	-0.2776
KoreaPark	0.1825	-0.1941	-0.2975
Mitsubishi	0.2110	-0.1719	-0.2793
Nihon	0.1914	-0.1796	-0.2821
Poulenc	0.1906	-0.2295	-0.2487
Sumitomo	0.2511	-0.1943	-0.2805
TNO	0.3095	-0.2361	-0.2305
WIL	0.2051	-0.1797	-0.2407
Zeneca	0.1774	-0.1758	-0.2838