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 random effects of the models are conditioned on the labs to account for lab-to-lab variability. Each iteration of the model differs in transformations of the predictors and response, including Gaussian kernels on the dose 1 predictor, until we arrive at a model that we deem most appropriate for the dataset. We then evaluate the results of the linear mixed effects model to determine whether they display large variation in responses to the dosages between labs, or if they predict a consistent measured response to the chemicals. We do this by drawing samples of the random effect of dose 1 from the final mixed effects model, and determining if the dose-response curves across labs display homogeneous trends.

Model-Fitting

The structure of our linear mixed effects model is described below:

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

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

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

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 dose 1 and dose 2 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. $x_{ij,w}$ is the body weight for x_{ij}. Uterus weight is 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 means 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.

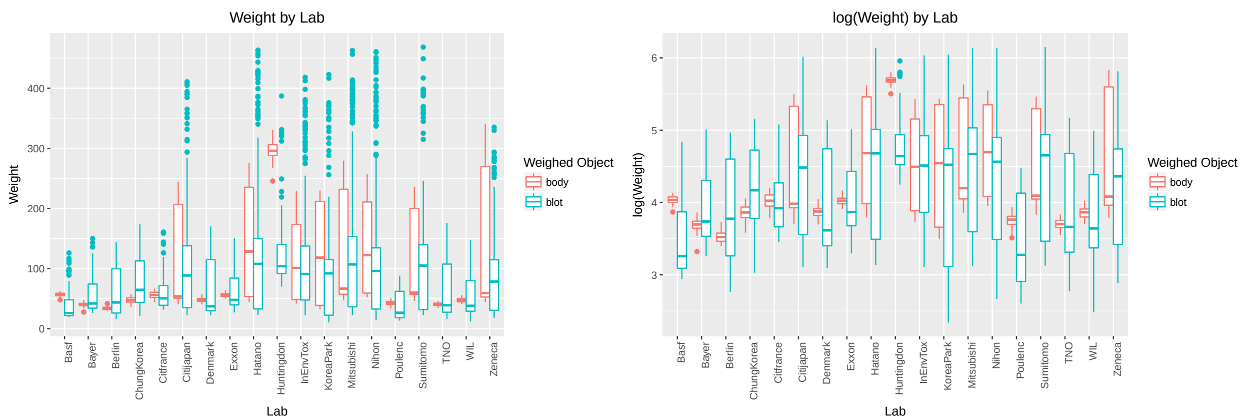

Figure 1: Log Transformations of Weights

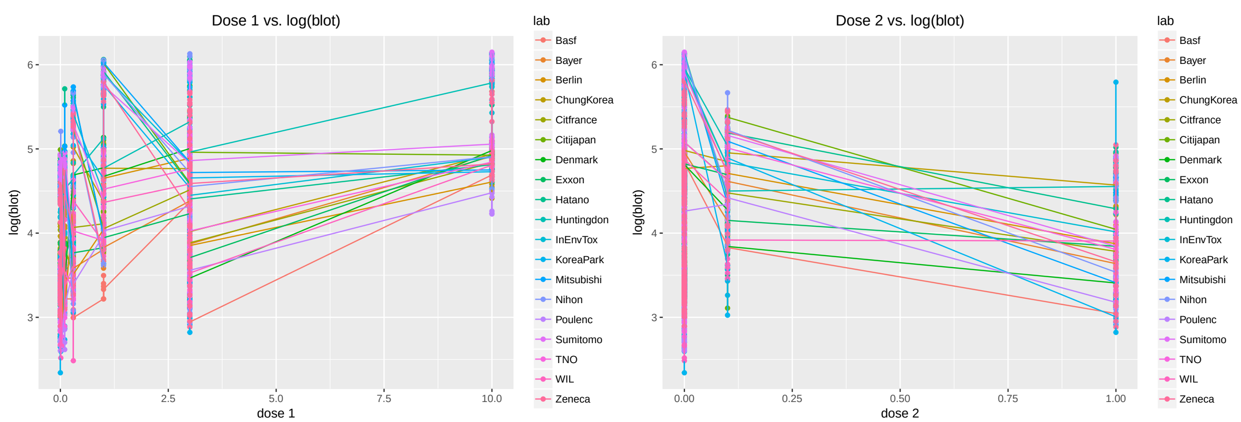

Figure 2: Lab-to-Lab Variability in Intercepts and Slopes

Tables 1-4: Summary Statistics of Initial Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	4.8887	0.0017
protoB	0.0422	0.0007
protoC	1.4555	0.0115
protoD	1.4618	0.0128
body	-0.0012	0.0000

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	-1.5448	-1.1764	-1.2169	...	-1.1342	-1.2671	-1.2367
dose1	0.1837	0.1419	0.1528	...	0.1409	0.1521	0.1444
dose2	-0.6617	-0.4441	-0.2799	...	-0.3054	-0.4999	-0.6099

	Variance Random Effects
(Intercept)	1.4818
dose1	0.0211
dose2	0.2710

	Variance Residuals
residual	0.1918

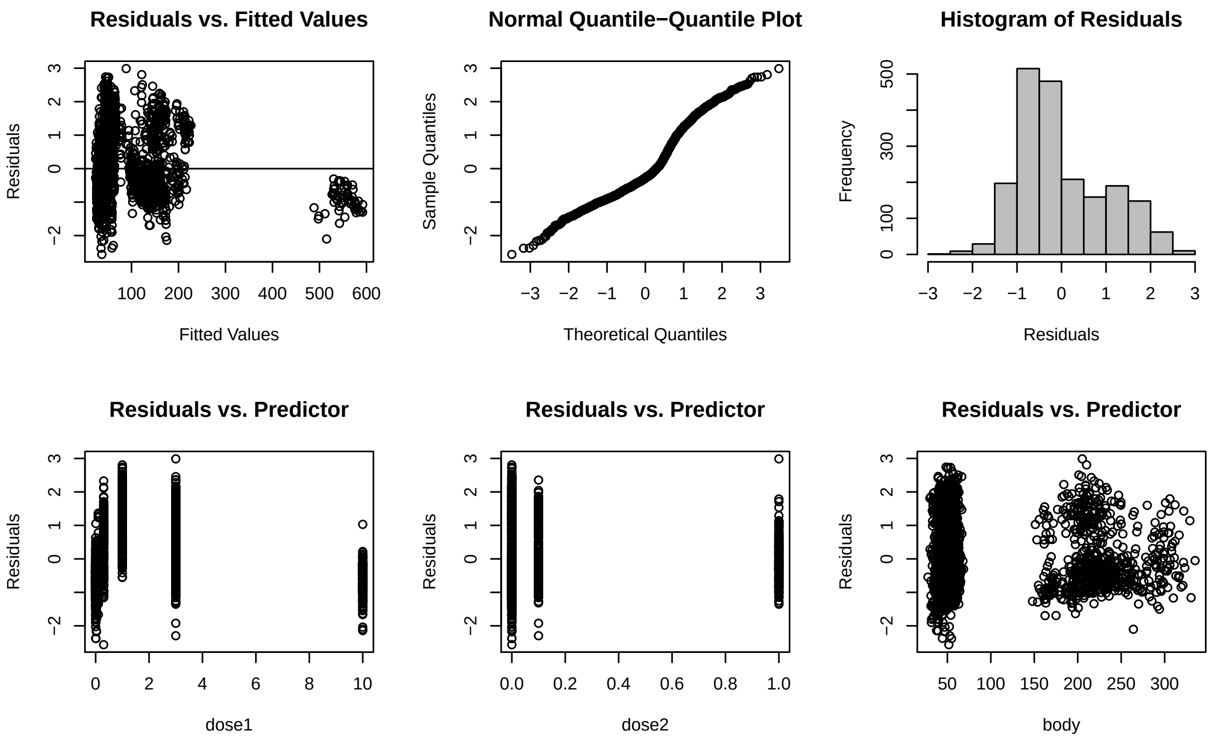

Figure 3: Diagnostic Plots of Initial Model

Among the problems in these diagnostic plots is the nonlinearity in the Residuals vs. Predictor plot for dose 1. To counter this, we transformed dose 1 by the reciprocal of (dose1 + 1/2) to reduce the leverage of observations with high dosage (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 Reciprocal Dose 1 Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	3.8298	0.0024

	Mean Fixed Effects	Variance Fixed Effects
protoB	0.0415	0.0003
protoC	1.1270	0.0054
protoD	1.1254	0.0062
body	0.0008	0.0000

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	0.5518	0.6745	1.1657	...	1.1933	0.8175	0.7862
I(1/(dose1 + 1/2))	-0.718	-0.5587	-0.9204	...	-0.9378	-0.7147	-0.7224
dose2	-1.0138	-0.6829	-0.8091	...	-0.9651	-0.9721	-1.1541

	Variance Random Effects
(Intercept)	0.7984
I(1/(dose1 + 1/2))	0.5341
dose2	1.0495

	Variance Residuals
residual	0.0803

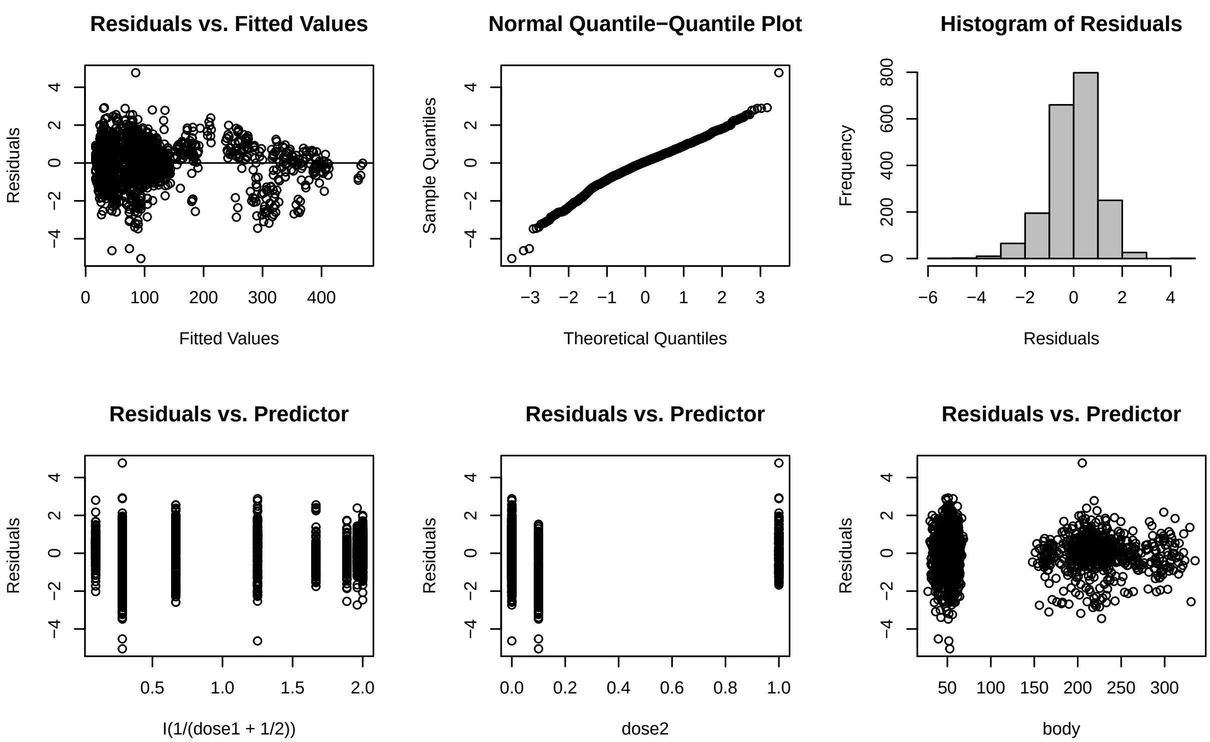

Figure 4: Diagnostic Plots of Reciprocal Dose 1 Model

Table 9: Predictive Error of First Two Models

	MAE	RMSE
Initial	36.4207	61.1562
Reciprocal Dose 1	21.8325	37.5289

For this model, the residual plots indicate a better fit, the predictive error improves dramatically, and the residual variance decreases from 0.1918 to 0.0803, which means that this model is better at explaining variance in uterus weight. We also see that the reciprocal transformation of dose 1 improved its Residuals vs. Predictor plot. However, the Residual vs. Fitted Values plot contains signs of slight arching. This suggests that a non-linear approach may be helpful, so we replace the reciprocal of (dose1 + 1/2) with Gaussian kernels on the log of (dose1 + 1/2). We take the log instead of the reciprocal because it reduces the influence of high dosage observations, and it also preserves the order of the observations.

Tables 10-13: Summary Statistics of Kernels on Log Dose 1 Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	4.6750	0.0015
protoB	0.0431	0.0003
protoC	1.1119	0.0047
protoD	1.1035	0.0053
body	0.0009	0.0000

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	-1.5417	-1.1261	-0.6403	...	-0.6332	-0.847	-0.6417
knot1	-0.0684	-0.1239	2.2168	...	2.257	1.4736	2.4292
knot2	1.4134	1.1322	1.0694	...	1.0221	0.7068	0.1738
knot3	1.0561	0.783	0.4535	...	0.5037	0.6276	0.4724
dose2	-0.9679	-0.71	-0.8437	...	-0.976	-0.9479	-1.0048

	Variance Random Effects
(Intercept)	0.6196
knot1	4.8074
knot2	0.5103
knot3	0.3214
dose2	0.9113

	Variance Residuals
residual	0.0693

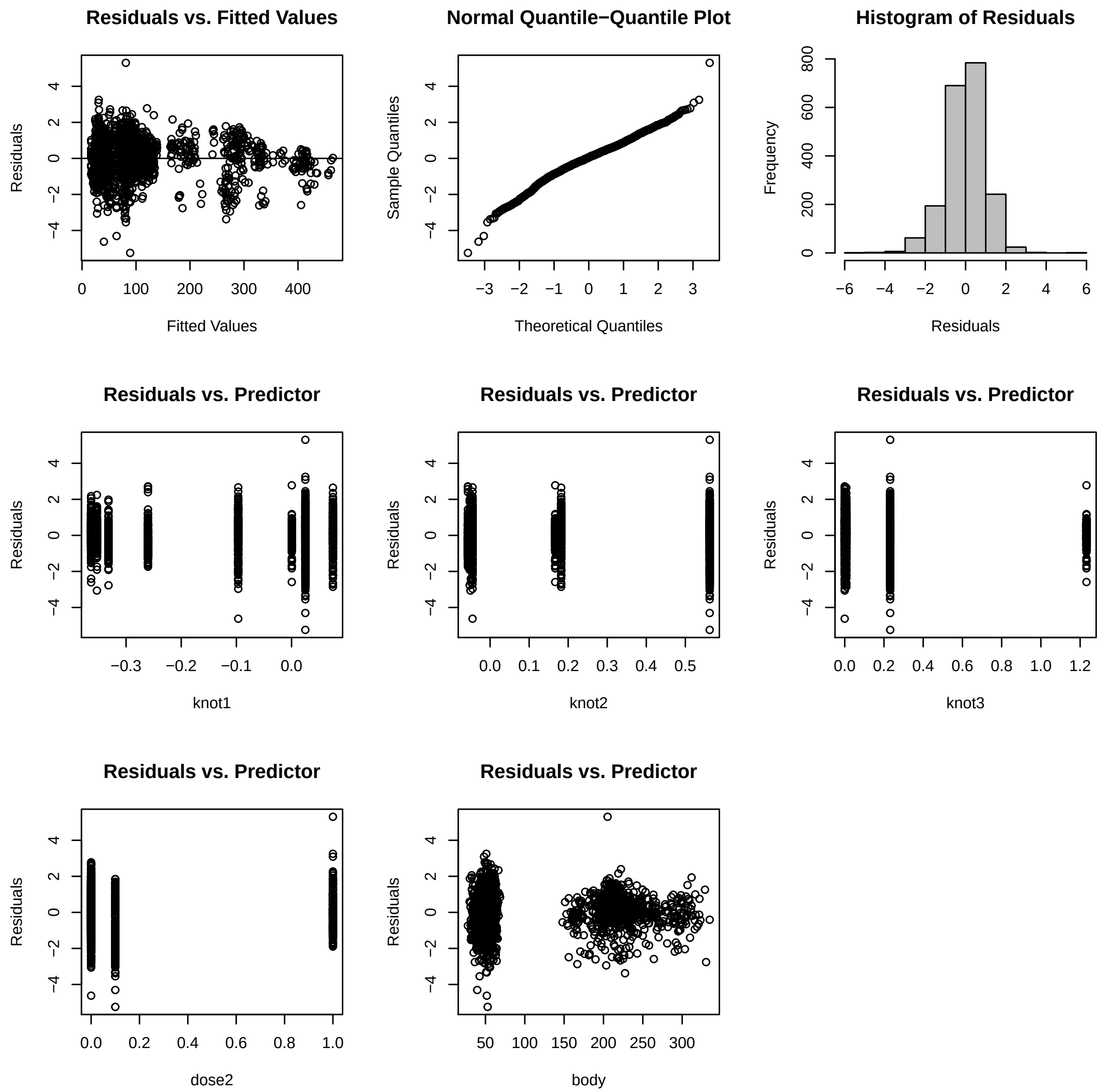

Figure 5: Diagnostic Plots of Kernels on Log Dose 1 Model

Table 14: Predictive Error of All Models

	MAE	RMSE
Initial	36.4207	61.1562
Reciprocal Dose 1	21.8325	37.5289
Kernels on Log Dose 1	19.3146	32.5493

Based on the residual plots, this mixed model with kernels on dose 1 best satisfies the assumptions of linear regression. This model is our best fit for the blotted uterus weight with reasonable residual plots, the lowest residual variance, and the lowest predictive error.

Results

The figure below takes samples of the random dose 1 effects (slopes) and lab effects (intercepts) from our final mixed model, holding all other predictors constant, and plots the resulting dose response curve for each lab.

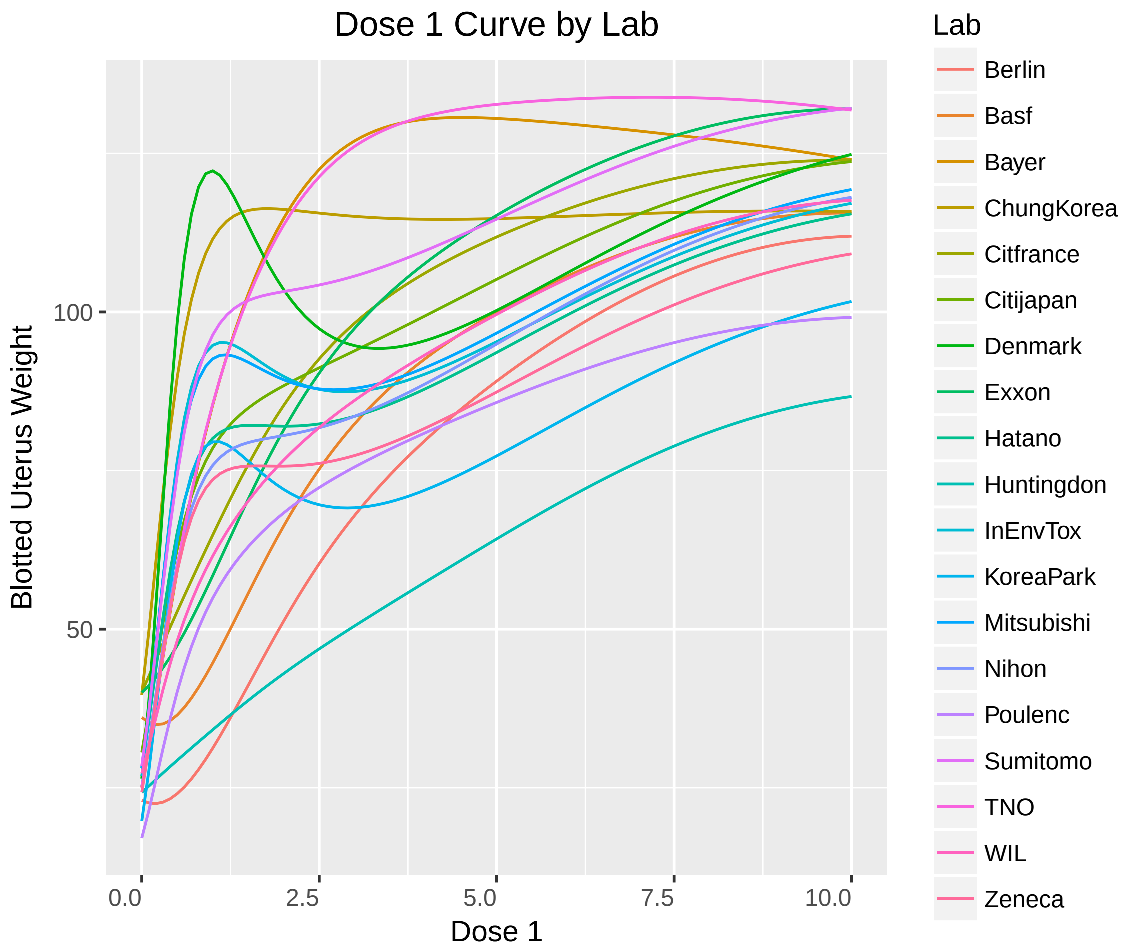

Figure 6: Dose 1 Response Curves for Each Lab

Although the dose effect curves of each lab mostly trend positive, the variance in the dose 1 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 residuals do show a slight decrease in variance for large fitted values, which may indicate that the response better fits a different model, 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 and tables 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 file and wrote the accompanying explanations for the EDA and approaches to analysis. William Yang helped evaluate the models and compile analyses and explanations into a report. Approaches to analysis and implementation of mixed effects models were a joint effort by all members of the group.

Appendix

Complete Results of Random Means for Each Model:

Table 2: Mean Random Effects for Initial Model

	(Intercept)	dose1	dose2
Basf	-1.5448	0.1837	-0.6617
Bayer	-1.1764	0.1419	-0.4441
Berlin	-1.2169	0.1528	-0.2799
ChungKorea	-0.8105	0.1006	-0.2224
Citfrance	-0.9818	0.1187	-0.3630
Citijapan	-1.0933	0.1291	-0.4945
Denmark	-1.0500	0.1225	-0.5197
Exxon	-1.0081	0.1197	-0.4366
Hatano	-1.1445	0.1341	-0.5477
Huntingdon	-1.4032	0.1684	-0.5532
InEnvTox	-1.2162	0.1421	-0.5945
KoreaPark	-1.4711	0.1735	-0.6710
Mitsubishi	-1.1182	0.1299	-0.5688
Nihon	-1.2808	0.1505	-0.6026
Poulenc	-1.5753	0.1895	-0.6096
Sumitomo	-1.0888	0.1300	-0.4514
TNO	-1.1342	0.1409	-0.3054
WIL	-1.2671	0.1521	-0.4999
Zeneca	-1.2367	0.1444	-0.6099

Table 6: Mean Random Effects for Reciprocal Dose 1 Model

	(Intercept)	I(1/(dose1 + 1/2))	dose2
Basf	0.5518	-0.7180	-1.0138
Bayer	0.6745	-0.5587	-0.6829
Berlin	1.1657	-0.9204	-0.8091
ChungKorea	1.0607	-0.6207	-0.6105
Citfrance	0.8547	-0.5514	-0.7382
Citijapan	0.9280	-0.6944	-1.0560
Denmark	1.1087	-0.8130	-1.3937

	(Intercept)	I(1/(dose1 + 1/2))	dose2
Exxon	0.8556	-0.5728	-0.8956
Hatano	0.8828	-0.7078	-1.0931
Huntingdon	0.1761	-0.4471	-0.5872
InEnvTox	0.9440	-0.7450	-1.1890
KoreaPark	0.7510	-0.7928	-1.2135
Mitsubishi	0.9484	-0.7249	-1.1953
Nihon	0.8619	-0.7508	-1.1911
Poulenc	0.6874	-0.8595	-0.9547
Sumitomo	1.0987	-0.8060	-1.1813
TNO	1.1933	-0.9378	-0.9651
WIL	0.8175	-0.7147	-0.9721
Zeneca	0.7862	-0.7224	-1.1541

Table 11: Mean Random Effects for Kernels on Log Dose 1 Model

	(Intercept)	knot1	knot2	knot3	dose2
Basf	-1.5417	-0.0684	1.4134	1.0561	-0.9679
Bayer	-1.1261	-0.1239	1.1322	0.7830	-0.7100
Berlin	-0.6403	2.2168	1.0694	0.4535	-0.8437
ChungKorea	-0.2255	2.2128	0.2740	0.1693	-0.6145
Citfrance	-0.7558	0.6448	0.8368	0.5788	-0.7367
Citijapan	-0.5850	1.9175	0.4312	0.4932	-0.9754
Denmark	-0.1330	3.6639	-0.3293	0.2362	-1.1864
Exxon	-0.8632	0.3360	0.9775	0.6983	-0.8441
Hatano	-0.5438	2.3038	0.1551	0.4411	-0.9926
Huntingdon	-1.3440	0.4372	0.6200	0.7953	-0.7108
InEnvTox	-0.3845	2.8921	-0.0351	0.3489	-1.0513
KoreaPark	-0.5630	3.2636	-0.1724	0.3973	-1.0970
Mitsubishi	-0.4004	2.7092	0.0017	0.3718	-1.0502
Nihon	-0.6238	2.3642	0.2666	0.5090	-1.0821
Poulenc	-1.0301	2.2520	0.7655	0.6294	-0.9486
Sumitomo	-0.4137	2.6018	0.3362	0.4203	-1.0989
TNO	-0.6332	2.2570	1.0221	0.5037	-0.9760
WIL	-0.8470	1.4736	0.7068	0.6276	-0.9479
Zeneca	-0.6417	2.4292	0.1738	0.4724	-1.0048