Analyzing Rat Bioassays

Nathaniel Brown, Annie Tang, William Yang

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GOALS FOR FINAL REPORT:

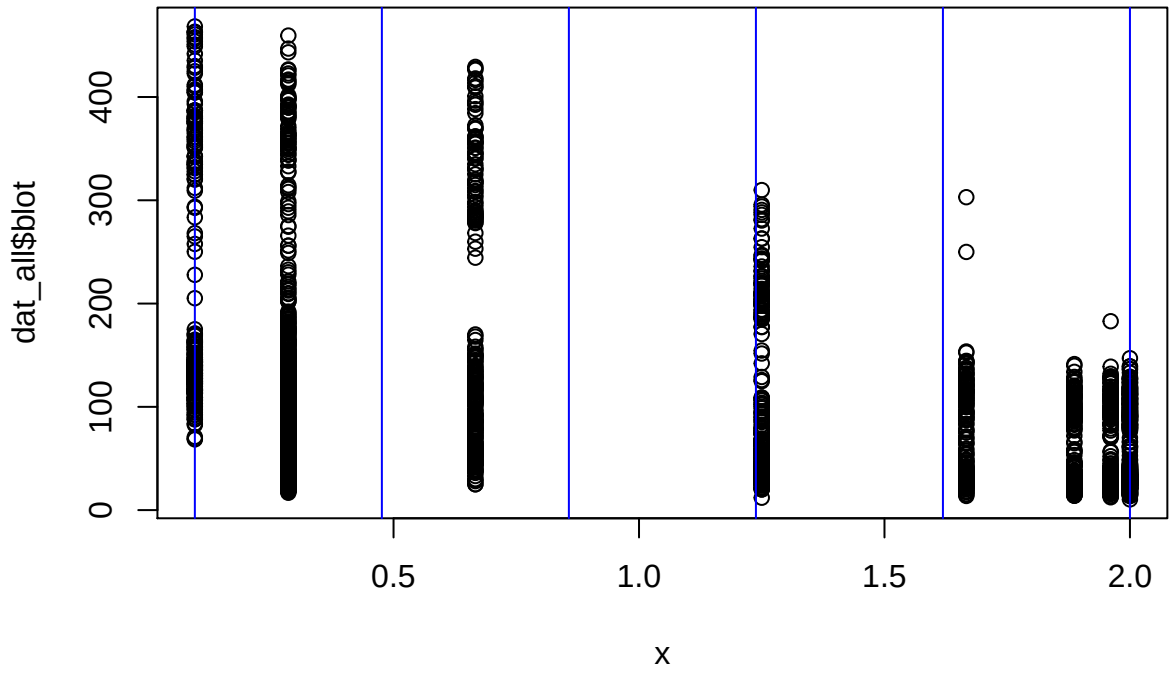
take out the sqrt model from back in the day

add the kernel model (m5 here)

the residual variance decreases for higher fitted values. let's further investigate

update those simulation plots

proofread




```
## pdf
##   2
```

```
## $abs_err
## [1] 19.9118
##
```

```
## $rms_err
## [1] 34.4649
```

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 slightly in transformations of the predictors and response until we arrive at a model that we deem most appropriate for the dataset. We then evaluate the final model to determine whether it displays 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 effect of dose 1 from the final model, and determining if the dose-response curves across labs display homogeneous trends.

Model-Fitting

The structure of our 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. $x_{ij,log(w)}$ 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 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

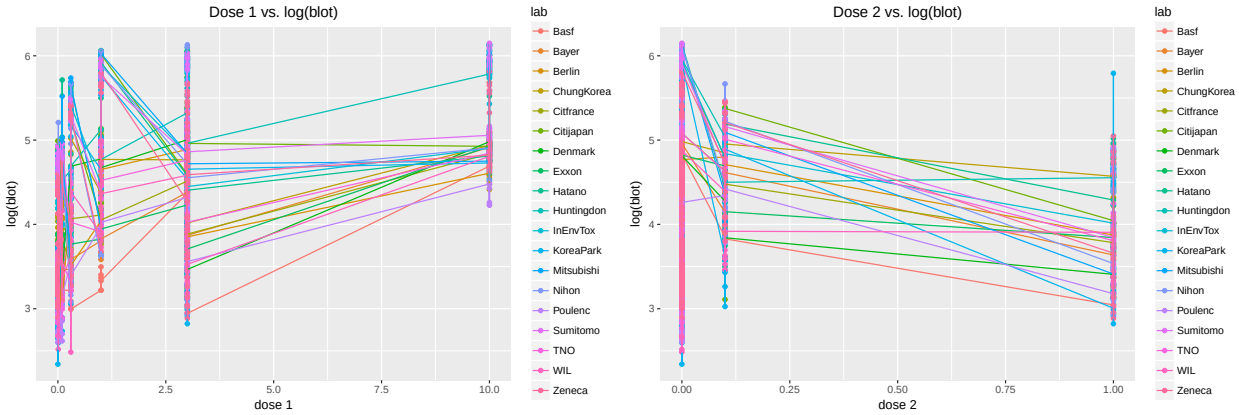


Figure 2: Lab-to-Lab Variability in Intercepts and Slopes

Tables 1-4: Summary Statistics of Initial Model

	Mean Fixed Effects	Variance Fixed Effects
(Intercept)	4.8875	0.1421
protoB	0.0429	0.0007
protoC	1.2723	0.0193
protoD	1.2716	0.0204
log(body)	-0.0065	0.0092

	Basf	Bayer	Berlin		TNO	WIL	Zeneca
(Intercept)	-1.5851	-1.1978	-1.2306	...	-1.1556	-1.2962	-1.2994
dose1	0.1838	0.1411	0.1513	...	0.1406	0.1519	0.1476
dose2	-0.6631	-0.442	-0.2795	...	-0.3045	-0.4994	-0.6272

	Variance Random Effects
(Intercept)	1.5746
dose1	0.0214
dose2	0.2730

	Variance Residuals
residual	0.192

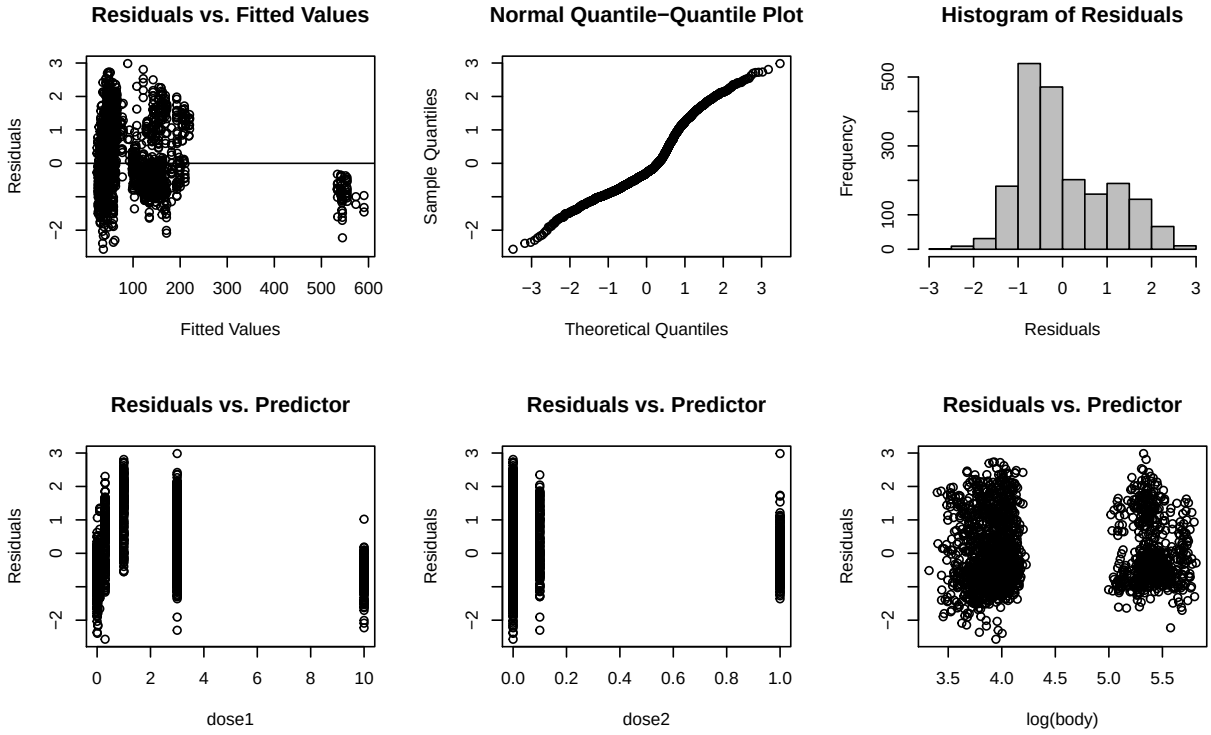


Figure 3: Diagnostic Plots of Initial Model

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) (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)	2.6378	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	0.817	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	-0.7941	...	-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

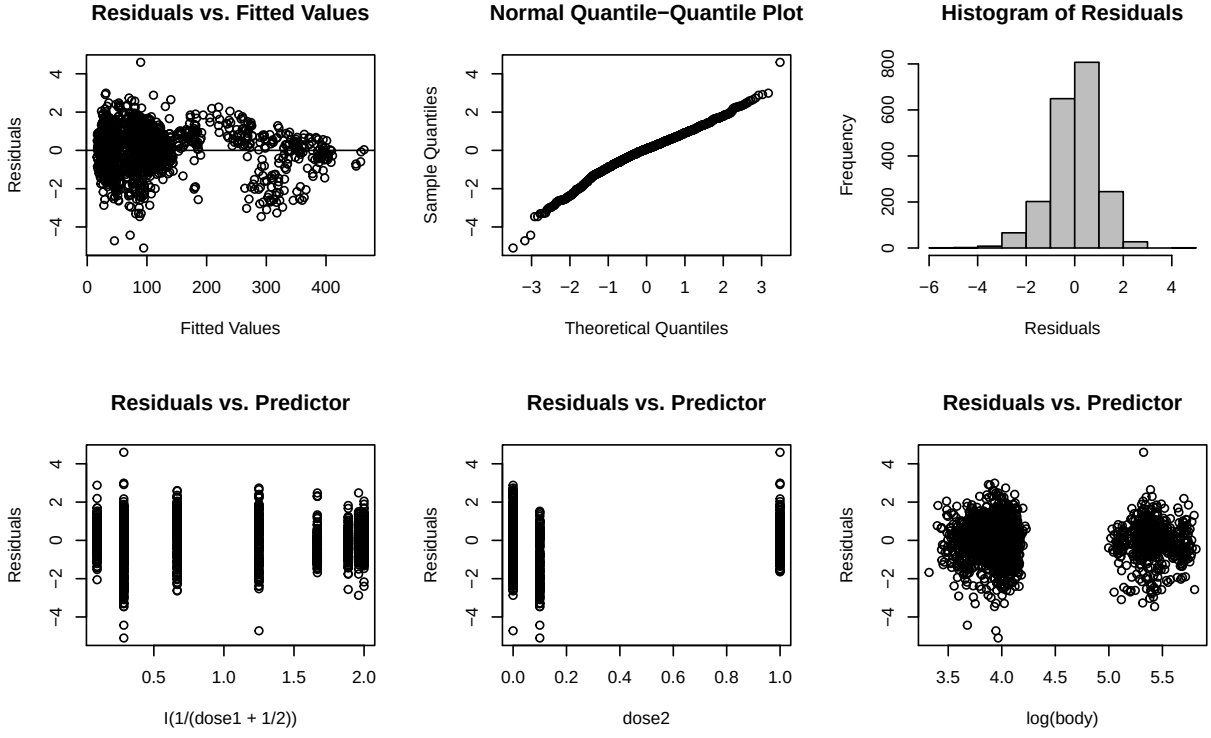


Figure 4: Diagnostic Plots of Model with Transformed Dose 1

Table 9: Predictive Error of First Two Models

	MAE	RMSE
Initial	36.4126	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:

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

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

Table 14: Predictive Error of All Models

	MAE	RMSE
Initial	36.4126	60.8907
Reciprocal Dose 1	21.7968	37.7587

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) reduces the arching in the residual plot at the cost of slightly lower predictive accuracy on the hold-out sample dataset.

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 effect curve. The black curves represent the means of the random effects from the model output.

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 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 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 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	-1.5851	0.1838	-0.6631
Bayer	-1.1978	0.1411	-0.4420
Berlin	-1.2306	0.1513	-0.2795
ChungKorea	-0.8401	0.1018	-0.2309
Citfrance	-1.0196	0.1203	-0.3709
Citijapan	-1.1273	0.1299	-0.4933
Denmark	-1.0810	0.1229	-0.5177
Exxon	-1.0462	0.1212	-0.4416
Hatano	-1.1899	0.1360	-0.5521
Huntingdon	-1.5308	0.1789	-0.6030
InEnvTox	-1.2238	0.1396	-0.5754
KoreaPark	-1.4955	0.1721	-0.6610
Mitsubishi	-1.1646	0.1320	-0.5689
Nihon	-1.3158	0.1509	-0.5968
Poulenc	-1.6002	0.1878	-0.6067
Sumitomo	-1.1252	0.1312	-0.4503
TNO	-1.1556	0.1406	-0.3045
WIL	-1.2962	0.1519	-0.4994
Zeneca	-1.2994	0.1476	-0.6272

Table 6: Mean Random Effects for Transformed Dose Model

	(Intercept)	I(1/(dose1 + 1/2))	dose2
Basf	0.6012	-0.7142	-1.0239
Bayer	0.8170	-0.5655	-0.6824
Berlin	1.3398	-0.9166	-0.7941
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

	(Intercept)	I(1/(dose1 + 1/2))	dose2
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: Mean Random Effects for Transformed Dose and Blotted Uterus Weight Model