

APPENDIX XVI

Statistical Analysis of Post-Mating Body Weights - Males

Statistical Report

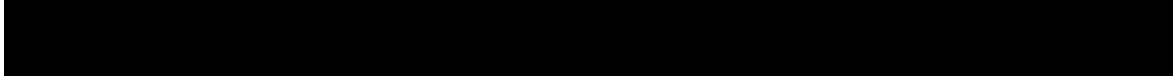
Project #: E02186.01
Project Title: Effect of oxybenzone on fertility and early embryonic development in Sprague-Dawley rats (Segment I)
PI: Amy Inselman
Task: Statistical Analysis of Sire Body Weight
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
Reviewer: Paul Felton, Division of Bioinformatics and Biostatistics


Statistician

Date


Reviewer

Date


Team Leader – Statistical Support Group

Date

Statistical Analysis of Sire Body Weight Data

1. Objectives

1.1 Project Objectives

The objective of the study is to examine the reproductive toxicity of oxybenzone in male and female rats and is designed to focus specifically on fertility and early embryonic development to implantation [ICH Guideline S5(R2) 4.1.1]. An additional objective is to compare the results of a typical Segment I, II, III study design with results from a modified one-generation study proposed by the NTP.

1.2 Analysis Objectives

The goal of this analysis is to determine the effects of oxybenzone on sire body weight.

2. Experimental Design

A total of 262 rats were to be requested for this study. Of this number 125 male rats were to be requested along with 125 female rats. Males were to be approximately 5-7 weeks old when delivered to the NCTR, and females were to be approximately 9-11 weeks of age when delivered. All males were to be delivered in one shipment, and all females were to be delivered in a separate shipment. After a two week quarantine period the animals were to be weighed and allocated to the study.

The test article in this study is 2-hydroxy-4-methoxybenzophenone (synonyms: HMB, benzophenone-3, oxybenzone). The animals were to be divided into five treatment groups with 25 male and 25 female rats assigned to each group. The treatment groups were to be four oxybenzone dose levels 0 ppm (control), 3000 ppm, 10,000 ppm, and 30,000 ppm and estrogen ethinyl estradiol (EE₂) 0.05 ppm treatment.

Males were to be dosed for 10 weeks and females for approximately 2 weeks prior to mating. Dosing was to continue until gestational day (GD) 6 for all animals. From GD 6 to GD 15, dams were to receive control chow. All dams were to be sacrificed on GD 15; males were to be sacrificed soon after breeding (approximately dam GD 6).

All animals were to be housed in pairs in cages prior to breeding. For breeding, males and females were to be housed one male: one female for up to 15 days or until animals have mated. Males and females were to be housed individually upon indication of mating (GD 0) until the time of sacrifice.

Body weights of males were to be determined twice per week from their day of allocation until the determination of pregnancy. Body weights of females were to be determined twice per week from their day of allocation until the beginning of pregnancy (GD 0).

3. Statistical Method

Analysis was performed using data from males in breeder pairs where the plug was detected and mating resulted in pregnancy of females. Pairwise comparisons of sires' body

weight means were performed using contrasts within a two-way repeated measures, mixed model analysis of variance (ANOVA), with terms for treatment group, dams' gestational day, and interaction. Analysis was performed on sire body weight data collected at dam's GD 0 and GD 6. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Test of trend, increasing treatment effect with increasing dose, was performed for the oxybenzone and control groups. Comparisons of treatment groups to control were performed with Dunnett's method for adjusted contrasts using two-sided tests.

4. Results

Tables are included in Appendix A1 and figures are included in Appendix A2.

Summary statistics of sire body weight are given in Table 1. For thirteen breeding pairs resulting in pregnancies with unknown GD 0 (unmonitored or missing plug dates), sires remained in the breeding cage with the dams instead of being removed. These animals were excluded from the analysis (UIN=5A000002448, 5A000002436, 5A000002437, 5A000002445, 5A000002483, 5A000002487, 5A000002489, 5A000002496, 5A000002516, 5A000002526, 5A000002528, 5A000002538, and 5A000002549). Of 88 sires where the plug date was known and mating resulted in pregnancy of females, there were 7 missing body weights, although every sire had data at either dam's GD 0 or GD 6.

The ANOVA omnibus test results are given in Table 2 for the null hypothesis that all of the control, oxybenzone, and EE₂ treatment means for sire body weight are equal. Treatment and dam's GD effects were significant (both $p < 0.001$).

Comparisons of least squares mean sire body weights are presented in Table 3. There were significant trends overall and at dam's GD 0 and GD 6 (all $p < 0.001$). For oxybenzone 30,000 ppm and EE₂ 0.05 ppm, there were significant differences overall and at dams' GD 0 and GD 6 (all $p < 0.001$), with the treatment groups showing lower means compared to control (ranging from 9.2% to 10.9% less than control).

5. Conclusions

For oxybenzone 30,000 ppm and EE₂ 0.05 ppm, there were significant differences overall and at dams' GD 0 and GD 6, with the treatment groups showing lower means compared to control.

Appendices

A1 Tables

Table 1. Summary Statistics for Sire Body Weight by Treatment and GD

<i>Treatment</i>															
<i>CTRL</i>				<i>OXY 3,000</i>			<i>OXY 10,000</i>			<i>OXY 30,000</i>			<i>EE2 0.05</i>		
<i>Dam GD</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>
0	10	402.5	7.9	19	389.1	4.4	17	389.3	6.5	17	359.2	3.2	20	354.3	4.3
6	13	406.7	8.0	18	397.1	5.1	19	399.8	6.2	16	370.3	3.0	20	363.2	4.6

1. There were 7 missing values (N=13 for control, N=19 for OXY 3,000 ppm, N=19 for OXY 10,000 ppm, N=17 for OXY 30,000 ppm, and N=20 for EE2 0.05 ppm).

Table 2. ANOVA Results for Sire Body Weights

<i>Effect</i>	<i>NumDF</i>	<i>DenDF</i>	<i>Fvalue</i>	<i>P value</i>
Treatment	4	83	14.387	<.001
Dam GD	1	76	121.203	<.001
Treatment*Dam GD	4	76	0.703	0.592

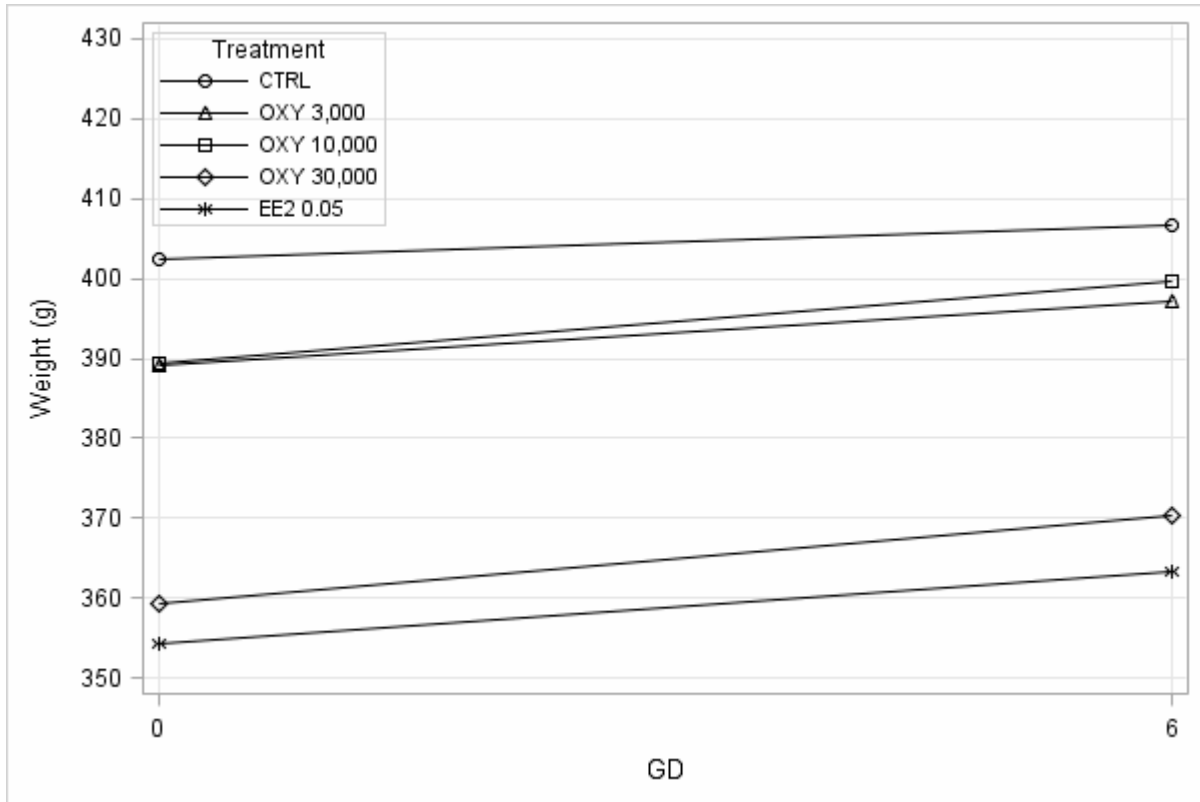
Table 3. Comparisons of Least Squares Mean Sire Body Weights Across Treatments¹

<i>Treatment</i>																			
<i>CTRL</i>				<i>OXY 3,000</i>				<i>OXY 10,000</i>				<i>OXY 30,000</i>				<i>EE2 0.05</i>			
<i>Dam GD</i>	<i>Mean</i>	<i>SE</i>	<i>P value</i>	<i>Mean</i>	<i>SE</i>	<i>Pct</i>	<i>P value</i>	<i>Mean</i>	<i>SE</i>	<i>Pct</i>	<i>P value</i>	<i>Mean</i>	<i>SE</i>	<i>Pct</i>	<i>P value</i>	<i>Mean</i>	<i>SE</i>	<i>Pct</i>	<i>P value</i>
All	402.2	6.0	<.001	393.0	4.9	97.7	0.545	393.8	4.9	97.9	0.617	364.3	5.2	90.6	<.001	358.8	4.8	89.2	<.001
0	397.6	6.2	<.001	389.1	5.0	97.9	0.666	387.8	5.1	97.5	0.556	359.2	5.3	90.4	<.001	354.3	4.9	89.1	<.001
6	406.9	6.0	<.001	397.0	4.9	97.6	0.521	399.8	4.9	98.3	0.774	369.4	5.2	90.8	<.001	363.2	4.8	89.3	<.001

1. All p-values and % are relative to the control group, except p-value for trend (excluding the EE2 treatment) shown below control.

A2 Figures

Figure 1. Sire Body Weight by Dam Gestational Day



A3 Data

Sire body weight data were extracted from the Genesis database using SAS Proc SQL, utilizing the Vortex ODBC driver.

Statistical Analysis of Sire Body Weight Data – QC

1. Data Verification

The extraction of the data into SAS was verified by the reviewer, Paul Felton, by review of the SAS code used to extract and verify the data.

2. Computer Program Verification

SAS programs were used to extract the data, explore the distributional properties of the data, and perform the statistical analysis.

The SAS programs were verified by detailed review of the program code, the program log, and the program output, and by independent verification of the results.

3. Statistical Report Review

3.1 Statistical Report Text

The statistical report was reviewed for logic, internal completeness, technical appropriateness, technical accuracy, and grammar. Technical appropriateness was reviewed based on statistical expertise.

Comments and questions were provided from the reviewer to the statistician. The statistician made appropriate changes and returned the report to the reviewer for final verification.

The text of the final statistical report was considered by the reviewer to be logical, internally complete, and technically appropriate and accurate. The statistical results stated in the text accurately presented those presented in the tables.

3.2 Table Verification

Analysis results were output from SAS to an .rtf file using PROC REPORT, which were then copied into the statistical report.

Statistical report tables were verified by independent verification of the numerical results.

3.3 Graph Verification

Graphs were verified by review of the SAS code used to generate them, and by calculation of summary statistics and checking numbers sufficiently to conclude that the graphs are correct. Graphs appear to be appropriate and correct.

4. Conclusions

The final statistical report has been fully reviewed and is considered by the reviewer to be logical, internally complete, and technically appropriate and accurate.