

APPENDIX XIV

Statistical Analysis of Gestational Body Weights - Females

Statistical Report

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

Signatures:

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Statistician Date

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Reviewer Date

[Redacted Signature]

Team Leader – Statistical Support Group Date

Statistical Analysis of Gestational 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 gestational 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 one 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 gestation 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 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 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). Body weights of pregnant females were to be determined on GD 0, 6 (end of dosing), 10 and 15 (sacrifice).

3. Statistical Method

Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model analysis of variance (ANOVA), with terms for treatment group, gestational day, and interaction. Analysis was performed on body weight data collected at GD 0, 6, 10, and GD 15 receiving weight at sacrifice. 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 gestational body weight are given in Table 1. Two dams, sacrificed early in the oxybenzone 10,000 ppm treatment at GD 14 (UIN=5A000002589) and in the EE₂ 0.05 ppm treatment at GD 13 (UIN=5A000002581), are presented as GD 15 (analysis excluding receiving weight data for these two dams did not result in any differences in conclusions). Due to missing plug dates, there were thirteen pregnant dams with unknown GD 0 that were excluded from the analysis (UIN=5A000002579, 5A000002580, 5A000002600, 5A000002607, 5A000002630, 5A000002636, 5A000002639, 5A000002648, 5A000002652, 5A000002653, 5A000002661, 5A000002671, and 5A000002678). Dosing for four dams was not stopped until GD 8 (UIN=5A000002650, 5A000002658, 5A000002664, 5A000002667), and their gestational weights were excluded at GD 10 and GD 15. There were 24 females that were not pregnant.

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 gestational body weight are equal. Treatment effect, GD effect, and the interaction were significant (all $p < 0.001$).

Comparisons of least squares mean gestational body weights are presented in Table 3. For oxybenzone 10,000 ppm, there were significant differences compared to the control group overall and at GD 15 ($p = 0.032$ and $= 0.029$). There were significant differences compared to control overall and at all gestational days for oxybenzone 30,000 ppm (all $p < 0.001$ except $p = 0.002$ at GD 0) and for EE₂ 0.05 ppm (all $p < 0.001$ except $p = 0.001$ at GD 0). Means in the treatment groups were lower than control means in all comparisons.

5. Conclusions

For oxybenzone 10,000 ppm, there was a significant difference compared to the control group at GD 15. There were significant differences for oxybenzone 30,000 ppm and EE₂ 0.05 ppm compared to control overall and at all gestational days. Means in the treatment groups were lower than control means in all comparisons.

Appendices

A1 Tables

Table 1. Summary Statistics for Gestational 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>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	11	267.1	6.2	19	260.1	3.4	18	260.5	3.7	17	246.7	2.2	20	246.8	4.2
6	13	286.1	1.7	19	283.9	2.3	19	279.1	1.7	17	264.3	2.1	20	260.3	2.0
10	12	300.3	1.7	18	297.8	2.3	18	293.1	1.8	17	286.9	1.9	19	287.5	2.0
15	12	327.5	2.1	18	321.4	2.9	18	317.6	2.1	17	309.3	2.2	19	305.2	2.5

Receiving weight is reported for gestational weight at GD 15 (2 females, sacrificed early at GD 13 and 14, are included as GD 15).

Table 2. ANOVA Results for Gestational Body Weights¹

<i>Effect</i>	<i>NumDF</i>	<i>DenDF</i>	<i>Fvalue</i>	<i>P value</i>
Treatment	4	83	21.787	<.001
GD	3	238	687.958	<.001
Treatment*GD	12	238	3.830	<.001

1. Two females, sacrificed early at GD 13 and 14, are included in the analysis.

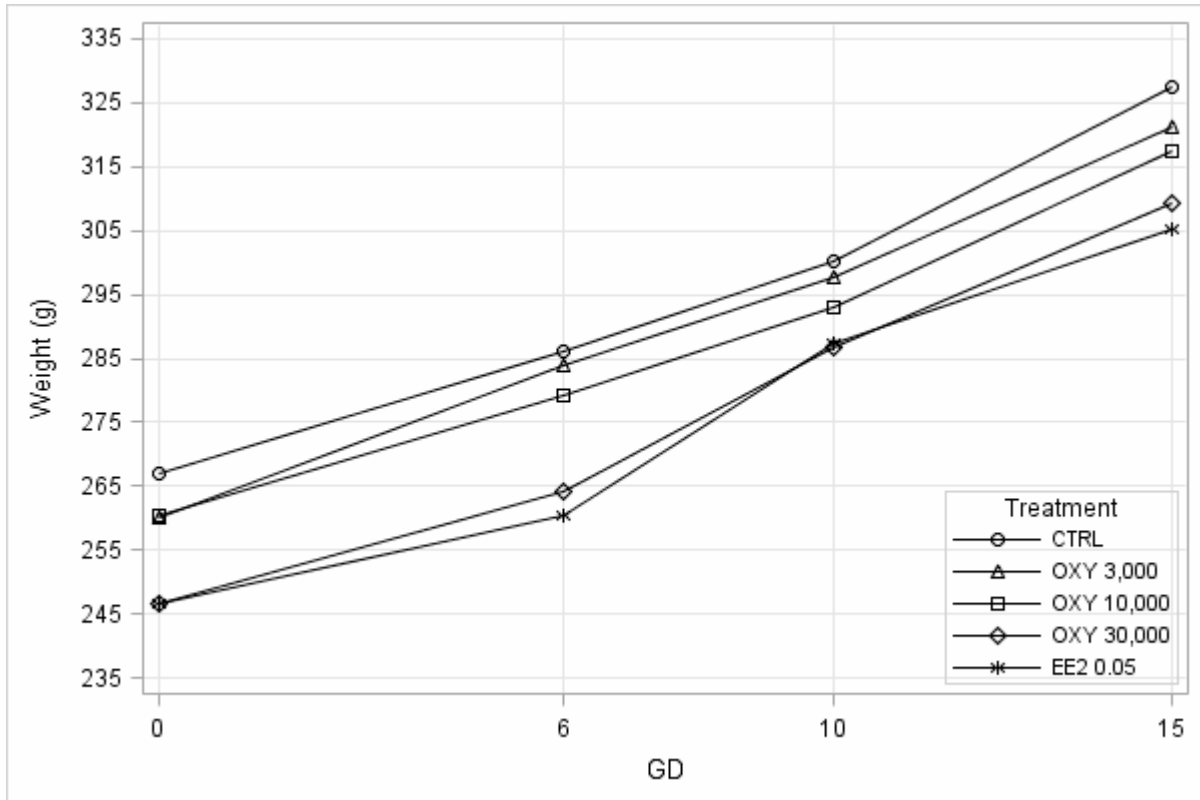
Table 3. Comparisons of Least Squares Mean Gestational Body Weights Across Treatments¹

<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>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	294.9	2.2	<.001	290.4	1.8	98.5	0.277	287.6	1.8	97.5	0.032	276.8	1.9	93.8	<.001	274.6	1.7	93.1	<.001
0	266.4	4.3	<.001	259.2	3.4	97.3	0.487	260.4	3.5	97.8	0.648	246.7	3.8	92.6	0.002	246.0	3.3	92.3	0.001
6	286.0	2.5	<.001	283.8	2.0	99.2	0.894	278.8	2.0	97.5	0.087	264.3	2.1	92.4	<.001	260.1	1.9	90.9	<.001
10	300.2	2.4	<.001	297.6	2.0	99.1	0.813	293.3	2.0	97.7	0.086	286.9	2.0	95.6	<.001	287.4	1.9	95.7	<.001
15	327.1	2.7	<.001	320.9	2.3	98.1	0.234	317.7	2.3	97.1	0.029	309.3	2.4	94.6	<.001	304.8	2.2	93.2	<.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. Gestational Body Weight by Gestational Day



A3 Data

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

Statistical Analysis of Gestational 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.