APPENDIX XI

Statistical Analysis of Maternal Body Weight

Statistical Report

Project #: E02187.01
Project Title: Effect of oxybenzone on fertility and early embryonic development in Sprague-Dawley rats (Segment II)
PI: Amy Inselman

Task: Statistical Analysis of Maternal 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

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Team Leader – Statistical Support Group Date

Statistical Analysis of Maternal Body Weight

1. Objectives

1.1 Project Objectives

This experiment is a study of embryo/fetal development [ICH Guideline S5(R2) 4.1.3] to determine the potential developmental toxicity of oxybenzone.

1.2 Analysis Objectives

The goal of this analysis is to assess effects of oxybenzone on maternal body weight.

2. Experimental Design

Oxybenzone is used in sunscreens and many commercial products to absorb UV radiation and prevent UV-induced photodecomposition in plastics and cosmetics. There has been recent interest in the biological activity of oxybenzone due to its high volume of use and its detection in the urine of a large percentage of the population. This study is designed to address concerns expressed by CDER that oxybenzone may have endocrine disruptor activity.

The test article in this study is 2-hydroxy-4-methoxybenzophenone (synonyms: HMB, benzophenone-3, oxybenzone). Dose levels were 0 ppm (control), 3,000 ppm, 10,000 ppm, and 30,000 ppm with approximately 25 animals per treatment group.

Date-mated females (approximately 11- 13 weeks old) were to be delivered in 5 loads to the NCTR on GD 3 or 4 (day of vaginal plug detection= GD 0). They were to be placed on control chow initially, and randomized to treatment groups. All animals were to be placed on dosed chow on GD 6 continuing to GD 15; all animals were to be fed control chow from GD 15 until sacrifice at GD 21. Feed and water were to be provided *ad libitum*. All animals were to be individually housed.

At sacrifice, the uterus was to be removed and the fetuses were to be separated from the placenta, individually weighed, sexed, and examined prior to sacrifice. Each fetus was to be given a complete fetal evaluation.

Dam body weights were to be measured on GD 3 (arrival), GD 6 (start of dosing), and GD 10, 14, 17 and 21 (removal).

3. Statistical Methods

Summary statistics are presented for each GD by treatment. Maternal weight at GD 3 prior to dosing was considered to be baseline weight. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model analysis of covariance (ANOCOVA) with terms for treatment group, GD, interaction, and baseline weight. 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. Comparisons of treatment groups to control

were performed with Dunnett's method for adjusted contrasts. Tests were conducted as two-sided at the 0.05 significance level

4. Results

Tables are presented in appendix A1 and Figures are presented in appendix A2.

Summary statistics for maternal body weight at each GD by treatment are given in Table 1, and body weight gain, defined as weight at GD 6 through 21 minus weight at GD 3, are presented in Table 2.

Results of the ANOCOVA for body weight are given in Table 3. All terms in the model were significant ($p<0.001$).

Least square mean comparisons of treatments to the control group are presented in Table 4. There were significant trends overall and at GD 10, 14, 17, and 21. In pairwise comparisons of treatments to control, there were significant differences for treatments 10,000 ppm and 30,000 ppm overall and at GD 10, 14, and 17. For both high dose treatments, means in significant comparisons were lower compared to the control means (ranging from 3.8% to 2.5% lower for 10,000 ppm and from 5.7% to 4.0% lower for 30,000 ppm, respectively).

5. Conclusions

There were significant differences for treatments 10,000 ppm and 30,000 ppm compared to the control group overall and at GD 10, 14, and 17. For both high dose treatments, means in significant comparisons were lower compared to the control means.

A1. Tables

Table 1. Summary Statistics of Maternal Body Weight (g)												
	Treatment											
	CTRL			OXY 3,000			OXY 10,000			OXY 30,000		
GD	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
3	19	217.22	3.30	21	215.07	2.09	22	215.28	1.71	19	214.45	2.24
6	19	238.32	3.02	21	236.13	2.76	22	236.52	2.30	19	239.06	4.63
10	19	255.29	3.05	21	250.88	1.85	22	247.41	1.62	19	238.80	1.72
14	18	274.83	3.70	21	269.74	2.29	21	263.68	1.98	18	254.04	1.69
17	19	306.66	3.90	21	299.40	3.91	22	295.01	2.30	19	294.21	2.61
21	19	353.22	5.14	21	349.58	3.97	22	342.27	3.11	19	341.39	3.29

Table 2. Summary Statistics of Maternal Weight Gain (g)												
	Treatment											
	CTRL			OXY 3,000			OXY 10,000			OXY 30,000		
GD	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
6	19	21.11	1.51	21	21.06	1.73	22	21.24	2.03	19	24.61	4.73
10	19	38.08	1.19	21	35.81	1.12	22	32.13	1.45	19	24.35	2.06
14	18	57.52	1.58	21	54.68	1.22	21	48.02	1.78	18	39.25	2.49
17	19	89.44	2.04	21	84.33	3.14	22	79.73	2.48	19	79.75	2.94
21	19	136.01	3.55	21	134.51	3.28	22	126.99	3.12	19	126.94	2.73

1. Body weight gain was calculated as body weight minus weight at GD 3.

Table 3. ANOVA Results for Maternal Body Weight[1]				
Effect	***NumDF***	***DenDF***	***Fvalue***	***P value***
Treatment	3	76	9.238	<.001
GD	4	305	938.637	<.001
Treatment*GD	12	305	4.285	<.001
Baseline	1	76	146.186	<.001

1. Body weight analysis was adjusted for baseline weight at GD 3 prior to dosing at GD 6.

Table 4. Comparison of Least Square Mean Maternal Body Weight Across Treatments[1]														
	Treatment													
	CTRL			***OXY 3,000***				***OXY 10,000***				***OXY 30,000***			
GD	***Mean***	***SE***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***
All	284.9	1.6	<.001	281.5	1.5	98.8	0.277	277.1	1.5	97.3	0.002	274.0	1.6	96.2	<.001
6	237.0	2.8	0.356	236.5	2.6	99.8	0.998	236.7	2.6	99.9	1.000	239.9	2.8	101.2	0.808
10	254.0	1.4	<.001	251.2	1.3	98.9	0.325	247.6	1.3	97.5	0.002	239.6	1.4	94.3	<.001
14	274.2	1.8	<.001	270.1	1.7	98.5	0.219	263.7	1.6	96.2	<.001	254.3	1.8	92.7	<.001
17	306.8	2.6	0.005	299.7	2.6	97.7	0.144	295.2	2.4	96.2	0.004	294.5	2.6	96.0	0.003
21	352.7	3.3	0.022	349.9	3.2	99.2	0.874	342.5	3.1	97.1	0.065	342.0	3.3	97.0	0.058

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

A2. Figures

Figure 1. Maternal Body Weight (g)

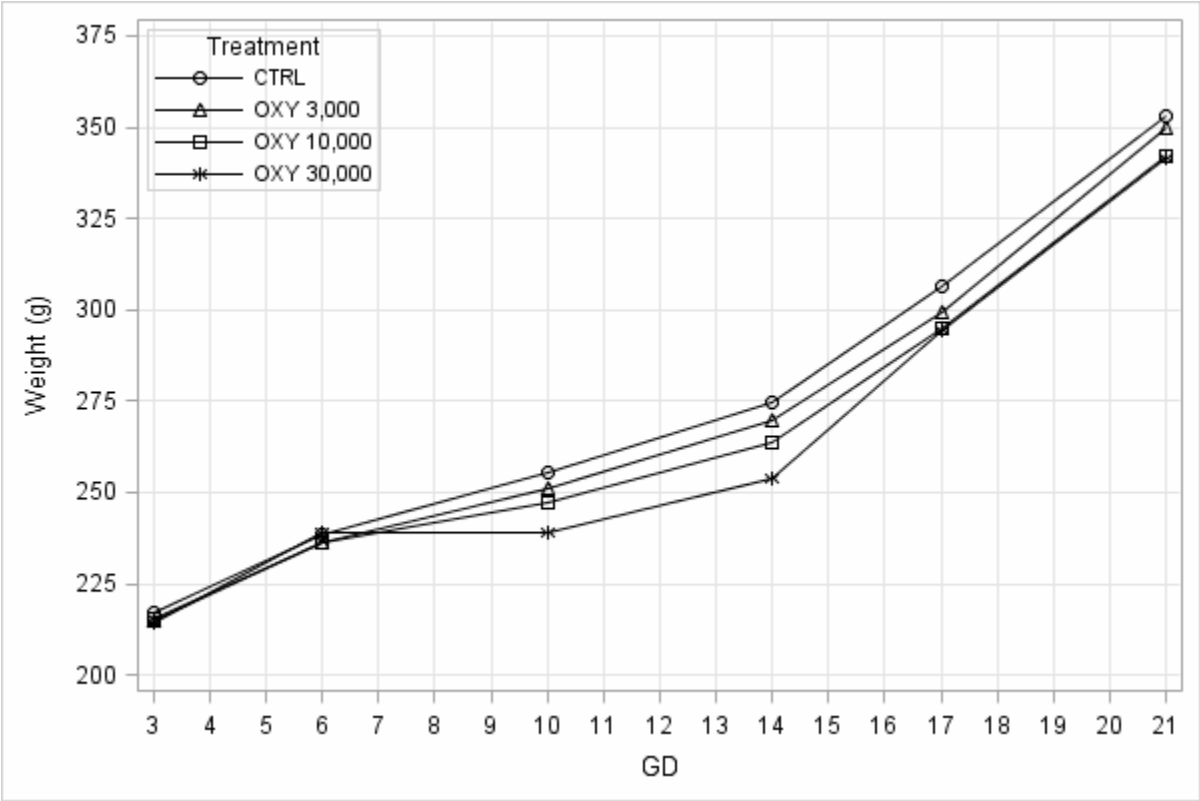
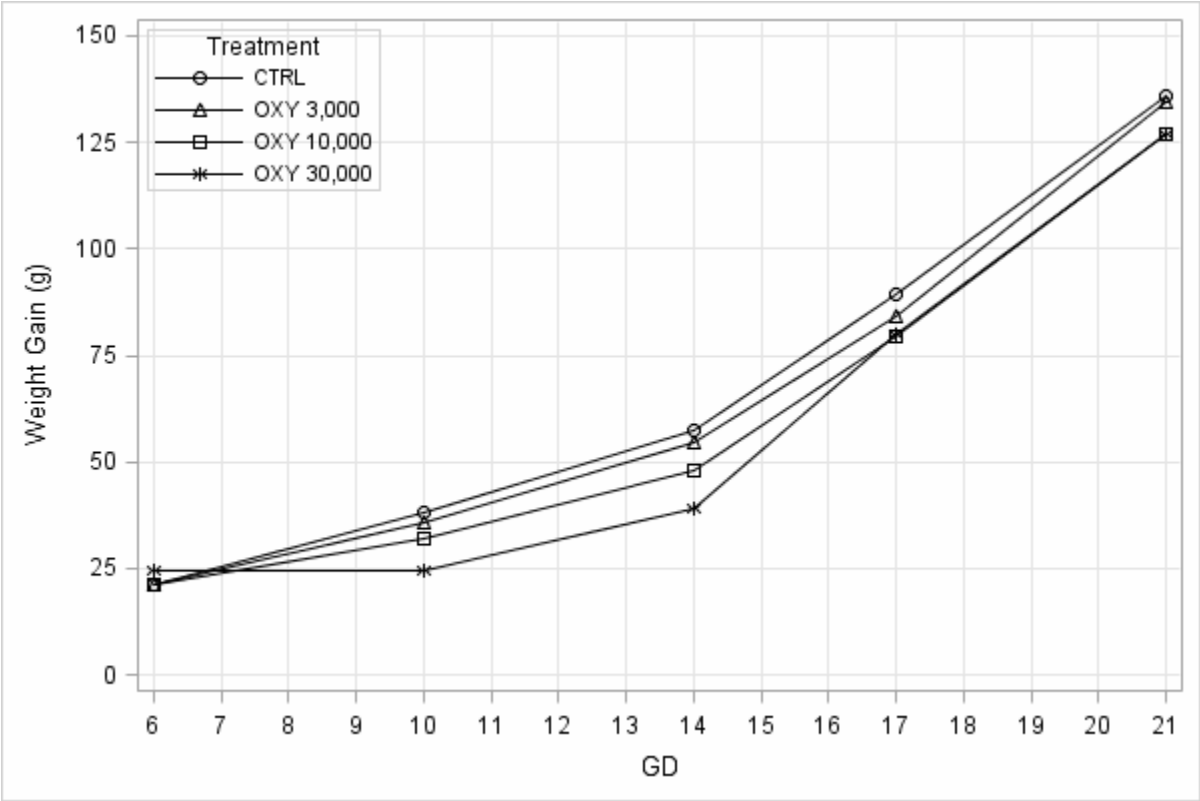


Figure 2. Maternal Body Weight Gain (g)



A3 Data

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

Statistical Analysis of Maternal 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.

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 checking the procedure used to create the tables and, additionally, by conducting a number of “spot-checks”.

3.3 Graph Verification

Graphs were verified by review of the SAS code used to generate them, and by calculation of summary statistics to use for “spot-checks” of the graphs. 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.

Statistical Report Addendum

Project #: E02187.01
Project Title: Effect of oxybenzone on fertility and early embryonic development in Sprague-Dawley rats (Segment II)
PI: Amy Inselman

Title: Maternal Body Weight Statistical Report Addendum 1
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
Reviewer: Paul Felton, Division of Bioinformatics and Biostatistics

Signatures:

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Statistician Date

[redacted]

Reviewer Date

[redacted]

Team Leader – Statistical Support Group Date

Maternal Body Weight Statistical Report Addendum 1

1. Purpose

Post hoc analyses were performed at the request of the Principle Investigator. The purpose of Addendum 1 is to provide summary statistics of maternal interval weight gain for body weight data collection at GD 3, 6, 10, 14, 17, and 21.

2. Statistical Methods

Maternal weight gain was introduced as an endpoint for summary statistics over the time ranges GD 3-6, GD 6-10, GD 10-14, GD 14-17, and GD 17-21. There are no other changes in endpoints or analysis methods introduced in *Addendum 1*.

3. Results

Summary statistics for maternal interval weight gain from GD 3 to 6, GD 6 to 10, GD 10 to 14, GD 14 to 17, and GD 17 to 21 are given in Addendum Table 1 and in Figure 1.

Tables

Table 1. Summary Statistics of Maternal Interval Body Weight Gain (g)

	Treatment											
	CNTL			*OXY 3,000*			*OXY 10,000*			*OXY 30,000*		
GD	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***
3-6	19	21.11	1.51	21	21.06	1.73	22	21.24	2.03	19	24.61	4.73
6-10	19	16.97	1.17	21	14.75	2.13	22	10.90	1.91	19	-0.26	4.49
10-14	18	19.67	1.03	21	18.86	1.14	21	16.11	1.06	18	14.83	1.06
14-17	18	30.98	1.14	21	29.66	2.37	21	31.48	1.45	18	40.80	1.65
17-21	19	46.56	2.87	21	50.18	4.60	22	47.26	1.50	19	47.18	2.51

Figures

Figure 1. Maternal Interval Body Weight Gain (g)