APPENDIX XII

Statistical Analysis of Pre-Mating Body Weights – Females and 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 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 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 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 EE_2 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 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 the day of allocation until the determination of pregnancy. Body weights of females were to be determined twice per week from the 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

Treatment group means of body weight were analyzed using twice weekly observations for each animal (days 1, 5, 9, and 12 for females and weeks 1 through 10 for males). Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model analysis of variance (ANOCOVA) for females and males separately. Model terms were treatment group, days on study, interaction and covariate baseline (defined as pre-dosing 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.

4. Results

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

Summary statistics of body weight are given in Table 1 for females and Table 2 for males. For females, data was collected by day. For males, the last data collected for each animal by week was used for analysis. Due to study logistics, dose durations prior to pairing were greater than the per protocol durations of 2 weeks for some females and 10 weeks for some males. However, the dose duration ranges were balanced across treatment groups for both females and males (ranges in all groups were 13 through 37 days for females and 69 through 93 days for males).

The ANOCOVA omnibus test results are given in Table 3 for females for the null hypothesis that all of the control, oxybenzone, and EE_2 treatment means for body weight are equal. Treatment effect, days on study effect, the interaction term, and the baseline covariate were all significant for females (all $p<0.001$).

Comparisons of least squares mean female body weights are presented in Table 4. There were significant trends overall and at days 5, 9, and 12 (all $p<0.001$). For oxybenzone 10,000 ppm, there were significant differences compared to the control group overall and at days 5 and 9 ($p=0.015$, $=0.001$, and $=0.011$, respectively). There were significant differences compared to control overall and at days 5, 9, and 12 for oxybenzone 30,000 ppm and EE_2 0.05 ppm (all $p<0.001$). Means in the dosed groups were lower than control means in all significant comparisons.

The ANOCOVA omnibus test results are given in Table 5 for males for the null hypothesis that all of the control, oxybenzone, and EE_2 treatment means for body weight are equal. Treatment effect, week effect, the interaction term, and baseline covariate were significant (all $p<0.001$).

Comparisons of least squares mean male body weights are presented in Table 6. Trends were significant overall and at all weeks (all $p<0.001$). There were significant differences compared to control overall and at all weeks for oxybenzone 30,000 ppm and EE_2 0.05 ppm (all $p<0.001$, with two exceptions with $p<0.01$). Means in the treatment groups were lower than control means in all significant comparisons.

5. Conclusions

For females, there were significant differences for oxybenzone 10,000 ppm compared to the control group overall and at days 5 and 9. There were significant differences compared to control overall and at days 5, 9, and 12 for oxybenzone 30,000 ppm and EE_2 0.05 ppm. For males, there were significant differences compared to control overall and at all weeks for oxybenzone 30,000 ppm and EE_2 0.05 ppm. Means in the dosed groups for females and males were lower than control means in all significant comparisons.

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Appendices

A1 Statistical Tables

Table 1. Summary Statistics for Female Body Weight by Treatment and Day															
	Treatment														
	CTRL			OXY 3,000			OXY 10,000			OXY 30,000			EE2 0.05		
Day	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Baseline	25	236.6	1.5	25	238.0	1.6	25	238.3	1.4	25	240.7	2.0	25	237.5	1.8
1	25	241.0	1.9	25	243.8	1.9	25	242.1	1.7	25	245.0	2.4	25	242.4	2.1
5	25	244.7	1.7	25	246.4	2.2	25	239.4	1.4	25	237.1	2.1	25	229.4	1.7
9	25	248.2	1.9	25	247.6	1.9	25	243.7	1.3	25	238.6	1.7	25	229.2	1.5
12	25	251.3	1.8	25	250.9	2.1	25	247.7	1.5	25	239.9	1.9	25	232.4	1.7

Table 2. Summary Statistics for Male Body Weight by Treatment and Week															
	Treatment														
	CTRL			OXY 3,000			OXY 10,000			OXY 30,000			EE2 0.05		
Week	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Baseline	25	225.5	1.6	25	225.1	1.5	25	224.9	1.2	25	226.9	2.2	25	227.2	1.5
1	25	260.1	2.3	25	256.6	1.6	25	255.2	1.6	25	239.5	1.8	25	251.9	1.4
2	25	280.5	2.6	25	275.8	2.1	25	277.2	2.3	25	266.7	1.9	25	263.3	1.8
3	25	302.1	3.0	25	297.7	2.4	25	298.7	2.5	25	290.3	1.8	25	281.0	1.9
4	25	321.5	3.5	25	318.1	2.6	25	314.4	3.3	25	305.7	2.5	25	295.7	2.3
5	25	334.9	3.7	25	330.9	2.6	25	327.8	3.5	25	314.6	2.4	25	306.7	2.5
6	25	346.4	3.9	25	341.4	2.9	25	340.3	3.7	25	327.0	2.6	25	315.3	2.5
7	25	358.5	4.1	25	354.3	2.9	25	352.6	3.8	25	337.4	2.7	25	325.1	2.6
8	25	366.5	4.3	25	362.2	2.9	25	360.5	4.1	25	341.9	2.8	25	329.5	2.7
9	25	378.4	4.5	25	370.2	3.2	25	367.8	4.4	25	349.5	2.9	25	336.2	2.9
10	25	383.4	4.4	25	376.2	3.2	25	373.9	4.6	25	354.7	2.9	25	342.7	3.1

Table 3. ANOCOVA Results for Female Body Weight				
Effect	*NumDF*	*DenDF*	*Fvalue*	*P value*
Treatment	4	119	29.974	<.001
DaysOn	3	360	29.845	<.001
Treatment*DaysOn	12	360	18.581	<.001
Baseline	1	119	229.416	<.001

Table 4. Comparisons of Least Squares Mean Body Weights for Females Across Treatments[1]																			
Treatment																			
CTRL				OXY 3,000				OXY 10,000				OXY 30,000				EE2 0.05			
Days On	*Mean*	*SE*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*
All	247.7	1.1	<.001	247.3	1.1	99.9	0.998	243.2	1.1	98.2	0.015	238.1	1.1	96.1	<.001	233.9	1.1	94.4	<.001
1	242.4	1.2	0.934	244.0	1.2	100.6	0.764	242.0	1.2	99.8	0.997	242.9	1.2	100.2	0.995	242.9	1.2	100.2	0.995
5	246.1	1.3	<.001	246.6	1.3	100.2	0.996	239.3	1.3	97.2	0.001	235.0	1.3	95.5	<.001	229.9	1.3	93.4	<.001
9	249.6	1.4	<.001	247.8	1.4	99.3	0.760	243.7	1.4	97.6	0.011	236.5	1.4	94.7	<.001	229.8	1.4	92.1	<.001
12	252.7	1.6	<.001	251.0	1.6	99.4	0.887	247.7	1.6	98.0	0.094	237.8	1.6	94.1	<.001	233.0	1.6	92.2	<.001

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

Table 5. ANOCOVA Results for Male Body Weight				
Effect	*NumDF*	*DenDF*	*Fvalue*	*P value*
Treatment	4	119	24.015	<.001
Week	9	1080	914.631	<.001
Treatment*Week	36	1080	6.402	<.001
Baseline	1	119	12.574	<.001

Table 6. Comparisons of Least Squares Mean Body Weights for Males Across Treatments[1]																		
Treatment																		
CTRL				OXY 3,000				OXY 10,000				OXY 30,000				EE2 0.05			
Week	*Mean*	*SE*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*	*Mean*	*SE*	*Pct*	*P value*
All	333.4	2.5	<.001	328.6	2.5	98.6	0.463	327.2	2.5	98.1	0.237	312.4	2.5	93.7	<.001	304.3	2.5	91.3	<.001
1	260.3	1.7	<.001	256.9	1.7	98.7	0.423	255.5	1.7	98.2	0.142	239.2	1.7	91.9	<.001	251.5	1.7	96.6	0.001
2	280.7	2.1	<.001	276.0	2.1	98.4	0.343	277.5	2.1	98.9	0.667	266.4	2.1	94.9	<.001	262.9	2.1	93.7	<.001
3	302.3	2.3	<.001	298.0	2.3	98.6	0.485	299.0	2.3	98.9	0.713	290.0	2.3	95.9	0.001	280.6	2.3	92.8	<.001
4	321.6	2.8	<.001	318.4	2.8	99.0	0.830	314.7	2.8	97.9	0.245	305.4	2.8	95.0	<.001	295.3	2.8	91.8	<.001
5	335.0	2.9	<.001	331.1	2.9	98.8	0.755	328.1	2.9	97.9	0.278	314.3	2.9	93.8	<.001	306.3	2.9	91.4	<.001
6	346.6	3.2	<.001	341.6	3.2	98.6	0.640	340.7	3.2	98.3	0.484	326.7	3.2	94.3	<.001	314.9	3.2	90.9	<.001
7	358.7	3.3	<.001	354.6	3.3	98.9	0.788	352.9	3.3	98.4	0.538	337.1	3.3	94.0	<.001	324.7	3.3	90.5	<.001
8	366.6	3.4	<.001	362.4	3.4	98.9	0.802	360.8	3.4	98.4	0.563	341.6	3.4	93.2	<.001	329.1	3.4	89.8	<.001
9	378.5	3.6	<.001	370.4	3.6	97.9	0.328	368.1	3.6	97.2	0.137	349.2	3.6	92.3	<.001	335.8	3.6	88.7	<.001
10	383.5	3.7	<.001	376.4	3.7	98.1	0.456	374.3	3.7	97.6	0.227	354.4	3.7	92.4	<.001	342.3	3.7	89.2	<.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. Body Weight by Days on Study for Females

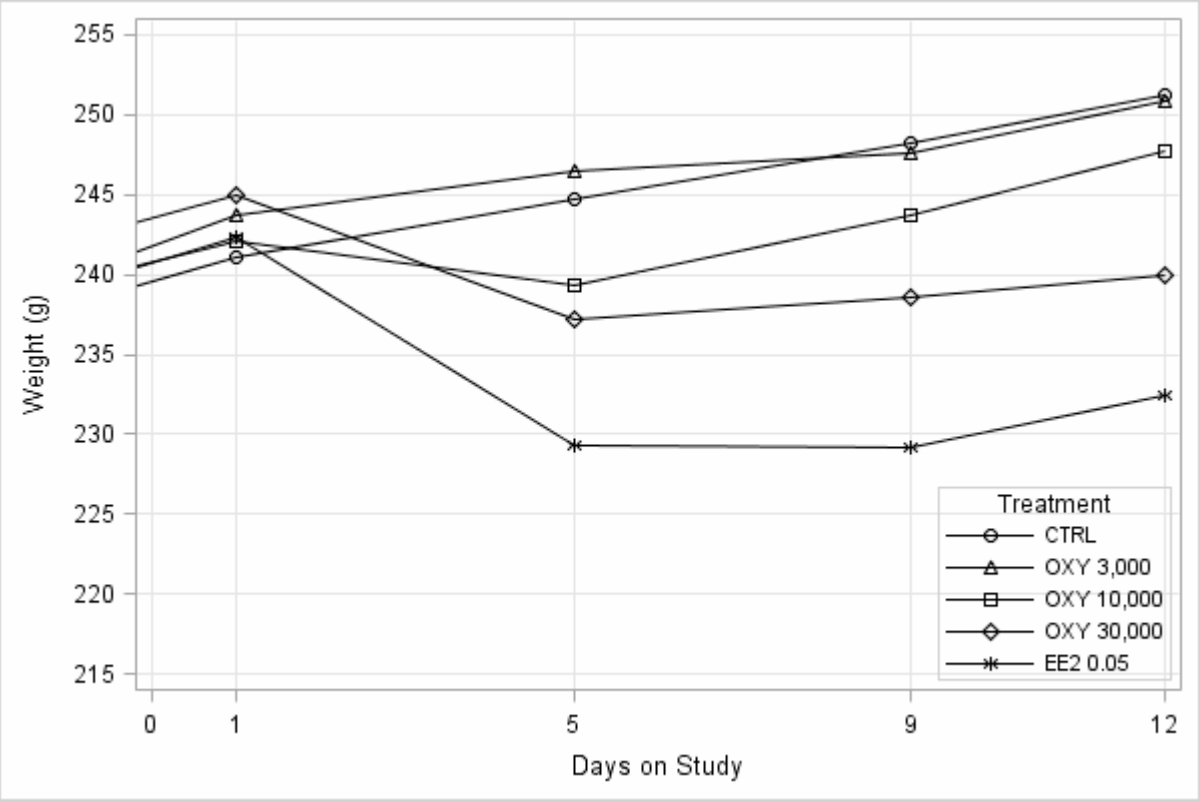
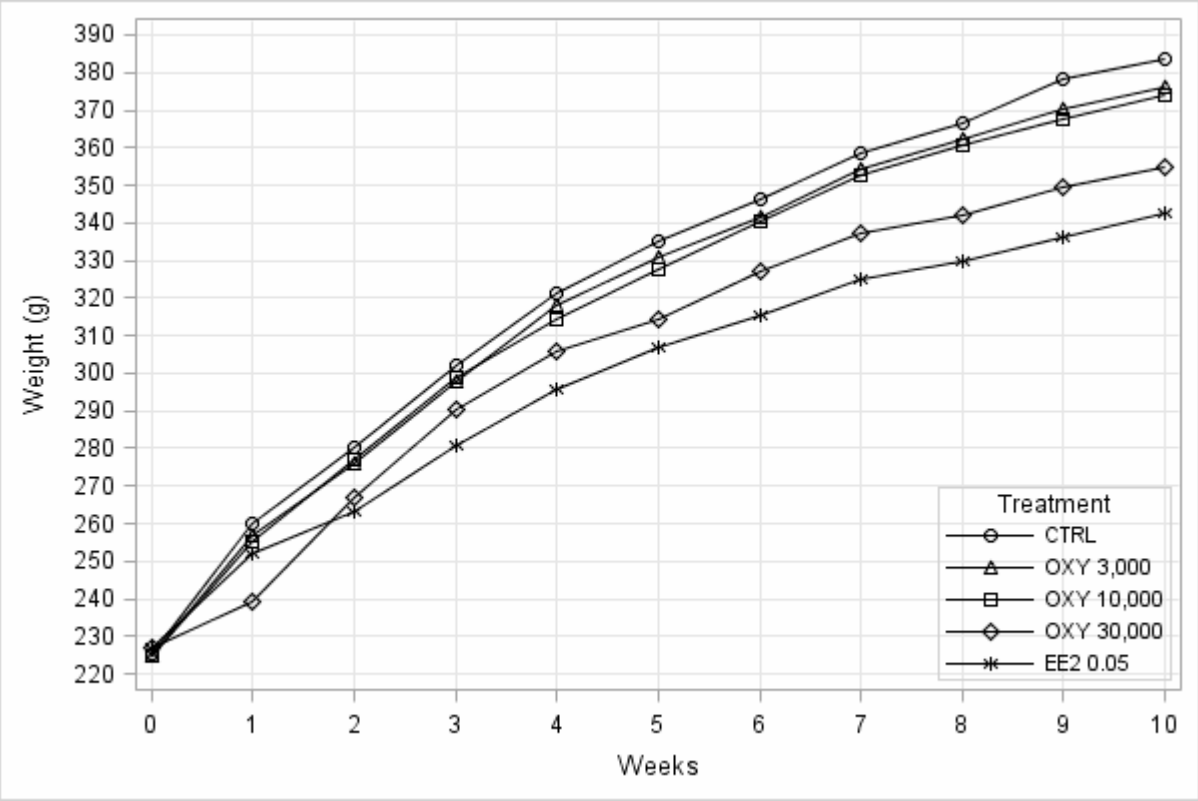


Figure 2. Body Weight by Week for Males



A3 Data

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

Statistical Analysis of 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.