APPENDIX XIII

Statistical Analysis of Maternal Organ Weights

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 Organ Weights
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 Organ Weights

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 test effects of oxybenzone on organ weights.

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 liver, kidneys (separate), and ovaries (separate) were to be removed from the dams and weighed.

3. Statistical Methods

Summary statistics of weights are presented for each organ by treatment, with left, right and combined weight for paired organs. Analysis was conducted on combined weight of paired organs. Pairwise comparisons of dosed treatments to the control were performed using contrasts within an analysis of covariance (ANOCOVA) with terms for treatment group and receiving weight. 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.

Summary statistics for organ weight (g) are given in Table 1, and relative organ weights to receiving weight (mg/g) are presented in Table 2.

The ANOCOVA omnibus test results are given in Table 3 for the null hypothesis that all of the oxybenzone treatment and control means are equal. There were no significant treatment effect for liver, kidneys (combined weight), or ovaries (combined weight). The covariate receiving weight was significant for liver, kidneys (combined weight), and ovaries (combined weight) ($p<0.001$, $=0.001$, and $=0.049$).

Least square mean comparisons of oxybenzone treatments to the control group are presented in Table 4. In pairwise comparisons of dosed treatments to control, there were no significant differences, although there was a significant trend for liver ($p=0.025$).

5. Conclusions

.In comparisons of dosed treatments to the vehicle control, there were no significant differences for organ weights.

A1. Tables

Table 1. Summary Statistics of Organ Weight (g)

		Treatment (ppm)										
		CTRL			OXY 3,000			OXY 10,000			OXY 30,000	
Organ	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Liver	19	10.70	0.25	21	10.34	0.25	22	10.65	0.23	19	10.75	0.21
Kidney Left	19	0.76	0.02	21	0.73	0.01	22	0.74	0.01	19	0.72	0.01
Kidney Right	19	0.76	0.02	21	0.72	0.01	22	0.73	0.01	19	0.73	0.01
Kidney Paired	19	1.52	0.04	21	1.45	0.02	22	1.47	0.03	19	1.45	0.02
Ovary Left	19	0.087	0.007	21	0.086	0.004	22	0.080	0.005	19	0.085	0.005
Ovary Right	19	0.087	0.004	21	0.096	0.004	22	0.091	0.004	19	0.088	0.004
Ovary Paired	19	0.174	0.008	21	0.181	0.005	22	0.172	0.007	19	0.173	0.006
Receiving Wt	19	352.7	4.5	21	347.8	3.7	22	339.6	3.1	19	338.7	3.2

Table 2. Summary Statistics of Relative Organ to Receiving Weight (mg/g)

		Treatment (ppm)										
		CTRL			OXY 3,000			OXY 10,000			OXY 30,000	
Organ	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Liver	19	30.34	0.62	21	29.67	0.48	22	31.39	0.70	19	31.72	0.51
Kidney Left	19	2.16	0.05	21	2.09	0.03	22	2.17	0.04	19	2.12	0.03
Kidney Right	19	2.16	0.05	21	2.08	0.03	22	2.16	0.04	19	2.16	0.03
Kidney Paired	19	4.32	0.09	21	4.17	0.06	22	4.32	0.07	19	4.29	0.06
Ovary Left	19	0.244	0.019	21	0.246	0.010	22	0.237	0.015	19	0.252	0.015
Ovary Right	19	0.249	0.013	21	0.276	0.011	22	0.268	0.011	19	0.259	0.012
Ovary Paired	19	0.493	0.021	21	0.522	0.013	22	0.505	0.019	19	0.511	0.018

Table 3. ANOVA Results for Analysis of Organ Weight[1]					
Organ	**Effect**	**NumDF**	**DenDF**	**Fvalue**	**P**
Liver	Receiving Wt	1	76	30.117	**<.001**
	Treatment	3	76	2.597	0.058
Kidney Paired	Receiving Wt	1	76	20.004	**<.001**
	Treatment	3	76	1.114	0.349
Ovary Paired	Receiving Wt	1	76	4.019	**0.049**
	Treatment	3	76	0.401	0.753

Table 4. ANOCOVA Comparison of Least Square Mean Organ Weights Across Treatment Groups																
	Treatment (ppm)															
	Control			**OXY 3,000**				**OXY 10,000**				**OXY 30,000**				
Organ	**Mean**	**SE**	**Pvalue**	**Mean**	**SE**	**Pct**	**Pvalue**	**Mean**	**SE**	**Pct**	**Pvalue**	**Mean**	**SE**	**Pct**	**Pvalue**	
Liver	10.41	0.22	**0.025**	10.23	0.20	98.2	0.848	10.82	0.20	103.9	0.376	10.95	0.21	105.2	0.196	
Kidney Paired	1.50	0.03	0.985	1.44	0.02	96.0	0.222	1.48	0.02	99.3	0.980	1.47	0.03	98.3	0.839	
Ovary Paired	0.17	0.01	0.909	0.18	0.01	105.6	0.581	0.17	0.01	101.7	0.978	0.18	0.01	102.9	0.918	

1. All p-values and % are relative to the control group, except p-values for the linear trend are presented under the control group.

A2. Data

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

Statistical Analysis of Organ Weights 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".

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.