APPENDIX XV

Statistical Analysis of Pregnancy Parameters

Statistical Report

Project #: E02187.01
Project Title: Effect of oxybenzone on fertility and early embryonic development in Sprague-Dawley rats (Segment II)
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Task: Statistical Analysis of Pregnancy Parameters
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
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Statistical Analysis of Pregnancy Parameters

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 the effect of oxybenzone on pregnancy parameters.

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 of dams on GD 21, the uterus was to be removed and weighed, and the ovaries were to be removed for counting corpora lutea. The number and status of each implantation site was to be recorded (live, dead, early or late resorption). 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.

3. Statistical Methods

Counts and percentages of pregnant dams in each treatment group were calculated. Summary statistics were performed for gravid weight. Mean counts by treatment were calculated for corpora lutea, implants, resorptions, and number of live fetuses per litter. Mean percentages were computed for pre-implantation loss and post-implantation loss. Pre-implantation loss was defined as the percentage of corpora lutea that did not result in implantation. Post-implantation loss was defined as the percentage of implantations that were resorbed.

Pregnancy proportions were analyzed using Fisher's Exact test for comparisons of treatments to control and using Cochran-Armitage test for trend. Analysis of gravid uterine weight was performed using contrasts within a one-way analysis of variance (ANOVA). Counts of implantation sites were analyzed using Poisson regression with terms for treatment and covariate number of corpora lutea. Counts of resorptions were analyzed using Poisson regression with terms for treatment and covariate number of implantation sites.

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.

Counts and percentages of pregnant dams in each treatment are given in Table 1 with analysis results using Fisher's Exact test and Cochran-Armitage trend test. The test of trend was not statistically significant, and there were no significant differences in pairwise comparisons.

Summary statistics for gravid weight are given in Table 2. Results of the ANOVA for gravid uterine weight are given in Table 3. There was no statistically significant treatment effect. Least square mean comparisons of treatments to the control group are presented in Table 4. There was no statistically significant trend, and there were no significant differences in pairwise comparisons.

Summary statistics for counts of corpora lutea, implants, resorptions, and live fetuses, and for pre-implantation loss and post-implantation loss are presented in Table 5.

In the analysis of implant counts adjusted for corpora lutea, there was no significant treatment effect. The covariate corpora lutea was statistically significant ($p=0.011$). Results for trend and pairwise comparisons are presented in Table 6. The test for trend was not significant, and there were no significant differences comparing treatments to control.

There was no significant treatment or covariate effect in the analysis of resorptions adjusted for implants. Results for trend and pairwise comparisons are presented in Table 6. There was no statistically significant trend, and there were no significant differences in pairwise comparisons.

5. Conclusions

There were no significant differences for treatments compared to the control group for pregnancy parameters, including proportion of pregnant dams, gravid weights, and counts of implantation sites and resorptions.

A1. Tables

Table 1. Summary Statistics and Analysis for Pregnancies by Treatment				
Treatment (ppm)	N	Pregnancy Count	Percentages	P-value[1]
CTRL	25	19	76.0	1.000
OXY 3,000	25	21	84.0	0.725
OXY 10,000	25	22	88.0	0.463
OXY 30,000	25	19	76.0	1.000

1. Fisher's exact test was used for comparisons of treatments to control; Cochran-Armitage trend test across treatments is shown for control. P-values are unadjusted for multiple comparisons

Table 2. Summary Statistics for Gravid Uterine Weights (g)											
Treatment (ppm)											
CTRL			OXY 3,000			OXY 10,000			OXY 30,000		
N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
19	86.9	3.0	21	90.0	2.6	22	84.9	4.2	19	92.6	3.1

Table 3. ANOVA Test of Treatment on Gravid Uterine Weight (g)				
Effect	NumDF	DenDF	Fvalue	P value
Treatment	3	77	1.037	0.380

Table 4. ANOVA Pairwise Comparisons for Gravid Uterine Weight (g)[1]															
Treatment (ppm)															
Control			OXY 3,000				OXY 10,000				OXY 30,000				
LS Mean	SE	Trend	LS Mean	SE	Pct	P	LS Mean	SE	Pct	P	LS Mean	SE	Pct	P
86.9	3.4	0.285	90.0	3.3	103.6	0.843	84.9	3.2	97.7	0.949	92.6	3.4	106.5	0.509

1. Dunnett adjusted p-values and % are relative to control except p-value for trend.

Table 5. Summary Statistics for Pregnancy Parameters												
	Treatment (ppm)											
	CTRL			OXY 3,000			OXY 10,000			OXY 30,000		
Parameter[1]	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
Corpora Lutea	19	14.8	0.4	21	15.0	0.3	22	14.6	0.5	19	15.1	0.5
Implants	19	13.1	0.4	21	13.4	0.5	22	12.6	0.6	19	13.7	0.5
Resorptions	19	0.6	0.2	21	0.4	0.2	22	0.3	0.1	19	0.4	0.2
Alive	19	12.5	0.5	21	13.0	0.5	22	12.3	0.6	19	13.4	0.6
Post-Implantation Loss %	19	4.6	2.0	21	2.8	1.4	22	2.8	1.1	19	3.1	2.1
Pre-Implantation Loss %	19	11.4	2.4	21	10.9	2.8	22	12.5	3.8	19	8.4	2.1

1. Parameters are counts for corpora lutea, implants, resorptions, and live fetuses.

Table 6. Poisson Regression Pairwise Comparisons for Pregnancy Parameters[1]															
	Treatment (ppm)														
	CTRL			OXY 3,000				OXY 10,000				OXY 30,000			
Analysis	LS Mean	SE	Trend[1]	LS Mean	SE	Pct	P-value[1]	LS Mean	SE	Pct	P-value[1]	LS Mean	SE	Pct	P-value[1]
Implants[2]	13.1	0.8	0.671	13.3	0.8	101.3	0.998	12.7	0.8	96.9	0.968	13.6	0.8	103.7	0.956
Resorptions[3]	0.6	0.2	0.624	0.4	0.1	66.9	0.740	0.3	0.1	51.6	0.415	0.4	0.1	66.8	0.761

1. Dunnett adjusted p-values and % are relative to the control group except the p-value for trend.
2. “Implants”analysis was performed with covariate corpora lutea.
3. “Resorptions” analysis was performed with covariate implants.

A2 Data

Pregnancy parameter data were provided in an Excel spreadsheet from the Principle Investigator.

Statistical Analysis of Pregnancy Parameter 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.