APPENDIX XVII

Statistical Analysis of Post-Mating Food Consumption - Males

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 Sire Food Consumption
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
Reviewer: Paul Felton, Division of Bioinformatics and Biostatistics

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Team Leader – Statistical Support Group Date

Statistical Analysis of Sire Food Consumption 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 sire food consumption.

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 estrogen ethinyl estradiol (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 dam 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.

Food consumption of males was to be determined twice per week from the day of allocation. This report presents data from each male after removal from breeding cages from dam’s GD 0 through GD 6.

3. Statistical Method

Analysis was performed using data from males in breeder pairs where the plug was detected and mating resulted in pregnancy of females. Pairwise comparisons of sires' daily food consumption means were performed using contrasts within an analysis of variance (ANOVA) to test for treatment effect. Analysis was performed on sire food consumption data collected from dam's GD 0 through GD 6. 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.

Summary statistics of sire food consumption are given in Table 1. For thirteen breeding pairs resulting in pregnancies with unknown GD 0 (unmonitored or missing plug dates), sires remained in the breeding cage with the dams instead of being removed. These animals were excluded from the analysis (UIN=5A000002448, 5A000002436, 5A000002437, 5A000002445, 5A000002483, 5A000002487, 5A000002489, 5A000002496, 5A000002516, 5A000002526, 5A000002528, 5A000002538, and 5A000002549). Of 88 sires where the plug date was known and mating resulted in pregnancy of females, there were 2 with missing food consumption data (UIN=5A000002533 in the oxybenzone 3000 ppm treatment and UIN=5A000002474 in the oxybenzone 30,000 ppm treatment).

The ANOVA omnibus test results are given in Table 2 for the null hypothesis that all of the control, oxybenzone, and EE_2 treatment means for sire food consumption are equal. Treatment effect was not significant for food consumption.

Comparisons of least squares mean sire food consumption are presented in Table 3. There was no significant trend and no pairwise differences for any dosed group compared to the control.

5. Conclusions

There were no significant results in the analysis of mean daily food consumption for sires from GD 0 through GD 6.

Appendices

A1 Tables

Table 1. Summary Statistics for Daily Sire Food Consumption by Treatment															
Treatment															
CTRL			OXY 3,000			OXY 10,000			OXY 30,000			EE2 0.05			
N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	
13	26.3	1.6	18	26.5	0.8	19	25.2	1.2	16	26.9	1.4	20	26.8	1.5	

1. There were 2 missing values (N=13 for control, N=19 for OXY 3,000 ppm, N=19 for OXY 10,000 ppm, N=17 for OXY 30,000 ppm, and N=20 for EE2 0.05 ppm).

Table 2. ANOVA Results for Sire Food Consumption				
Effect	NumDF	DenDF	Fvalue	P value
Treatment	4	81	0.294	0.880

Table 3. Comparisons of Least Squares Mean Sire Daily Food Consumption[1]																		
Treatment																		
Control			OXY 3,000				OXY 10,000				OXY 30,000				EE2 0.05			
Mean	SE	P value	Mean	SE	Pct	P value	Mean	SE	Pct	P value	Mean	SE	Pct	P value	Mean	SE	Pct	P value
26.3	1.5	0.737	26.5	1.3	100.7	0.999	25.2	1.3	95.6	0.930	26.9	1.4	102.3	0.994	26.8	1.2	101.8	0.997

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

A2 Data

Sire food consumption data were extracted from the Genesis database using SAS Proc SQL, utilizing the Vortex ODBC driver.

Statistical Analysis of Sire Food Consumption 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.

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.