

APPENDIX XV

Statistical Analysis of Gestational Food Consumption - Females

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

Signatures:

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Reviewer Date

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Team Leader – Statistical Support Group Date

Statistical Analysis of Gestational Food Consumption

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 test the effects of oxybenzone on gestational food consumption of females and males prior to breeding.

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 estrogen ethinyl estradiol (EE₂) 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.

Food consumption was to be measured twice weekly throughout the study beginning at the time of allocation. Water consumption was not to be measured. Food consumption was not to be determined while the animals were housed together for mating.

3. Statistical Methods

Treatment group means of gestational food consumption were analyzed using daily food consumption of each dam for intervals GD 1-6 (dosed chow) and GD 7-15 (control chow). Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model analysis of variance (ANOVA), with terms for treatment group, GD interval; and interaction. 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. Test of trend, increasing treatment effect with increasing dose, was performed for the oxybenzone and control groups. Comparisons of dosed groups to control were performed with Dunnett's method for adjusted contrasts using two-sided tests.

4. Results

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

Summary statistics for mean daily gestational food consumption per animal by treatment are given in Table 1. Due to missing plug dates, there were thirteen pregnant dams with unknown GD 0 that were excluded from the analysis (UIN=5A000002579, 5A000002580, 5A000002600, 5A000002607, 5A000002630, 5A000002636, 5A000002639, 5A000002648, 5A000002652, 5A000002653, 5A000002661, 5A000002671, and 5A000002678). Dosing for four dams was not stopped until GD 8 (UIN=5A000002650, 5A000002658, 5A000002664, 5A000002667), and their maternal weights were excluded for the interval GD 7-15. There were 24 females that were not pregnant.

The ANOVA omnibus test results are given in Table 2 for the null hypothesis that all of the oxybenzone treatment and control means for gestational food consumption are equal. The effect of treatment, GD interval, and the interaction were significant (all $p < 0.001$).

Least squares mean comparisons of dosed treatments to the control group are presented in Table 3. In analyses of oxybenzone 30,000 ppm and EE₂ 0.05 ppm, there were significant trends and pairwise comparisons to control overall and at GD 1-6 (all $p < 0.001$). The oxybenzone 30,000 ppm and EE₂ 0.05 ppm treated animals showed higher means at GD 1-6 compared to the control group (73.1% and 98.0% higher than control, respectively). There were no other significant trends or pairwise differences.

5. Conclusions

There were significant pairwise comparisons to control at GD 1-6 for oxybenzone 30,000 ppm and EE₂ 0.05 ppm, with the treated animals showing higher means compared to the control group.

A1. Tables

Table 1. Summary Statistics for Daily Gestational Food Consumption by Treatment

Day Interval	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
1-6	13	25.9	1.1	19	25.6	0.7	19	25.8	0.8	17	44.9	2.0	20	51.3	1.9
7-15	12	24.5	0.6	18	23.7	0.5	18	25.7	0.5	17	27.2	0.7	19	25.3	0.5

Table 2. ANOVA Results for Gestational Food Consumption

Effect	NumDF	DenDF	Fvalue	P value
Treatment	4	83	14.434	<.001
Interval	1	79	67.446	<.001
Treatment*Interval	4	79	22.442	<.001

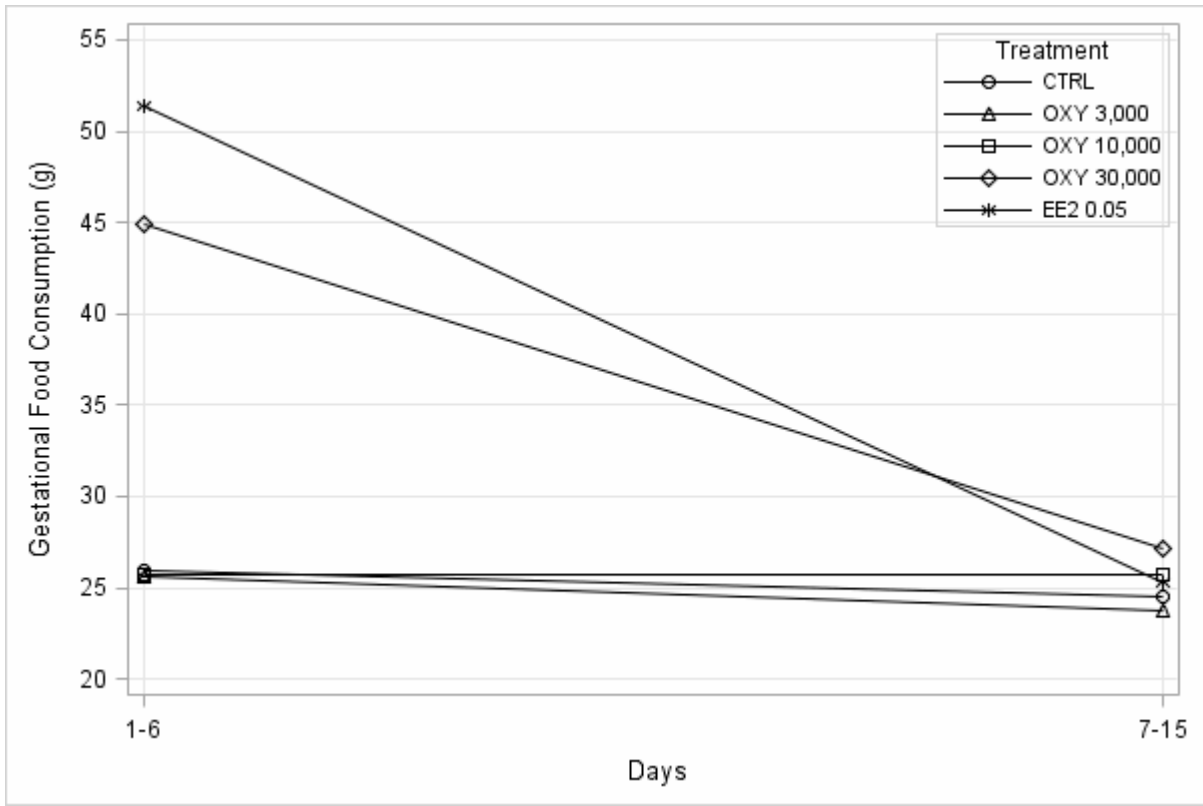
Table 3. Comparisons of Least Squares Mean Daily Gestational Food Consumption Across Treatments¹

Day Interval	Treatment																		
	CTRL				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
All	25.2	2.1	<.001	24.6	1.7	97.9	0.999	25.7	1.7	102.1	0.999	36.0	1.8	143.2	0.001	38.4	1.7	152.5	<.001
1-6	25.9	3.2	<.001	25.6	2.7	98.6	1.000	25.8	2.7	99.3	1.000	44.9	2.8	173.1	<.001	51.3	2.6	198.0	<.001
7-15	24.4	1.6	0.085	23.7	1.3	97.1	0.991	25.7	1.3	105.1	0.936	27.2	1.4	111.4	0.495	25.5	1.3	104.3	0.963

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. Mean Daily Gestational Food Consumption for Dams



A3. Data

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

Statistical Analysis of Gestational 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.

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 checking numbers sufficiently to conclude that the tables are correct.

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.