# APPENDIX XII

# Statistical Analysis of Maternal Food Consumption

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

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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 Food Consumption

# 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 effects of oxybenzone on food consumption.

# 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 uterus was to be removed and the 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.

Food consumption was to be measured as least twice weekly.

# 3. Statistical Methods

Summary statistics are presented for mean daily food consumption per dam by treatment. 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, 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. 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.

Food consumption data was collected for gestational ranges GD 4-6, GD 7-10, GD 11-15, GD 16-17, and GD 18-21. Summary statistics for mean food consumption per animal by treatment are given in Table 1.

The ANOVA omnibus test results are given in Table 2 for the null hypothesis that all of the oxybenzone treatment and control means for food consumption are equal. Treatment effect, GD effect, and the interaction were significant ($p<0.001$, $<0.001$, and $=0.013$).

Least squares means comparisons of dosed treatments to the control group are presented in Table 3. In pairwise comparisons of dosed treatments to control, there were significant differences for treatment 30,000 ppm during all GD ranges (all $p<0.001$ except $p=0.020$ for GD 18-21), with the dosed treatment showing overall mean 41.1% higher compared to control. There were significant trends overall and at all GD ranges although there were no other significant pairwise differences.

# 5. Conclusions

There were significant differences for treatment 30,000 ppm compared to the control group, with consistently higher means in the dosed group compared to the control group.

## *A1. Tables*

| *Table 1. Summary Statistics for Mean Daily Food Consumption per Animal (g)* [1] | | | | | |
|---|---|---|---|---|---|
| ***Treatment*** | ***GD*** | ***Days*** | ***N*** | ***Mean*** | ***SE*** |
| CTRL | 4-6 | 3 | 19 | 21.1 | 1.9 |
| | 7-10 | 4 | 19 | 17.7 | 0.5 |
| | 11-15 | 5 | 19 | 20.9 | 0.6 |
| | 16-17 | 2 | 19 | 20.5 | 0.8 |
| | 18-21 | 4 | 19 | 25.5 | 0.9 |
| OXY 3,000 | 4-6 | 3 | 21 | 21.5 | 2.3 |
| | 7-10 | 4 | 21 | 18.5 | 1.7 |
| | 11-15 | 5 | 21 | 20.0 | 0.5 |
| | 16-17 | 2 | 21 | 22.3 | 0.9 |
| | 18-21 | 4 | 21 | 24.0 | 0.9 |
| OXY 10,000 | 4-6 | 3 | 22 | 20.4 | 1.7 |
| | 7-10 | 4 | 22 | 15.7 | 0.5 |
| | 11-15 | 5 | 22 | 18.7 | 0.5 |
| | 16-17 | 2 | 22 | 21.1 | 0.9 |
| | 18-21 | 4 | 22 | 24.3 | 0.9 |
| OXY 30,000 | 4-6 | 3 | 19 | 20.6 | 1.6 |
| | 7-10 | 4 | 19 | 29.6 | 3.0 |
| | 11-15 | 5 | 19 | 32.7 | 3.2 |
| | 16-17 | 2 | 19 | 27.5 | 2.0 |
| | 18-21 | 4 | 19 | 29.6 | 1.4 |

1. Animals were fed control chow until GD 6, dosed chow from GD 6 to GD 15, and control chow from GD 15 until sacrifice at GD 21.

| Table 2. ANOVA Results for Food Consumption | | | | |
|---|---|---|---|---|
| *Effect* | *NumDF* | *DenDF* | *Fvalue* | *P value* |
| Treatment | 3 | 77 | 32.204 | <.001 |
| GD | 3 | 231 | 11.857 | <.001 |
| Treatment*GD | 9 | 231 | 2.393 | 0.013 |

| Table 3. Comparisons of Least Squares Means Food Consumption Across Treatments[1] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Treatment | | | | | | | | | | | | | |
| | CTRL | | | OXY 3,000 | | | | OXY 10,000 | | | | OXY 30,000 | | | |
| *GD*[2] | *Mean* | *SE* | *P value* | *Mean* | *SE* | *Pct* | *P value* | *Mean* | *SE* | *Pct* | *P value* | *Mean* | *SE* | *Pct* | *P value* |
| All | 21.1 | 0.8 | <.001 | 21.2 | 0.8 | 100.3 | 1.000 | 19.9 | 0.8 | 94.3 | 0.569 | 29.8 | 0.8 | 141.1 | <.001 |
| 7-10 | 17.7 | 1.8 | <.001 | 18.5 | 1.7 | 104.4 | 0.978 | 15.7 | 1.7 | 88.3 | 0.728 | 29.6 | 1.8 | 166.7 | <.001 |
| 11-15 | 20.9 | 1.6 | <.001 | 20.0 | 1.5 | 95.8 | 0.961 | 18.7 | 1.5 | 89.4 | 0.619 | 32.7 | 1.6 | 156.2 | <.001 |
| 16-17 | 20.5 | 1.3 | <.001 | 22.3 | 1.2 | 108.9 | 0.596 | 21.1 | 1.2 | 103.1 | 0.967 | 27.5 | 1.3 | 134.5 | <.001 |
| 18-21 | 25.5 | 1.1 | <.001 | 24.0 | 1.0 | 94.2 | 0.626 | 24.3 | 1.0 | 95.5 | 0.777 | 29.6 | 1.1 | 116.1 | 0.020 |

1. All p-values and % are relative to the control group, except p-value for trend shown below control.
2. Animals were fed control chow until GD 6, dosed chow from GD 6 to GD 15, and control chow from GD 15 until sacrifice at GD 21.

## *A3. Data*

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

# Statistical Analysis of 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 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.

# Statistical Report Addendum

Project #: E02187.01
Project Title: Effect of oxybenzone on fertility and early embryonic development in Sprague-Dawley rats (Segment II)
PI: Amy Inselman

Title: Food Consumption Statistical Report Addendum 1
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
Reviewer: Paul Felton, Division of Bioinformatics and Biostatistics

Signatures:

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Statistician Date

[redacted]

Reviewer Date

[redacted]

Team Leader – Statistical Support Group Date

# Food Consumption Statistical Report Addendum 1

## 1. Purpose

Post hoc analyses were performed at the request of the Principle Investigator. The purpose of Addendum 1 is to provide statistical analysis for maternal food consumption using intervals consistent with GD presented for analysis of maternal body weight, i.e., food consumption for GD 14-15 is included in range GD 14-17 instead of GD 10-15.

## 2. Statistical Methods

The ranges for maternal food consumption are GD 6-10, GD 10-14, GD 14-17, and GD 17-21. There are no other changes in endpoints or analysis methods introduced in *Addendum 1*.

## 3. Results

Food consumption data was collected for gestational ranges GD 3-6, GD 6-10, GD 10-14, GD 14-17, and GD 17-21. Summary statistics for mean food consumption per animal by treatment are given in Addendum Table 1.

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

Least squares means comparisons of dosed treatments to the control group are presented in Addendum Table 3. In pairwise comparisons of dosed treatments to control, there were significant differences for oxybenzone 30,000 ppm overall and for GD 6-10, GD 10-14, GD 14-17, and GD 17-21 ($p<0.001$, $p<0.001$, $p=0.004$, and $p=0.020$, respectively), with the dosed treatment showing overall mean 41.8% higher compared to control. There were significant trends overall and at all GD ranges although there were no other significant pairwise differences.

## 4. Conclusions

There were significant differences for oxybenzone 30,000 ppm compared to the control group, with consistently higher means in the dosed group compared to the control group.

These results are completely consistent with the results from the a priori analyses.

# Tables

***Table 1. Summary Statistics for Mean Daily Food Consumption per Animal (g)***

| *Treatment* | *GD* | *Days* | *N* | *Mean* | *SE* |
|---|---|---|---|---|---|
| CTRL | 4-6 | 3 | 19 | 21.1 | 1.9 |
| | 6-10 | 4 | 19 | 17.7 | 0.5 |
| | 10-14 | 4 | 19 | 20.3 | 0.7 |
| | 14-17 | 3 | 19 | 21.4 | 0.7 |
| | 17-21 | 4 | 19 | 25.5 | 0.9 |
| OXY 3,000 | 4-6 | 3 | 21 | 21.5 | 2.3 |
| | 6-10 | 4 | 21 | 18.5 | 1.7 |
| | 10-14 | 4 | 21 | 19.2 | 0.6 |
| | 14-17 | 3 | 21 | 22.6 | 0.8 |
| | 17-21 | 4 | 21 | 24.0 | 0.9 |
| OXY 10,000 | 4-6 | 3 | 22 | 20.4 | 1.7 |
| | 6-10 | 4 | 22 | 15.7 | 0.5 |
| | 10-14 | 4 | 22 | 18.4 | 0.6 |
| | 14-17 | 3 | 22 | 20.7 | 0.7 |
| | 17-21 | 4 | 22 | 24.3 | 0.9 |
| OXY 30,000 | 4-6 | 3 | 19 | 20.6 | 1.6 |
| | 6-10 | 4 | 19 | 29.6 | 3.0 |
| | 10-14 | 4 | 19 | 34.6 | 3.6 |
| | 14-17 | 3 | 19 | 26.7 | 2.0 |
| | 17-21 | 4 | 19 | 29.6 | 1.4 |

***Table 2. ANOVA Results for Food Consumption***

| *Effect* | *NumDF* | *DenDF* | *Fvalue* | *P value* |
|---|---|---|---|---|
| Treatment | 3 | 77 | 28.814 | <.001 |
| GD | 3 | 231 | 12.741 | <.001 |
| Treatment*GD | 9 | 231 | 3.358 | <.001 |

***Table 3. Comparison of Least Square Mean Food Consumption Across Treatments[1]***

| | ***Treatment*** | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***CTRL*** | | | ***OXY 3,000*** | | | | ***OXY 10,000*** | | | | ***OXY 30,000*** | | | |
| ***GD[2]*** | ***Mean*** | ***SE*** | ***P value*** | ***Mean*** | ***SE*** | ***Pct*** | ***P value*** | ***Mean*** | ***SE*** | ***Pct*** | ***P value*** | ***Mean*** | ***SE*** | ***Pct*** | ***P value*** |
| All | 21.2 | 0.9 | <.001 | 21.1 | 0.9 | 99.3 | 0.999 | 19.8 | 0.8 | 93.1 | 0.491 | 30.1 | 0.9 | 141.8 | <.001 |
| 6-10 | 17.7 | 1.8 | <.001 | 18.5 | 1.7 | 104.4 | 0.978 | 15.7 | 1.7 | 88.3 | 0.728 | 29.6 | 1.8 | 166.7 | <.001 |
| 10-14 | 20.3 | 1.8 | <.001 | 19.2 | 1.7 | 94.5 | 0.945 | 18.4 | 1.7 | 90.3 | 0.767 | 34.6 | 1.8 | 170.0 | <.001 |
| 14-17 | 21.4 | 1.2 | 0.001 | 22.6 | 1.1 | 105.8 | 0.781 | 20.7 | 1.1 | 96.9 | 0.955 | 26.7 | 1.2 | 124.9 | 0.004 |
| 17-21 | 25.5 | 1.1 | <.001 | 24.0 | 1.0 | 94.2 | 0.626 | 24.3 | 1.0 | 95.5 | 0.777 | 29.6 | 1.1 | 116.1 | 0.020 |

1. All p-values and % are relative to the control group, except p-value for trend shown below control.
2. Animals were fed control chow until GD 6, dosed chow from GD 6 to GD 15, and control chow from GD 15 until sacrifice at GD 21.