APPENDIX XVII

Statistical Analysis of Fetal Variations/Malformations

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 Fetal Abnormalities
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 Fetal Abnormalities

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 assess potential effects of oxybenzone on fetal abnormalities.

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 to be oxybenzone (OXY) 0 ppm (control), 3,000 ppm, 10,000 ppm, and 30,000 ppm with approximately 25 animals per treatment group.

Date-mated female Sprague-Dawley rats (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 time of sacrifice on GD 21, the uterus was to be removed and the fetuses were to be separated from the placenta, individually identified, weighed, sexed, examined for gross abnormalities and sacrificed by decapitation. Each fetus was to be given a complete fetal evaluation including external, visceral, head and skeletal examinations.

3. Statistical Methods

Skeletal and visceral abnormalities are summarized and analyzed separately. Due to sparce data, counts were combined across sex. Fetal counts were calculated within litter for fetuses with skeletal and visceral abnormalities. Each fetus was counted only once for a specific abnormality.

Skeletal abnormality count data within litter are presented for rib, sternebrae, centra, and other abnormality. The total count of skeletal abnormalities in each litter was calculated

as the sum of rib, sternebrae, centra, and other abnormalities. Counts for any malformation were calculated as the number of fetuses with one or more abnormality. Counts for each skeletal abnormality, total abnormalities, and number of malformed fetuses in each litter were analyzed using Poisson regression with terms for treatment and covariate number of live fetuses.

Visceral abnormality count data within litter are presented for cleft palate, hydroureter, malformed testes, and other abnormality. The total count of visceral abnormalities in each litter was calculated as the sum of cleft palate, hydroureter, malformed testes, and other abnormalities. Counts for hydroureter and total abnormalities in each litter were analyzed using Poisson regression with terms for treatment and covariate number of live fetuses. Due to sparse data for cleft palate, malformed testes, and other abnormality counts, analysis was not performed.

Comparisons were performed with Dunnett's method for adjusted contrasts for treatment groups versus the control group. Tests were conducted as two-sided at the 0.05 significance level

4. Results

Tables are presented in appendix A1.

4.1 Analyses of Skeletal Abnormalities

Summary statistics are given by treatment group in Table 1 for total counts of fetuses with skeletal abnormalities. In Table 2, the mean number of abnormalities per litter is presented for each treatment, calculated as the sum of abnormalities in the treatment divided by the total number of litters. The mean percentage of live fetuses with skeletal abnormalities in the litter is presented by treatment in Table 3.

Comparisons of treatments to the control group for skeletal abnormalities are presented in Table 7 (estimated counts from the analysis correspond to the summary statistics in Table 2). There was a significant trend for rib abnormalities (p=0.012), but no treatment was significantly different from the control group. There were no statistically significant differences for any treatment compared to control for skeletal abnormalities.

4.2 Analysis of Visceral Abnormalities

Summary statistics are given by treatment group in Table 4 for total counts of fetuses with visceral abnormalities. Of 78 total visceral abnormalities, 84.6% were hydroureter. In Table 5, the mean number of abnormalities per litter is presented for each treatment, calculated as the sum of abnormalities in the treatment divided by the total number of litters. The mean percentage of live fetuses with visceral abnormalities in the litter is presented by treatment in Table 6.

Results of pairwise comparisons of treatments to control are presented for total and hydroureter abnormalities in Table 8 (estimated counts from the analysis correspond to the summary statistics in Table 5). There was a significant trend for total and for

hydroureter abnormalities (p=0.020 and =0.010, respectively). There were no statistically significant pairwise comparisons of treatments to the control group.

5. Conclusions

There were no significant pairwise differences for treatment groups compared to the control for skeletal or visceral abnormalities.

A1. Tables

Table 1. Skeletal Abnormality Counts across Litters within Treatments[1]							
Treatment	*Litters (N)*	*Total*	*Rib*	*Sternebrae*	*Centra*	*Other*	*Any[2]*
CTRL	19	34	23	5	6	0	31
OXY 3,000	21	60	38	3	18	1	53
OXY 10,000	22	47	25	13	8	1	44
OXY 30,000	19	29	12	6	11	0	27

1. Each abnormality is summed across litters within treatment.
2. Any is defined as the count of fetuses with one or more malformation.

Table 2. Mean Number of Skeletal Abnormalities per Litter[1]						
Treatment	*Litters (N)*	*Total[2]*	*Rib*	*Sternebrae*	*Centra*	*Any[3]*
CTRL	19	1.79	1.21	0.26	0.32	1.63
OXY 3,000	21	2.86	1.81	0.14	0.86	2.52
OXY 10,000	22	2.14	1.14	0.59	0.36	2.00
OXY 30,000	19	1.53	0.63	0.32	0.58	1.42

1. For each treatment, mean was calculated as the sum of abnormalities divided by the number of litters.
2. Total is defined as the sum of rib, sterebrae, centra, and other abnormalities.
3. Any is defined as the number of fetuses with one or more malformations (rib, sterebrae, centra, and other).

Table 3. Mean Litter Percentages[1] of Live Fetuses with Skeletal Abnormalities					
Treatment	*Mean Live Fetuses (N)*	*Rib (%)*	*Sternebrae (%)*	*Centra (%)*	*Any (%)[2]*
CTRL	12.5	9.5	1.8	2.7	12.9
OXY 3,000	13.0	14.0	1.0	6.5	19.3
OXY 10,000	12.3	9.5	5.2	3.0	16.8
OXY 30,000	13.4	4.7	2.4	4.2	10.6

1. For each litter, percentage was calculated as the number of fetuses with abnormalities divided by the number of live fetuses.
2. Any is defined as the number of fetuses with one or more malformation (rib, sterebrae, centra, and other).

Table 4. Visceral Abnormality Counts within Treatments[1]						
Treatment	Litters (N)	Total	Hydroureter	CleftPalate	Malformed Testis	Other
CTRL	19	12	10	0	1	1
OXY 3,000	21	18	14	0	2	2
OXY 10,000	22	20	16	1	1	2
OXY 30,000	19	28	26	2	0	0

1. Each abnormality is summed across litters within treatment.

Table 5. Mean Number of Visceral Abnormalities per Litter[1]			
Treatment	Litters (N)	Total[2]	Hydroureter
CTRL	19	0.00	0.00
OXY 3,000	21	0.00	0.00
OXY 10,000	22	0.05	0.05
OXY 30,000	19	0.32	0.32

1. For each treatment, mean was calculated as the sum of abnormal fetuses divided by the number of litters.
2. Total is defined as the total number of fetuses with malformations (hydroureter, cleft palate, malformed fetuses, and other).

Table 6. Mean Litter Percentages[1] of Live Fetuses with Visceral Abnormalities		
Treatment	Mean Live Fetuses (N)	Hydroureter (%)
CTRL	12.5	4.5
OXY 3,000	13.0	5.3
OXY 10,000	12.3	5.4
OXY 30,000	13.4	9.8

1. For each litter, percentage was calculated as the number of fetuses with abnormalities divided by the number of live fetuses.

Table 7. Poisson Regression Test of Treatment Effect on Number of Fetuses with Skeletal Abnormalities															
	Treatment														
	CTRL			OXY 3,000				OXY 10,000				OXY 30,000			
Analysis[1]	Mean	SE	Trend[2]	Mean	SE	Pct	P	Mean	SE	Pct	P	Mean	SE	Pct	P
Total	1.802	0.309	0.076	2.810	0.365	155.9	0.104	2.169	0.317	120.4	0.734	1.471	0.277	81.6	0.751
Rib	1.216	0.254	0.012	1.798	0.293	147.8	0.327	1.147	0.230	94.3	0.994	0.622	0.181	51.1	0.160
Sternebrae	0.262	0.117	0.574	0.134	0.078	51.3	0.676	0.593	0.166	226.2	0.280	0.280	0.118	106.8	0.999
Centra	0.318	0.130	0.674	0.844	0.200	265.4	0.096	0.369	0.131	116.1	0.981	0.559	0.172	175.9	0.510
Any[3]	1.643	0.295	0.120	2.485	0.343	151.2	0.169	2.030	0.306	123.6	0.680	1.372	0.267	83.5	0.826

1. Analysis of fetal abnormalities was adjusted for litter size.
2. All p-values and % are relative to the control group, except the p-value for trend.
3. Any is defined as the number of fetuses with one or more malformation (rib, sterebrae, centra, and other).

Table 8. Poisson Regression Test of Treatment Effect on Number of Fetuses with Visceral Abnormalities[1]															
	Treatment														
	CTRL			OXY 3,000				OXY 10,000				OXY 30,000			
Analysis	Mean	SE	Trend[2]	Mean	SE	Pct	P	Mean	SE	Pct	P	Mean	SE	Pct	P
Total	0.634	0.183	0.020	0.821	0.195	129.6	0.797	0.920	0.206	145.3	0.571	1.351	0.267	213.3	0.077
Hydroureter	0.526	0.166	0.010	0.632	0.171	120.1	0.933	0.733	0.184	139.3	0.711	1.230	0.256	233.8	0.064

1. Analyses of fetal abnormalities were adjusted for litter size.
2. All p-values and % are relative to the control group, except the p-value for trend.

A2 Data

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

Statistical Analysis of Fetal Abnormality 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.