APPENDIX XIV

Statistical Analysis of Hematology/Clinical Chemistry Parameters

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 Clinical Chemistries
Statistician: Beth Juliar, Division of Bioinformatics and Biostatistics
Reviewer: Paul Felton, Division of Bioinformatics and Biostatistics

Signatures:

[redacted]

Statistician _____ Date

[redacted]

Reviewer _____ Date

[redacted]

Team Leader – Statistical Support Group _____ Date

Statistical Analysis of Clinical Chemistries

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 clinical chemistries and hematology.

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 oxybenzone 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.

The clinical chemistry panel performed on the dams was to include alkaline phosphatase, sorbitol dehydrogenase, total bile acids, BUN (blood urea nitrogen), ALT (alanine aminotransferase), creatinine, total protein, albumin, glucose, creatine kinase, cholesterol and triglycerides. AST (aspartate aminotransferase) was to be measured as an additional marker for liver toxicity. Ten animals from each treatment group were to be randomly selected for this analysis (40 animals total).

3. Statistical Methods

Analysis of variance (ANOVA) was performed to determine the effect of treatment on clinical chemistries using a nonparametric method with midranks (using the average of

left and right ranks for ties) and an unstructured covariance[1]. Comparisons of treatments versus the vehicle control group were performed with Dunnett's method for adjusted contrasts. Tests were conducted as two-sided at the alpha=0.05 level of significance.

Abbreviations

Abbreviations are presented in Table A for hematology and in Table B for clinical chemistries.

Table A.

Abbreviation	Hematology
WBC	white blood cell
NEU	neutrophils
LYM	lymphocytes
MON	monocytes
EOS	eosinophils
BAS	basophils
RBC	red blood cells
HGB	hemoglobin concentration
HCT	hematocrit
MCV	mean corpuscular volume
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
PLT	platelets
PCV	packed cell volume

Table B.

Abbreviation	Clinical Chemistry
SDH	sorbitol dehydrogenase
TBA	total bile acids
ALB	albumin
ALT	alanine aminotransferase
ALP	alkaline phosphatase
AST	aspartate aminotransferase
TRIG	triglycerides
CHOL	cholesterol
TP	total protein
CK	creatine kinase
CREAT	creatinine
BUN	blood urea nitrogen
GLU	glucose

4. Results

Tables are presented in appendix A1.

Summary statistics for clinical chemistries 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 are equal. There was a significant treatment effect for RBC, HCT, PLT, TBA, and CHOL (all $p<0.05$).

Least square mean comparisons of oxybenzone treatments to the control group are presented in Table 3. In pairwise comparisons of treatment 3,000 ppm oxybenzone to control, there were significant differences for HGB and HCT (p=0.039 and =0.026, respectively), with lower ranked values in the treated group relative to the control group. Treatment 10,000 ppm oxybenzone differed significantly compared to control for HCT (p=0.042), with relatively lower values in the treatment group. For the 30,000 ppm oxybenzone treatment, there were significant differences for RBC, HGB, HCT, PCV, and BUN, with lower values in the treated group relative to control (p=0.012, =0.036, =0.014, =0.024, and =0.039, respectively). There were significant pairwise comparisons for PLT, TBA, and CHOL, with higher values for 30,000 ppm oxybenzone compared to control (p=0.009, =0.001, and =0.017, respectively). There were significant trends for RBC, PLT, TBA, CHOL, and BUN (all $p<0.05$), but only the high oxybenzone dose differed from the control in pairwise comparisons.

5. Conclusions

In pairwise comparisons, all oxybenzone treatments differed from control for HCT. There was a significant difference for treatment 3,000 ppm oxybenzone compared to control for HGB. For the 30,000 ppm oxybenzone treatment, there were significant pairwise comparisons to control for RBC, HGB, PLT, PCV, TBA, CHOL, and BUN.

A1. Tables

Table 1. Summary Statistics for Clinical Chemistries													
		Treatment											
		CTRL			*OXY 3,000*			*OXY 10,000*			*OXY 30,000*		
Chemistry	***Unit***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***
WBC	$10^3/mm^3$	10	4.09	0.56	10	2.92	0.31	10	3.58	0.47	10	3.46	0.57
NEU	%	10	32.48	2.34	10	39.70	2.02	10	36.89	2.62	10	36.09	1.86
LYM	%	10	53.25	2.94	10	46.51	2.35	10	48.19	2.74	10	47.45	1.95
MON	%	10	13.45	0.97	10	13.18	1.03	10	13.98	0.96	10	15.65	1.19
EOS	%	10	0.67	0.09	10	0.47	0.09	10	0.81	0.16	10	0.66	0.09
BAS	%	10	0.15	0.02	10	0.14	0.02	10	0.13	0.02	10	0.15	0.02
NEU	$10^3/mm^3$	10	1.30	0.16	10	1.13	0.11	10	1.31	0.21	10	1.22	0.19
LYM	$10^3/mm^3$	10	2.22	0.36	10	1.38	0.19	10	1.78	0.30	10	1.67	0.31
MON	$10^3/mm^3$	10	0.54	0.08	10	0.38	0.05	10	0.47	0.04	10	0.53	0.09
EOS	$10^3/mm^3$	10	0.03	0.01	10	0.02	0.00	10	0.03	0.01	10	0.03	0.01
BAS	$10^3/mm^3$	10	0.01	0.00	10	0.01	0.00	10	0.00	0.00	10	0.00	0.00
RBC	$10^6/mm^3$	10	6.30	0.09	10	6.01	0.11	10	6.01	0.10	10	5.97	0.06
HGB	g/dL	10	12.34	0.15	10	11.72	0.27	10	11.84	0.18	10	11.73	0.15
HCT	%	10	34.98	0.41	10	33.08	0.77	10	33.37	0.52	10	33.16	0.50
MCV	μm^3	10	55.60	0.45	10	55.10	0.53	10	55.60	0.31	10	55.70	0.45
MCH	pg	10	19.57	0.15	10	19.51	0.17	10	19.70	0.13	10	19.68	0.10
MCHC	g/dL	10	35.23	0.08	10	35.44	0.14	10	35.47	0.09	10	35.42	0.14
PLT	$10^3/mm^3$	10	1044.0	29.6	10	1113.8	24.6	10	1087.6	44.2	10	1233.2	51.3
PCV	%	10	35.00	0.45	10	33.30	0.86	10	33.60	0.53	10	33.15	0.51
SDH	U/L	10	7.73	1.88	10	7.61	2.15	10	7.39	1.10	10	11.04	3.35
TBA	µmol/L	10	42.24	7.86	10	69.29	13.78	10	62.28	21.45	10	90.07	9.64
ALB	g/dL	10	2.97	0.07	10	2.87	0.06	10	2.97	0.07	10	2.85	0.10
ALT	U/L	10	56.80	3.33	10	52.90	3.50	10	49.70	3.00	10	58.30	2.91
ALP	U/L	10	111.50	11.40	10	90.30	8.31	10	94.20	10.17	10	96.40	8.32
AST	U/L	10	78.30	4.91	10	80.10	7.05	10	78.40	5.44	10	88.70	10.20
TRIG	mg/dL	10	194.50	44.41	10	198.60	51.14	10	172.50	30.42	10	277.10	68.12
CHOL	mg/dL	10	119.90	4.19	10	116.40	3.73	10	123.20	3.71	10	138.30	4.13
TP	g/dL	10	5.63	0.11	10	5.38	0.09	10	5.49	0.13	10	5.28	0.14
CK	U/L	10	263.50	34.74	10	203.80	33.10	10	199.70	39.57	10	255.40	50.02
CREAT	mg/dL	10	0.58	0.04	10	0.53	0.04	10	0.55	0.02	10	0.51	0.04
BUN	mg/dL	10	17.50	0.78	10	16.10	0.71	10	18.70	1.27	10	15.60	0.60
GLU	mg/dL	10	92.80	2.71	10	84.20	5.24	10	100.90	5.89	10	84.30	5.60

Table 2. ANOVA Results for Clinical Chemistries				
Clinical Chemistry	***NumDF***	***DenDF***	***Fvalue***	***P value***
WBC	3	34	0.723	0.540
NEU (%)	3	35	1.931	0.143
LYM (%)	3	35	1.055	0.380
MON (%)	3	36	1.278	0.297
EOS (%)	3	35	1.399	0.260
BAS (%)	3	36	0.080	0.970
NEU	3	34	0.159	0.918
LYM	3	35	1.336	0.278
MON	3	32	1.014	0.395
EOS	3	31	0.845	0.470
BAS	3	35	0.800	0.500
RBC	3	33	2.950	0.049
HGB	3	33	2.771	0.059
HCT	3	32	3.355	0.034
MCV	3	34	0.342	0.786
MCH	3	33	0.361	0.772
MCHC	3	33	0.900	0.446
PLT	3	34	3.715	0.022
PCV	3	34	2.723	0.062
SDH	3	33	0.126	0.937
TBA	3	29	4.239	0.017
ALB	3	33	0.832	0.480
ALT	3	35	1.624	0.202
ALP	3	33	0.810	0.492
AST	3	36	0.097	0.960
TRIG	3	36	0.500	0.683
CHOL	3	36	4.764	0.007
TP	3	34	1.204	0.322
CK	3	35	1.781	0.170
CREAT	3	33	0.913	0.441
BUN	3	31	2.732	0.065
GLU	3	34	2.411	0.085

Table 3. Comparison of Least Square Mean Clinical Chemistries Across Treatments							
	Trend	*OXY 3,000*		*OXY 10,000*		*OXY 30,000*	
Clinical Chemistry	*Pvalue*[1]	*Pct*[2]	*Pvalue*	*Pct*	*Pvalue*	*Pct*	*Pvalue*
WBC	0.727	69.0	0.263	87.7	0.904	80.1	0.717
NEU (%)	0.796	185.4	0.057	156.1	0.336	144.3	0.465
LYM (%)	0.411	69.2	0.336	71.3	0.357	73.0	0.369
MON (%)	0.070	95.6	0.997	109.3	0.975	145.6	0.281
EOS (%)	0.452	64.0	0.236	106.6	0.987	100.5	1.000
BAS (%)	0.970	99.0	1.000	90.5	0.955	100.0	1.000
NEU	0.840	84.0	0.810	92.7	0.980	88.6	0.947
LYM	0.601	61.1	0.097	78.9	0.600	74.1	0.459
MON	0.669	64.7	0.209	93.7	0.983	95.0	0.994
EOS	0.574	69.0	0.234	97.3	0.999	98.9	1.000
BAS	0.661	100.0	1.000	73.3	0.362	91.1	0.947
RBC	0.041	62.8	0.098	60.8	0.054	56.3	0.012
HGB	0.161	56.0	0.039	66.0	0.096	61.3	0.036
HCT	0.100	54.7	0.026	63.5	0.042	57.9	0.014
MCV	0.605	82.0	0.839	102.1	0.999	104.5	0.996
MCH	0.427	95.0	0.997	119.4	0.828	116.0	0.867
MCHC	0.500	136.3	0.588	153.1	0.166	137.9	0.557
PLT	0.008	152.2	0.225	130.8	0.701	211.2	0.009
PCV	0.096	58.2	0.055	68.4	0.133	58.2	0.024
SDH	0.704	90.5	0.973	102.7	0.999	105.8	0.994
TBA	0.001	159.6	0.143	114.9	0.962	207.1	0.001
ALB	0.379	77.8	0.571	97.9	0.999	71.8	0.521
ALT	0.611	83.2	0.767	60.2	0.148	101.9	1.000
ALP	0.626	69.4	0.334	76.1	0.584	78.7	0.590
AST	0.625	103.1	0.999	101.0	1.000	113.2	0.936
TRIG	0.351	92.4	0.984	89.3	0.950	118.3	0.829
CHOL	0.001	84.0	0.884	114.5	0.903	178.2	0.017
TP	0.188	73.0	0.322	84.1	0.781	63.8	0.230
CK	0.744	66.3	0.207	61.4	0.157	90.1	0.910
CREAT	0.202	80.4	0.720	93.3	0.972	67.9	0.327
BUN	0.018	71.5	0.337	102.4	0.999	55.4	0.039
GLU	0.502	61.8	0.173	110.5	0.907	72.3	0.415

1. Dunnett adjusted p-values and percent are relative to the control except p-value for trend.
2. Treatment percent of control is based on least square means from analysis of ranked data.

A2. Data

Clinical chemistries data were provided in an Excel spreadsheet from the Principle Investigator.

Statistical Analysis of Clinical Chemistries 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.