APPENDIX XXIII

Statistical Analysis of Hematology/Clinical Chemistry Parameters – Females and 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 Clinical Chemistries
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Statistical Analysis of Clinical Chemistries

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 effect of oxybenzone treatment on clinical chemistries.

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 (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 gestation 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.

Hematology and a basic clinical chemistry panel were done on both male and female rats; this panel includes 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 also measured as an additional marker for liver toxicity. Ten animals of each sex and from each treatment group will be randomly selected for this analysis.

3. Statistical Methods

Analysis of variance (ANOVA) was performed separately by sex for each endpoint to determine the effect of treatment on hematology and 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. Test of trend was performed for the oxybenzone and control treatments. Tests were conducted as two-sided at the alpha=0.05 level of significance.

1. *Nonparametric Analysis of Longitudinal Data in Factorial Experiments*, Edgar Brunner, Sebastian Domhof, and Frank Langer, Wiley Series in Probability and Statistics, 2002, ISBN 0-471-44166-X, p. 168.

Abbreviations are presented in Table A for hematology and 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
FSH	follicle stimulating hormone
LH	luteinizing hormone

4. Results

Tables are presented in Appendix 1.

Of 50 randomly selected males, 10 per treatment group, 6 animals were excluded from analysis due to undetermined plug dates (UIN=5A000002436, 5A000002445, 5A000002487, 5A000002489, 5A000002528, and 5A000002538). Instead of being removed after mating, these males remained in the breeding cages after dams' GD 0 and continued to be fed dosed chow after dams' GD 6. Of the 50 randomly selected females, female UIN=5A000002671 was excluded due to undetermined plug date, and female UIN=5A000002650 was excluded because dosed chow was not stopped until GD 8 although the breeding pair male had been removed.

Parametric summary statistics for clinical chemistries by treatment are presented in Table 1 for females and Table 2 for males, and nonparametric statistics are presented in Table 3 for females and Table 4 for males. The limit of quantification (LOQ) for luteinizing hormone was 0.1 ng/ml. Because luteinizing hormone was above the LOQ for only four males (0.1 and 0.2 in the oxybenzone 3000 ppm treatment and 0.2 and 0.3 in the EE_2 ppm treatment), analysis was not performed.

The ANOVA omnibus test results for the null hypothesis that all of the oxybenzone and EE_2 treatment and control means are equal are given for females in Table 5. There were statistically significant treatment effects for NEU %, EOS % and EOS $10^3/mm^3$, LYM, RBC, HGB, SDH, cholesterol, and glucose (p=0.025, =0.027, =0.023, =0.044, =0.027, =0.029, =0.002, =0.001, and =0.026, respectively).

Least squares mean comparisons of the oxybenzone and EE_2 treatments to the control group are presented in Table 6 for females. Treatment percent of control, from nonparametric analysis of ranked data, shows mean treatment rank relative to mean control. There was a significant dose trend for NEU% (p=0.007) and a significant difference for oxybenzone 30,000 ppm compared to control (p=0.003), with treatment mean rank 55.9% lower than control. There were significant dose trends for SDH, AST, CK, creatinine, and glucose (p=0.010, =0.031, =0.022, =0.007, =0.005). Oxybenzone 3000 ppm was significantly different from control for SDH (p=0.003), with treatment mean rank 55.6% lower than control. For SDH, AST and CK, and glucose, there were significant differences for oxybenzone 30,000 ppm compared to control (p=0.002, =0.032, =0.038, and =0.002, respectively). The treatment mean ranks for SDH, AST, and CK were 54.0%, 44.7% and 44.7% lower and the mean rank for glucose was 98.2% higher compared to control. For RBC, HGB, and cholesterol, there were significant differences for the EE_2 ppm group compared to control, with treatment mean ranks 70.7%, 106.6%, and 135.8% higher than control (p=0.027, =0.007, and <0.001, respectively).

The ANOVA omnibus test results are given for males in Table 7. There were statistically significant treatment effects for HGB, HCT, MCV, PCV, TBA, ALT, AST, cholesterol, and TP (p=0.003, =0.004, =0.048, =0.004, <0.001, <0.001, =0.020, =0.049, and =0.035, respectively).

Least squares mean comparisons of the oxybenzone and EE_2 treatments to the control group are presented in Table 8 for males. Treatment percent of control, from nonparametric analysis of ranked data, shows treatment mean rank relative to control mean. There were significant trends for RBC (p=0.027), HGB (p=0.005), HCT (p=0.001), PCV (p=0.001), TBA (p=0.001), AST (p=0.016), cholesterol (p=0.033), and TP (p=0.001). In pairwise comparisons of oxybenzone 3000 ppm to control, there was a significant difference for MCV (p=0.003). For oxybenzone 10,000 ppm compared to control, there was a significant difference for AST (p=0.018). Oxybenzone 30,000 ppm differed from control for HGB, HCT, PCV, and AST (p=0.013, =0.005, =0.004, and =0.018, respectively). Mean ranks of the dosed group were 58.7%, 62.9%, 61.9%, and 54.7% lower than control, respectively. The EE_2 ppm group differed significantly from control for TBA (p=0.007) and ALT (p<0.001), with higher treatment mean ranks relative to control (77.4% greater for TBA and 72.7% greater for ALT).

5. Conclusions

In pairwise comparisons of dosed groups to control for females, oxybenzone 3000 ppm was significantly different for SDH, with the mean treatment rank lower than control. For NEU%, SDH, AST and CK, and glucose, there were significant differences for oxybenzone 30,000 ppm compared to control. The oxybenzone mean treatment ranks for NEU%, SDH, AST, and CK were relatively lower than control and greater for glucose. For RBC, HGB, and cholesterol, there were significant differences for the EE_2 ppm group compared to control, with mean treatment ranks higher than control.

For males, oxybenzone 3000 ppm differed significantly from control for MCV. For oxybenzone 10,000 ppm compared to control, there was a significant difference for AST. Oxybenzone 30,000 ppm differed from control for HGB, HCT, PCV, and AST, with mean treatment ranks lower than control. The EE_2 ppm group differed from control for TBA and ALT, with higher treatment mean rank relative to control.

Appendixes

1. Statistical Tables

Table 1. Parametric Summary Statistics for Female Clinical Chemistries																
		Treatment (ppm)														
		Control			*OXY 3000*			*OXY 10000*			*OXY 30000*			*EE2 0.05*		
Chemistry	*Unit*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*
WBC	$10^3/mm^3$	8	8.1	0.5	10	9.2	0.6	10	8.2	0.6	10	8.6	0.6	10	7.0	0.8
NEU	%	8	28.7	2.0	10	23.4	2.2	10	23.8	1.3	10	20.8	1.2	10	25.5	2.2
LYM	%	8	63.2	2.6	10	66.4	2.6	10	67.4	2.4	10	70.1	1.7	10	65.3	2.5
MON	%	8	7.2	1.2	10	8.9	1.4	10	8.0	1.4	10	7.8	0.9	10	8.2	1.3
EOS	%	8	0.9	0.1	10	1.1	0.1	10	0.8	0.1	10	1.1	0.0	10	0.9	0.1
BAS	%	8	0.11	0.02	10	0.19	0.06	10	0.11	0.02	10	0.13	0.02	10	0.11	0.03
NEU	$10^3/mm^3$	8	2.35	0.26	10	2.13	0.19	10	1.93	0.14	10	1.79	0.15	10	1.88	0.30
LYM	$10^3/mm^3$	8	5.08	0.33	10	6.15	0.48	10	5.59	0.48	10	6.03	0.35	10	4.47	0.44
MON	$10^3/mm^3$	8	0.59	0.11	10	0.83	0.15	10	0.66	0.12	10	0.72	0.12	10	0.61	0.14
EOS	$10^3/mm^3$	8	0.07	0.01	10	0.11	0.01	10	0.06	0.01	10	0.09	0.01	10	0.07	0.01
BAS	$10^3/mm^3$	8	0.01	0.00	10	0.02	0.01	10	0.01	0.00	10	0.01	0.00	10	0.01	0.00
RBC	$10^6/mm^3$	8	6.73	0.09	10	6.86	0.11	10	6.63	0.08	10	6.71	0.09	10	7.05	0.07
HGB	g/dL	8	13.3	0.2	10	13.6	0.2	10	13.3	0.1	10	13.4	0.2	10	14.0	0.1
HCT	%	8	38.3	0.8	10	38.9	0.6	10	37.9	0.4	10	38.4	0.6	10	40.1	0.5
MCV	μm^3	8	57.0	0.5	10	56.6	0.6	10	57.2	0.4	10	57.0	0.6	10	56.9	0.5
MCH	pg	8	19.7	0.1	10	19.9	0.2	10	20.0	0.2	10	20.0	0.2	10	19.8	0.2
MCHC	g/dL	8	34.6	0.3	10	35.1	0.2	10	35.0	0.2	10	35.0	0.1	10	34.9	0.2
PLT	$10^3/mm^3$	8	751.0	21.7	10	735.1	38.9	10	793.2	27.7	10	794.4	36.9	10	795.3	16.8
PCV	%	8	38.5	0.7	10	38.9	0.6	10	38.0	0.4	10	38.7	0.6	10	40.2	0.4
Retic	%	8	3.5	0.1	10	3.3	0.1	10	3.3	0.1	10	3.4	0.1	10	3.3	0.1
SDH	U/L	8	16.6	3.5	10	5.8	1.3	10	12.9	2.2	10	6.0	1.0	10	9.5	1.6
TBA	µmol/L	8	62.2	11.4	10	69.9	5.7	10	69.1	7.9	10	68.3	9.6	10	85.3	8.3
ALB	g/dL	8	3.6	0.1	10	3.6	0.1	10	3.7	0.0	10	3.6	0.0	10	3.6	0.1
ALT	U/L	8	78.0	4.7	10	74.2	4.5	10	72.5	3.9	10	85.1	5.1	10	85.8	3.6
ALP	U/L	8	179.4	13.4	10	160.7	7.9	10	171.2	3.9	10	176.8	6.3	10	167.1	10.5
AST	U/L	8	106.8	8.7	10	105.9	14.1	10	107.7	14.4	10	87.7	6.5	10	103.6	17.7
TRIG	mg/dL	8	162.0	12.5	10	145.2	14.4	10	141.5	11.8	10	148.1	14.9	10	153.7	14.5
CHOL	mg/dL	8	79.8	3.7	10	83.0	3.1	10	83.0	4.1	10	90.2	3.7	10	105.2	4.3
TP	g/dL	8	6.6	0.2	10	6.5	0.2	10	6.6	0.1	10	6.4	0.1	10	6.6	0.1
CK	U/L	8	293.1	51.5	10	353.8	115.0	10	346.7	105.4	10	190.0	17.3	10	357.4	141.7
CREAT	mg/dL	8	0.48	0.02	10	0.44	0.03	10	0.49	0.02	10	0.51	0.01	10	0.50	0.03
BUN	mg/dL	8	20.6	1.2	10	20.3	0.9	10	20.0	0.9	10	21.5	0.5	10	21.3	0.8
GLU	mg/dL	8	137.4	4.9	10	164.3	12.2	10	134.5	7.8	10	170.9	10.2	10	152.2	8.9

Table 2. Parametric Summary Statistics for Male Clinical Chemistries																
		Treatment (ppm)														
		Control			*OXY 3000*			*OXY 10000*			*OXY 30000*			*EE2 0.05*		
Chemistry	*Unit*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*	*N*	*Mean*	*SE*
WBC	$10^3/mm^3$	9	6.6	0.5	10	6.1	0.8	8	8.0	0.8	7	6.8	1.0	10	6.8	0.4
NEU	%	9	14.6	1.5	10	14.0	1.6	8	14.4	1.6	7	12.1	1.1	10	15.6	1.9
LYM	%	9	79.9	1.8	10	80.7	1.8	8	79.4	1.6	7	83.4	1.3	10	79.3	2.0
MON	%	9	4.3	0.4	10	4.4	0.4	8	4.8	0.7	7	3.5	0.7	10	4.1	0.3
EOS	%	9	1.1	0.2	10	0.8	0.2	8	1.2	0.1	7	0.8	0.2	10	0.9	0.1
BAS	%	9	0.10	0.00	10	0.07	0.02	8	0.11	0.02	7	0.13	0.03	10	0.10	0.00
NEU	$10^3/mm^3$	9	0.97	0.12	10	0.86	0.16	8	1.13	0.15	7	0.84	0.18	10	1.07	0.19
LYM	$10^3/mm^3$	9	5.28	0.46	10	4.92	0.68	8	6.38	0.67	7	5.64	0.79	10	5.39	0.33
MON	$10^3/mm^3$	9	0.29	0.04	10	0.27	0.04	8	0.41	0.08	7	0.27	0.07	10	0.28	0.03
EOS	$10^3/mm^3$	9	0.07	0.01	10	0.06	0.02	8	0.11	0.02	7	0.06	0.02	10	0.06	0.01
BAS	$10^3/mm^3$	9	0.01	0.00	10	0.01	0.00	8	0.01	0.00	7	0.01	0.00	10	0.01	0.00
RBC	$10^6/mm^3$	9	8.63	0.10	10	8.77	0.08	8	8.84	0.15	7	8.43	0.10	10	8.79	0.16
HGB	g/dL	9	16.3	0.1	10	16.0	0.1	8	16.3	0.2	7	15.8	0.2	10	16.8	0.3
HCT	%	9	48.5	0.5	10	47.3	0.3	8	48.6	0.8	7	46.2	0.4	10	49.4	0.8
MCV	μm^3	9	56.2	0.6	10	54.1	0.3	8	55.0	0.6	7	54.9	0.9	10	56.4	0.5
MCH	pg	9	18.9	0.3	10	18.3	0.2	8	18.5	0.2	7	18.7	0.3	10	19.1	0.1
MCHC	g/dL	9	33.6	0.3	10	34.0	0.2	8	33.6	0.3	7	34.1	0.0	10	34.1	0.2
PLT	$10^3/mm^3$	9	673.9	29.6	10	654.0	14.2	8	670.5	16.2	7	695.4	24.8	10	638.5	14.5
PCV	%	9	48.7	0.5	10	47.3	0.3	8	48.6	0.9	7	46.4	0.4	10	49.5	0.8
Retic	%	9	1.2	0.1	10	1.3	0.1	8	1.2	0.1	7	1.3	0.1	10	1.3	0.1
SDH	U/L	9	7.6	1.8	10	5.5	1.3	8	8.7	2.2	7	5.0	1.3	10	7.4	2.0
TBA	µmol/L	9	48.3	3.7	10	40.4	1.5	8	45.4	4.6	7	60.1	4.7	10	68.7	4.5
ALB	g/dL	9	3.5	0.0	10	3.5	0.0	8	3.5	0.1	7	3.6	0.1	10	3.5	0.0
ALT	U/L	9	63.1	1.7	10	63.2	2.9	8	55.4	3.6	7	55.3	4.0	10	81.6	3.3
ALP	U/L	9	178.3	8.1	10	181.5	11.4	8	180.1	9.4	7	178.7	21.8	10	210.8	16.0
AST	U/L	9	84.6	4.5	10	76.9	3.1	8	72.5	2.1	7	69.9	2.5	10	84.1	5.0
TRIG	mg/dL	9	107.6	8.2	10	106.7	6.7	8	142.6	13.7	7	115.0	16.9	10	86.7	8.2
CHOL	mg/dL	9	97.7	3.5	10	103.3	4.4	8	108.4	3.4	7	112.3	4.7	10	96.0	4.4
TP	g/dL	9	7.0	0.1	10	6.9	0.1	8	7.0	0.1	7	7.3	0.1	10	7.0	0.1
CK	U/L	9	212.4	38.5	10	143.7	15.1	8	167.1	23.4	7	141.0	9.8	10	201.7	31.2
CREAT	mg/dL	9	0.52	0.03	10	0.46	0.02	8	0.48	0.02	7	0.46	0.02	10	0.47	0.02
BUN	mg/dL	9	19.4	1.1	10	20.3	0.7	8	18.6	1.1	7	21.4	0.9	10	19.8	1.0
GLU	mg/dL	9	177.1	11.9	10	166.5	10.2	8	172.4	12.2	7	170.7	11.5	10	178.4	12.0
Testosterone	ng/ml	9	1.9	0.4	10	2.7	1.2	8	3.6	1.3	7	4.5	2.2	10	3.1	0.7
FSH	ng/ml	9	15.7	1.2	10	16.8	0.9	8	14.8	1.0	7	16.8	0.8	9	16.1	0.7

Table 3. Nonparametric Summary Statistics for Female Clinical Chemistries (ppm)																				
		Control				OXY 3000				OXY 10000				OXY 30000				EE2 0.05		
Chemistry	Unit	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max	N	Median	Min	Max
WBC	$10^3/mm^3$	8	8.2	5.8	10.5	10	9.7	6.1	11.3	10	8.2	4.4	11.0	10	8.9	5.3	11.5	10	7.4	2.9	11.0
NEU	%	8	27.3	20.6	36.1	10	22.1	16.2	40.3	10	21.8	20.5	32.0	10	19.6	17.1	28.5	10	25.5	13.6	36.2
LYM	%	8	62.1	53.8	74.4	10	67.8	52.5	74.4	10	69.0	54.0	74.4	10	69.3	62.9	76.6	10	62.6	55.3	81.5
MON	%	8	5.7	4.0	14.6	10	8.0	4.6	19.5	10	7.6	2.7	15.7	10	7.2	4.6	12.1	10	6.7	4.8	16.8
EOS	%	8	1.0	0.6	1.1	10	1.2	0.6	1.7	10	0.7	0.5	1.1	10	1.1	0.9	1.2	10	0.9	0.1	1.5
BAS	%	8	0.10	0.00	0.20	10	0.10	0.10	0.70	10	0.10	0.00	0.30	10	0.10	0.10	0.20	10	0.10	0.00	0.30
NEU	$10^3/mm^3$	8	2.35	1.19	3.56	10	2.16	1.35	3.29	10	1.90	1.19	2.53	10	1.67	1.23	2.60	10	1.88	0.39	3.71
LYM	$10^3/mm^3$	8	4.85	4.05	6.45	10	6.45	4.05	8.39	10	5.63	2.91	8.09	10	6.25	3.45	7.62	10	4.55	2.34	6.46
MON	$10^3/mm^3$	8	0.53	0.23	1.21	10	0.69	0.39	1.91	10	0.61	0.20	1.26	10	0.64	0.30	1.27	10	0.48	0.14	1.50
EOS	$10^3/mm^3$	8	0.07	0.05	0.12	10	0.10	0.05	0.19	10	0.06	0.04	0.10	10	0.09	0.06	0.14	10	0.07	0.00	0.11
BAS	$10^3/mm^3$	8	0.01	0.00	0.02	10	0.01	0.01	0.08	10	0.01	0.00	0.02	10	0.01	0.01	0.02	10	0.01	0.00	0.02
RBC	$10^6/mm^3$	8	6.68	6.31	7.22	10	6.90	6.47	7.34	10	6.68	6.19	6.92	10	6.71	6.29	7.30	10	7.11	6.69	7.31
HGB	g/dL	8	13.1	12.6	14.2	10	13.5	12.9	15.0	10	13.3	12.7	13.7	10	13.4	12.6	14.7	10	14.0	13.5	14.7
HCT	%	8	37.8	35.8	41.9	10	39.0	35.5	42.7	10	38.2	35.8	39.5	10	38.3	36.1	41.9	10	40.2	37.7	43.0
MCV	μm^3	8	57.0	55.0	59.0	10	56.5	53.0	59.0	10	57.5	55.0	59.0	10	57.0	55.0	61.0	10	57.0	55.0	59.0
MCH	pg	8	19.7	19.2	20.3	10	20.0	18.3	21.0	10	20.0	19.1	20.9	10	20.0	18.7	21.4	10	19.9	19.0	20.7
MCHC	g/dL	8	34.9	33.3	35.8	10	35.0	34.5	36.2	10	35.3	33.2	35.6	10	35.0	34.0	35.6	10	35.2	33.5	35.9
PLT	$10^3/mm^3$	8	748.0	675.0	859.0	10	738.5	557.0	901.0	10	788.0	678.0	911.0	10	815.0	601.0	953.0	10	797.5	702.0	873.0
PCV	%	8	38.0	36.0	42.0	10	39.0	35.5	42.5	10	38.0	35.5	40.0	10	38.3	36.0	42.0	10	40.3	38.0	43.0
Retic	%	8	3.3	3.1	3.9	10	3.5	2.8	3.8	10	3.4	2.8	3.9	10	3.4	2.9	3.9	10	3.2	2.8	3.7
SDH	U/L	8	14.4	7.3	36.6	10	6.8	0.5	10.8	10	13.2	2.0	24.1	10	4.7	1.9	12.2	10	9.8	1.5	19.4
TBA	µmol/L	8	48.3	31.9	115.6	10	75.8	41.3	91.0	10	66.3	36.7	100.9	10	63.6	37.1	146.1	10	78.5	51.1	118.9
ALB	g/dL	8	3.7	3.4	4.0	10	3.7	3.1	3.8	10	3.7	3.5	3.9	10	3.6	3.4	3.8	10	3.7	3.2	3.8
ALT	U/L	8	76.5	62.0	99.0	10	69.5	54.0	95.0	10	71.5	56.0	92.0	10	83.5	64.0	120.0	10	89.0	64.0	98.0
ALP	U/L	8	185.5	123.0	231.0	10	168.5	109.0	187.0	10	166.5	157.0	198.0	10	179.0	144.0	210.0	10	176.5	103.0	211.0
AST	U/L	8	101.0	84.0	149.0	10	89.0	69.0	192.0	10	97.5	72.0	226.0	10	81.5	68.0	142.0	10	86.0	76.0	261.0
TRIG	mg/dL	8	160.0	125.0	205.0	10	139.0	79.0	211.0	10	136.0	100.0	205.0	10	135.5	88.0	256.0	10	148.0	97.0	237.0
CHOL	mg/dL	8	79.0	67.0	95.0	10	85.0	68.0	97.0	10	81.0	65.0	108.0	10	89.5	73.0	108.0	10	106.0	85.0	126.0
TP	g/dL	8	6.7	6.1	7.5	10	6.7	5.5	7.2	10	6.7	6.2	6.9	10	6.5	6.0	6.9	10	6.7	5.9	7.1
CK	U/L	8	255.0	172.0	632.0	10	199.0	109.0	1272.0	10	235.0	117.0	1224.0	10	160.0	139.0	295.0	10	235.5	144.0	1626.0
CREAT	mg/dL	8	0.50	0.40	0.50	10	0.50	0.30	0.50	10	0.50	0.40	0.60	10	0.50	0.50	0.60	10	0.50	0.40	0.70
BUN	mg/dL	8	21.0	15.0	26.0	10	20.0	15.0	24.0	10	19.5	17.0	25.0	10	21.5	19.0	25.0	10	21.5	17.0	26.0
GLU	mg/dL	8	136.0	118.0	161.0	10	153.0	117.0	254.0	10	135.5	95.0	169.0	10	164.0	138.0	251.0	10	149.5	112.0	191.0

Table 4. Nonparametric Summary Statistics for Male Clinical Chemistries (ppm)																				
		Control				*OXY 3000*				*OXY 10000*				*OXY 30000*				*EE2 0.05*		
Chemistry	*Unit*	*N*	*Median*	*Min*	*Max*	*N*	*Median*	*Min*	*Max*	*N*	*Median*	*Min*	*Max*	*N*	*Median*	*Min*	*Max*	*N*	*Median*	*Min*	*Max*
WBC	$10^3/mm^3$	9	6.2	3.6	9.3	10	5.6	3.3	10.2	8	7.8	4.0	11.4	7	5.7	4.3	11.9	10	6.3	5.3	9.0
NEU	%	9	12.7	10.6	23.9	10	12.7	9.1	22.4	8	14.4	9.0	22.5	7	11.6	8.3	15.5	10	15.7	8.8	30.1
LYM	%	9	80.9	70.0	86.2	10	82.6	72.1	86.7	8	79.2	71.8	86.3	7	82.3	78.6	88.1	10	80.5	65.2	86.7
MON	%	9	3.8	3.0	7.2	10	4.2	2.9	6.4	8	4.9	2.1	8.0	7	2.5	1.8	6.4	10	3.9	2.7	5.6
EOS	%	9	1.1	0.1	1.7	10	1.0	0.0	1.5	8	1.4	0.4	1.6	7	0.7	0.2	1.7	10	0.9	0.2	1.5
BAS	%	9	0.10	0.10	0.10	10	0.10	0.00	0.10	8	0.10	0.00	0.20	7	0.10	0.00	0.20	10	0.10	0.10	0.10
NEU	$10^3/mm^3$	9	0.91	0.38	1.52	10	0.83	0.34	2.09	8	1.01	0.71	1.74	7	0.73	0.48	1.85	10	0.94	0.56	2.69
LYM	$10^3/mm^3$	9	5.01	3.13	7.89	10	4.49	2.55	8.64	8	6.34	3.15	9.06	7	4.68	3.76	9.38	10	5.16	4.14	7.62
MON	$10^3/mm^3$	9	0.28	0.11	0.52	10	0.24	0.10	0.45	8	0.40	0.08	0.87	7	0.14	0.08	0.55	10	0.31	0.16	0.36
EOS	$10^3/mm^3$	9	0.08	0.00	0.10	10	0.07	0.00	0.15	8	0.11	0.02	0.17	7	0.04	0.01	0.13	10	0.06	0.01	0.09
BAS	$10^3/mm^3$	9	0.01	0.00	0.01	10	0.01	0.00	0.01	8	0.01	0.00	0.02	7	0.01	0.00	0.02	10	0.01	0.01	0.01
RBC	$10^6/mm^3$	9	8.64	8.10	9.00	10	8.83	8.33	9.06	8	8.83	8.01	9.34	7	8.40	8.09	8.90	10	8.71	8.21	9.89
HGB	g/dL	9	16.2	15.9	17.3	10	16.1	15.6	16.6	8	16.4	15.4	17.0	7	15.9	15.1	16.2	10	16.8	15.8	18.6
HCT	%	9	49.1	46.3	50.5	10	47.3	45.5	48.9	8	48.6	44.4	52.3	7	46.4	44.2	47.6	10	48.7	45.4	53.9
MCV	μm^3	9	57.0	52.0	58.0	10	54.5	52.0	55.0	8	55.0	52.0	57.0	7	55.0	52.0	58.0	10	56.5	55.0	59.0
MCH	pg	9	19.3	17.7	19.9	10	18.2	17.5	19.1	8	18.5	17.6	19.4	7	19.0	17.7	19.6	10	19.2	18.6	19.7
MCHC	g/dL	9	34.2	32.5	34.5	10	34.3	32.3	34.9	8	33.6	32.4	34.7	7	34.1	34.0	34.3	10	34.3	32.8	34.8
PLT	$10^3/mm^3$	9	661.0	563.0	822.0	10	645.0	578.0	742.0	8	669.5	597.0	724.0	7	676.0	618.0	792.0	10	617.5	594.0	721.0
PCV	%	9	49.0	46.0	50.5	10	47.5	45.5	48.5	8	48.3	44.0	52.5	7	46.5	44.5	47.5	10	48.8	45.5	54.0
Retic	%	9	1.2	0.8	1.7	10	1.4	0.9	1.6	8	1.2	0.8	1.6	7	1.1	1.0	1.7	10	1.4	0.8	1.8
SDH	U/L	9	6.9	1.4	15.4	10	4.2	2.0	15.9	8	9.4	1.4	17.3	7	4.4	0.9	10.6	10	6.9	1.2	21.6
TBA	µmol/L	9	47.7	35.1	65.9	10	42.1	33.1	46.4	8	41.4	31.0	68.1	7	59.6	45.4	81.4	10	65.7	53.1	94.2
ALB	g/dL	9	3.5	3.4	3.6	10	3.5	3.3	3.6	8	3.6	3.2	3.8	7	3.7	3.4	3.9	10	3.6	3.3	3.7
ALT	U/L	9	62.0	56.0	71.0	10	62.5	49.0	81.0	8	55.0	45.0	68.0	7	54.0	44.0	77.0	10	82.5	65.0	97.0
ALP	U/L	9	173.0	150.0	215.0	10	181.0	131.0	229.0	8	179.5	133.0	216.0	7	154.0	131.0	290.0	10	196.0	149.0	314.0
AST	U/L	9	79.0	72.0	114.0	10	75.0	65.0	98.0	8	72.5	66.0	85.0	7	72.0	61.0	78.0	10	81.0	65.0	116.0
TRIG	mg/dL	9	104.0	85.0	165.0	10	108.5	73.0	149.0	8	143.0	102.0	215.0	7	113.0	70.0	195.0	10	90.0	44.0	126.0
CHOL	mg/dL	9	95.0	82.0	112.0	10	102.5	90.0	136.0	8	108.5	91.0	119.0	7	111.0	91.0	125.0	10	92.0	80.0	120.0
TP	g/dL	9	7.1	6.5	7.3	10	6.9	6.6	7.4	8	7.1	6.4	7.4	7	7.2	7.1	7.5	10	7.0	6.7	7.3
CK	U/L	9	170.0	111.0	432.0	10	124.5	101.0	234.0	8	154.5	105.0	321.0	7	144.0	109.0	167.0	10	181.0	101.0	395.0
CREAT	mg/dL	9	0.50	0.40	0.60	10	0.50	0.40	0.50	8	0.50	0.40	0.50	7	0.50	0.40	0.50	10	0.50	0.40	0.60
BUN	mg/dL	9	20.0	14.0	25.0	10	20.5	17.0	23.0	8	18.5	15.0	23.0	7	21.0	18.0	25.0	10	21.0	14.0	23.0
GLU	mg/dL	9	186.0	123.0	244.0	10	155.0	139.0	243.0	8	170.0	117.0	230.0	7	163.0	130.0	217.0	10	185.0	122.0	234.0
Testosterone	ng/ml	9	1.8	0.6	5.1	10	1.3	0.7	13.0	8	2.5	1.0	12.5	7	1.5	1.1	15.9	10	2.6	0.8	7.3
FSH	ng/ml	9	14.4	13.1	24.2	10	16.3	13.3	22.7	8	13.9	11.8	20.6	7	16.4	14.1	19.2	9	15.5	14.3	20.9

Table 5. ANOVA Results for Female Clinical Chemistries				
Clinical Chemistry	***NumDF***	***DenDF***	***Fvalue***	***P value***
WBC	4	42	1.299	0.286
NEU (%)	4	40	3.184	0.025
LYM (%)	4	41	1.299	0.287
MON (%)	4	39	0.233	0.907
EOS (%)	3	36	3.265	0.027
BAS (%)	4	38	0.832	0.507
NEU	4	38	1.024	0.404
LYM	4	41	2.717	0.044
MON	4	41	0.566	0.685
EOS	4	38	3.271	0.023
BAS	4	37	1.328	0.279
RBC	4	38	3.194	0.027
HGB	3	32	3.262	0.029
HCT	3	33	2.477	0.071
MCV	4	42	0.132	0.969
MCH	4	42	0.727	0.576
MCHC	3	32	0.241	0.892
PLT	4	38	0.705	0.578
PCV	4	34	2.336	0.083
Retic	4	39	0.290	0.877
SDH	4	40	5.103	0.002
TBA	3	32	1.089	0.373
ALB	4	39	0.196	0.933
ALT	4	39	1.790	0.152
ALP	3	29	0.512	0.690
AST	4	41	1.501	0.222
TRIG	4	42	0.337	0.848
CHOL	4	39	5.737	0.001
TP	4	36	0.507	0.714
CK	4	39	1.253	0.305
CREAT	4	38	1.523	0.219
BUN	4	36	0.538	0.694
GLU	4	39	3.223	0.026

Table 6. Comparison of Least Squares Mean Female Clinical Chemistries Across Treatments (ppm)									
	Trend	***OXY 3000***		***OXY 10000***		***OXY 30000***		***EE2 0.05***	
Clinical Chemistry	***Pval[1]***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***
WBC	0.834	140.2	0.437	107.0	0.997	121.6	0.857	79.8	0.891
NEU (%)	0.007	59.7	0.080	72.1	0.182	44.1	0.003	80.7	0.662
LYM (%)	0.054	136.0	0.715	142.9	0.545	174.1	0.124	115.9	0.976
MON (%)	0.799	127.8	0.744	107.8	0.998	117.6	0.926	115.5	0.956
EOS (%)	0.157	137.0	0.483	58.9	0.202	137.6	0.170	107.7	0.996
BAS (%)	0.828	125.8	0.646	91.1	0.984	114.0	0.917	92.2	0.993
NEU	0.078	86.7	0.911	73.3	0.470	62.9	0.238	70.5	0.509
LYM	0.159	159.7	0.217	131.9	0.704	161.0	0.118	80.3	0.899
MON	0.953	139.3	0.410	107.7	0.998	117.7	0.940	102.3	1.000
EOS	0.407	157.7	0.153	74.2	0.751	140.1	0.371	93.4	0.998
BAS	0.902	138.9	0.293	99.8	1.000	117.8	0.805	92.1	0.994
RBC	0.559	129.1	0.756	82.4	0.905	96.1	1.000	170.7	0.027
HGB	0.996	147.8	0.491	110.5	0.990	119.9	0.950	206.6	0.007
HCT	0.723	124.7	0.844	87.6	0.975	99.8	1.000	169.6	0.086
MCV	0.902	90.3	0.987	107.4	0.995	93.5	0.998	95.7	0.999
MCH	0.405	139.7	0.626	151.6	0.318	143.5	0.521	120.8	0.924
MCHC	0.982	109.6	0.992	127.9	0.787	104.4	1.000	114.2	0.976
PLT	0.283	101.8	1.000	133.8	0.648	135.8	0.678	136.7	0.373
PCV	0.980	117.3	0.940	82.3	0.913	107.0	0.998	165.2	0.094
Retic	0.862	88.8	0.975	86.6	0.945	91.5	0.988	75.0	0.675
SDH	0.010	44.4	0.003	87.7	0.873	46.0	0.002	71.5	0.235
TBA	1.000	135.9	0.690	128.4	0.853	115.6	0.973	169.4	0.221
ALB	0.694	104.5	1.000	117.4	0.909	93.6	0.998	104.9	0.999
ALT	0.196	85.3	0.957	76.8	0.779	123.5	0.791	136.1	0.465
ALP	0.693	69.0	0.524	82.6	0.842	94.9	0.998	85.3	0.936
AST	0.031	73.7	0.482	83.6	0.819	55.3	0.032	70.7	0.270
TRIG	0.582	81.2	0.839	76.4	0.648	81.4	0.790	89.2	0.971
CHOL	0.072	116.5	0.957	115.9	0.973	161.8	0.239	235.8	<.001
TP	0.220	100.2	1.000	108.4	0.990	75.4	0.698	106.4	0.998
CK	0.022	80.5	0.771	83.8	0.875	55.3	0.038	84.4	0.806
CREAT	0.007	81.3	0.830	112.9	0.936	131.9	0.217	113.6	0.929
BUN	0.326	93.3	0.998	81.4	0.908	116.3	0.904	111.4	0.980
GLU	0.005	171.9	0.135	99.4	1.000	198.2	0.002	152.5	0.428

1. Except p-value for trend, Dunnett adjusted p-values and percent are relative to the control.
2. Treatment percent of control, from nonparametric analysis of ranked data, shows treatment mean rank relative to control mean rank.

Table 7. ANOVA Results for Male Clinical Chemistries				
Clinical Chemistry	***NumDF***	***DenDF***	***Fvalue***	***P value***
WBC	3	30	1.047	0.394
NEU (%)	4	38	0.479	0.749
LYM (%)	4	38	1.050	0.394
MON (%)	3	26	0.663	0.596
EOS (%)	4	31	1.664	0.187
BAS (%)	2	16	1.478	0.259
NEU	4	35	1.167	0.342
LYM	4	31	0.811	0.517
MON	3	23	0.695	0.566
EOS	4	30	2.733	0.053
BAS	3	19	1.160	0.346
RBC	4	35	2.064	0.110
HGB	4	34	5.166	0.003
HCT	4	34	4.854	0.004
MCV	3	24	3.020	0.048
MCH	3	28	2.296	0.092
MCHC	3	31	0.473	0.725
PLT	4	33	1.154	0.347
PCV	3	33	4.962	0.004
Retic	4	33	0.598	0.660
SDH	4	34	0.520	0.711
TBA	3	28	7.882	<.001
ALB	3	25	0.786	0.521
ALT	3	25	8.682	<.001
ALP	3	27	0.857	0.487
AST	4	36	3.377	0.020
TRIG	3	25	2.555	0.075
CHOL	4	36	2.676	0.049
TP	3	31	3.080	0.035
CK	4	37	0.901	0.469
CREAT	4	35	1.236	0.313
BUN	4	36	1.006	0.416
GLU	4	36	0.181	0.944
Testosterone	4	33	1.077	0.381
FSH	4	33	1.601	0.200

Table 8. Comparison of Least Squares Mean Male Clinical Chemistries Across Treatments (ppm)									
	Trend	***OXY 3000***		***OXY 10000***		***OXY 30000***		***EE2 0.05***	
Clinical Chemistry	***Pval[1]***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***	***Pct[2]***	***Pval***
WBC	0.892	84.5	0.959	138.0	0.402	91.3	0.996	104.7	0.999
NEU (%)	0.301	89.2	0.980	98.1	1.000	73.0	0.662	105.7	0.998
LYM (%)	0.118	112.7	0.981	92.6	0.997	145.8	0.356	98.9	1.000
MON (%)	0.303	101.9	1.000	114.9	0.962	70.0	0.783	94.5	0.998
EOS (%)	0.443	70.2	0.484	115.3	0.892	70.6	0.618	71.7	0.432
BAS (%)	0.177	74.0	0.171	110.8	0.950	124.8	0.713	100.0	1.000
NEU	0.316	77.0	0.785	112.5	0.968	67.1	0.559	105.7	0.997
LYM	0.940	82.6	0.938	132.0	0.603	96.3	1.000	101.1	1.000
MON	0.795	93.7	0.998	135.6	0.573	89.9	0.996	103.0	1.000
EOS	0.408	76.0	0.693	132.5	0.424	72.5	0.648	68.1	0.215
BAS	0.464	66.6	0.179	106.3	0.996	105.9	0.998	109.5	0.713
RBC	0.027	125.9	0.710	135.4	0.628	61.0	0.406	116.1	0.952
HGB	0.005	68.7	0.284	102.5	1.000	41.3	0.013	128.2	0.497
HCT	0.001	65.1	0.198	105.0	0.998	37.1	0.005	107.7	0.988
MCV	0.680	44.3	0.003	67.2	0.290	67.4	0.455	99.6	1.000
MCH	0.920	55.0	0.117	64.8	0.381	82.8	0.894	111.9	0.925
MCHC	0.943	118.4	0.925	93.6	0.999	108.4	0.988	130.3	0.701
PLT	0.265	89.1	0.977	107.2	0.996	123.1	0.815	70.2	0.618
PCV	0.001	62.6	0.127	103.2	1.000	38.1	0.004	107.9	0.986
Retic	0.999	145.6	0.437	114.9	0.982	119.0	0.965	142.5	0.518
SDH	0.358	85.7	0.918	108.4	0.995	73.6	0.719	93.9	0.998
TBA	0.001	63.5	0.316	83.6	0.942	159.1	0.085	177.4	0.007
ALB	0.156	91.5	0.987	118.1	0.954	143.1	0.557	113.5	0.962
ALT	0.060	98.7	1.000	66.7	0.431	55.3	0.203	172.7	<.001
ALP	0.579	107.6	0.997	108.5	0.993	84.9	0.982	140.4	0.346
AST	0.016	73.5	0.386	53.0	0.018	45.3	0.018	93.0	0.989
TRIG	0.983	102.0	1.000	149.5	0.127	103.4	1.000	63.8	0.354
CHOL	0.033	117.4	0.935	152.8	0.206	171.9	0.099	86.7	0.982
TP	0.001	73.4	0.583	100.5	1.000	152.7	0.066	83.0	0.888
CK	0.534	68.2	0.487	87.9	0.960	74.9	0.646	104.8	0.999
CREAT	0.212	66.5	0.224	76.4	0.500	64.6	0.241	71.4	0.398
BUN	0.191	117.9	0.920	84.1	0.964	142.0	0.479	115.0	0.965
GLU	0.997	81.0	0.851	95.1	0.999	92.3	0.995	100.8	1.000
Testosterone	0.617	98.1	1.000	152.2	0.229	117.8	0.968	141.2	0.462
FSH	0.186	143.4	0.505	85.1	0.983	154.3	0.311	136.3	0.572

1. Except p-value for trend, Dunnett adjusted p-values and percent are relative to the control.
2. Treatment percent of control, from nonparametric analysis of ranked data, shows treatment mean rank relative to control mean rank.

2. Data

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

3. Quality Control

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.

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.