APPENDIX XIX

Statistical Analysis of Vaginal Cytology

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 Vaginal Cytology
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Team Leader – Statistical Support Group Date

Statistical Analysis of Vaginal Cytology Data

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 on vaginal cytology.

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 pairing. 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 mated. Males and females were to be housed individually upon indication of mating (GD 0) until the time of sacrifice.

For vaginal cytology data, collected after completion of quarantine during the first 14 days of dosing prior to pairing, females were to be examined daily by vaginal lavage to determine the characteristics of the estrus cycle.

3. Statistical Methods

Daily vaginal swabs were collected for approximately 2 weeks from the initiation of dosing. Daily swabs were reported as estrus (E), proestrus (P), or diestrus (D).

Analyses were conducted on proportions of days in estrous stages, proportions of animals with abnormal cycles, daily cycling transitions (between normal, extended estrus, and extended diestrus), and cycle length. Pairwise comparison tests were two-sided and all tests were conducted at the 0.05 significance level. Tests for trend were performed for the oxybenzone and control treatments (excluding the EE_2 treatment).

Proportions of days spent in estrus, diestrus, and proestrus for each animal were analyzed using multivariate analysis of variance (MANOVA) using the arcsine-square root data transformation with treatment as the predictor. Dunnett's method of adjustment was performed for multiple comparisons.

For abnormal cycling defined by animal, the Cochran-Armitage method for binomial proportions was used to evaluate the pairwise differences in proportions. The two-sided p-value for the Fisher's exact test is reported for comparisons of dosed groups to control, and the Cochran-Armitage trend test was performed. The endpoints evaluated were any abnormal cycling, extended estrus, extended diestrus, and excessive proestrus. Extended estrus was defined by the principle investigator as more than 2 consecutive days of estrus; extended diestrus was defined as more than 4 consecutive days of diestrus; and excessive proestrus was defined as 2 or more consecutive days of proestrus in a cycle.

Transition matrices of treatment groups were compared to the control group for analyses of normal (N), extended estrus (EX) and extended diestrus (DX) based on the Markov chain model of Girard and Sager (1987). From the first day of estrus, transition to a second day of estrus was defined as normal. Subsequent transitions to estrus were defined as extended estrus. After four consecutive days of diestrus, subsequent transitions to diestrus were defined as extended diestrus. Although abnormal cycling transitions were not defined for proestrus (P), the analysis only considered the transition to the first P as "Normal". Subsequent transitions to P (2nd P or 3rd P, for instance) were treated as missing. Other transitions were defined as normal. Analysis was performed using Markov chains with the chi square statistic to test for differences between dosed groups and control. No adjustment for multiple comparisons was performed.

For analysis of estrous cycle length, cycle days were defined from the first day of estrus in a sequence of stages until the first day of estrus in the following sequence. Pairwise comparisons of means were performed using contrasts within a one-way repeated measures, mixed model to test for treatment effect accounting for animal effect and a compound symmetric correlation structure. Dunnett's method was performed for multiple comparisons adjustment.

References

Girard DM and Sager DB. "*The Use of Markov Chains to Detect Subtle Variation in Reproductive Cycling*." Biometrics 43 (1987): 225-234.

4. Results

Tables are included in Appendix A1 and Figures are presented in Appendix A2.

Summary statistics for daily vaginal swabs are given in Table 1 for the estrous stage data.

Results of analyses to compare stage proportions of dosed treatment groups to the control group are given in Table 2. There was no significant trend and there were no significant pairwise comparisons to control.

Results of vaginal cycling abnormality are presented in Table 3. There was a significant difference for extended diestrus abnormal cycling for EE_2 0.05 ppm compared to the control group (56.0% compared to 24.0% abnormal, p=0.042). There were no other significant results for abnormality.

Counts and percents are given in Table 4 for observed transitions and in Table 5 for expected transitions (assuming no difference between each treatment group and control). Results for pairwise comparisons of treatments to control are given in Table 6. There were no significant results overall for any of the dosed treatment to control comparisons.

In the analysis of estrous cycle length, cycles were considered censored if the last stage was either diestrus or proestrus. Summary and analysis were performed using non-censored cycles. Summary statistics of are given in Table 7. Results of the analysis are given in Table 8. There was no significant trend and there were no significant differences in cycle length for dosed treatments compared to control.

Estrus stages from daily swabs by animal and treatment are displayed in Figure 1.

5. Conclusions

There was a significant difference for diestrus abnormal cycling for EE_2 0.05 ppm compared to the control group (56.0% compared to 25.0% abnormal). There were no other significant results in analysis of stage proportions (diestrus, estrus, and proestrus), vaginal cycling abnormality, stage transitions (extended diestrus, extended estrus, and normal), or cycle length.

Appendices

A1. Statistical Tables

Table 1. Summary Statistics of Estrous Stage			
Treatment	**Estrous Stage**	**Count**	**Percent**
CTRL	Diestrus	216	63.9
	Proestrus	30	8.9
	Estrus	92	27.2
OXY 3,000	Diestrus	233	67.7
	Proestrus	25	7.3
	Estrus	86	25.0
OXY 10,000	Diestrus	220	63.6
	Proestrus	35	10.1
	Estrus	91	26.3
OXY 30,000	Diestrus	239	69.5
	Proestrus	25	7.3
	Estrus	80	23.3
EE2 0.05	Diestrus	236	68.2
	Proestrus	34	9.8
	Estrus	76	22.0

Table 2. MANOVA Comparison of Back-Transformed Least Squares Mean Stage Percents Across Treatments[1]										
	Control		**OXY 3,000**		**OXY 10,000**		**OXY 30,000**		**EE2 0.05**	
Stage (%)	**Mean**	**P Trend**	**Mean**	**Pvalue**	**Mean**	**Pvalue**	**Mean**	**Pvalue**	**Mean**	**Pvalue**
D	65.7	0.426	69.6	0.785	63.9	0.981	70.2	0.692	68.9	0.873
P	6.0	0.451	4.6	0.906	9.0	0.506	4.3	0.799	8.7	0.584
E	25.4	0.574	23.0	0.922	25.8	0.999	22.7	0.875	20.2	0.421

1. P-values were adjusted using Dunnett's method and are relative to the control group, except p-value for trend.
2. Analysis was performed using arcsine-squareroot transformed data.

Table 3. Comparison of Vaginal Cycling Abnormality by Animal Across Treatment Groups[1]															
	Treatment														
	CTRL			*OXY 3,000*			*OXY 10,000*			*OXY 30,000*			*EE2 0.05*		
Status	***Count***	***Pct***	***Trend***	***Count***	***Pct***	***Pvalue***	***Count***	***Pct***	***Pvalue***	***Count***	***Pct***	***Pvalue***	***Count***	***Pct***	***Pvalue***
Diestrus															
Abnormal	6	24.0	0.114	11	44.0	0.232	9	36.0	0.538	11	44.0	0.232	14	56.0	0.042
Normal	19	76.0		14	56.0		16	64.0		14	56.0		11	44.0	
Estrus															
Abnormal	5	20.0	0.115	3	12.0	0.701	3	12.0	0.701	2	8.0	0.417	2	8.0	0.417
Normal	20	80.0		22	88.0		22	88.0		23	92.0		23	92.0	
Proestrus															
Abnormal	1	4.0	0.094	2	8.0	1.000	0	0.0	1.000	0	0.0	1.000	1	4.0	1.000
Normal	24	96.0		23	92.0		25	100.0		25	100.0		24	96.0	
Total															
Abnormal	12	48.0	0.464	15	60.0	0.570	11	44.0	1.000	13	52.0	1.000	16	64.0	0.393
Normal	13	52.0		10	40.0		14	56.0		12	48.0		9	36.0	

1. All p-values are relative to the control group, except p-value for trend; percent are calculated within stage and treatment group.

Table 4. Estrus Stage Transition Counts and Proportions[1] By Treatment								
		To						
Treatment	*From*	*N*	*EX*	*DX*	*Total*	*N %*	*EX %*	*DX %*
CTRL	N	251	5	6	262	95.8	1.9	2.3
	EX	4	2	-	6	66.7	33.3	-
	DX	3	-	36	39	7.7	-	92.3
OXY 3,000	N	257	3	11	271	94.8	1.1	4.1
	EX	3	0	-	3	100.0	0.0	-
	DX	5	-	34	39	12.8	-	87.2
OXY 10,000	N	284	3	9	296	95.9	1.0	3.0
	EX	3	0	-	3	100.0	0.0	-
	DX	3	-	17	20	15.0	-	85.0
OXY 30,000	N	263	2	12	277	94.9	0.7	4.3
	EX	2	0	-	2	100.0	0.0	-
	DX	8	-	27	35	22.9	-	77.1
EE2 0.05	N	260	2	16	278	93.5	0.7	5.8
	EX	2	0	-	2	100.0	0.0	-
	DX	6	-	30	36	16.7	-	83.3

1. From EX to DX and from DX to EX are not possible transitions.

Table 5. Expected Transition Counts and Percentages[1] By Treatment[2]							
		To					
Treatment	***From***	***N***	***EX***	***DX***	***N %***	***EX %***	***DX %***
	N	249.7	3.9	8.4	95.3	1.5	3.2
CTRL	EX	4.7	1.3	0.0	77.8	22.2	0.0
	DX	4.0	0.0	35.0	10.3	0.0	89.7
	N	258.3	4.1	8.6	95.3	1.5	3.2
OXY 3,000	EX	2.3	0.7	0.0	77.8	22.2	0.0
	DX	4.0	0.0	35.0	10.3	0.0	89.7
	N	251.2	3.8	7.0	95.9	1.4	2.7
CTRL	EX	4.7	1.3	0.0	77.8	22.2	0.0
	DX	4.0	0.0	35.0	10.2	0.0	89.8
	N	283.8	4.2	8.0	95.9	1.4	2.7
OXY 10,000	EX	2.3	0.7	0.0	77.8	22.2	0.0
	DX	2.0	0.0	18.0	10.2	0.0	89.8
	N	249.8	3.4	8.7	95.4	1.3	3.3
CTRL	EX	4.5	1.5	0.0	75.0	25.0	0.0
	DX	5.8	0.0	33.2	14.9	0.0	85.1
	N	264.2	3.6	9.3	95.4	1.3	3.3
OXY 30,000	EX	1.5	0.5	0.0	75.0	25.0	0.0
	DX	5.2	0.0	29.8	14.9	0.0	85.1
	N	247.9	3.4	10.7	94.6	1.3	4.1
CTRL	EX	4.5	1.5	0.0	75.0	25.0	0.0
	DX	4.7	0.0	34.3	12.0	0.0	88.0
	N	263.1	3.6	11.3	94.6	1.3	4.1
EE2 0.05	EX	1.5	0.5	0.0	75.0	25.0	0.0
	DX	4.3	0.0	31.7	12.0	0.0	88.0

1. Within each pairwise comparison, expected transition percents are equal for the treatment and the control.
2. From EX to DX and from DX to EX are not possible transitions.

Table 6. Markov Chain Transition Matrix Analysis Results[1]			
Treatment	ChiSq	DF	P-Value
OXY 3,000	3.7	4	0.443
OXY 10,000	3.1	4	0.537
OXY 30,000	7.4	4	0.117
EE2 0.05	7.8	4	0.098

1. Abnormality is determined by differences in observed and expected transitions.

Table 7. Summary of Estrous Cycle Length (Days)														
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
20	4.98	0.39	20	4.50	0.11	24	4.98	0.36	21	5.26	0.35	19	5.03	0.19

1. N includes animals with at least one uncensored cycle.

Table 8. Comparison of Cycle Length (Days) Across Treatments[1]																		
Control			OXY 3,000				OXY 10,000				OXY 30,000				EE2 0.05			
Mean	SE	Trend	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval
4.83	0.23	0.101	4.57	0.23	94.5	0.827	4.81	0.21	99.5	1.000	5.22	0.25	107.9	0.624	5.04	0.24	104.1	0.934

1. All p-values and % are relative to the control group, except p-value for trend.

1

A2. Figures

Figure 1a. Control Daily Vaginal Cytology *(UIN by Sample Day)*

Treatment	UIN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
CTRL	5A000002562	D	D	D	D	E	E	D	D	P	E	E	D	D	P
CTRL	5A000002567	E	D	D	D	E	E	D	D	D	D	D	D	D	D
CTRL	5A000002572	E	E	E	D	D	D	P	E	D	D	P	E	E	D
CTRL	5A000002577	D	D	P	P	E	D	D		E	E	D	D		
CTRL	5A000002582	E	E	D	D		D	E	E	D	D	P	E	E	D
CTRL	5A000002587	D	D	P	E	D	D	D	D	E	D	D	D	P	E
CTRL	5A000002592	D	D	D	D	D	D	D	D	D	D	D	D	E	D
CTRL	5A000002597	E	D	D		P	E	D	D	P	E	E	D	D	P
CTRL	5A000002602	D	D	D	D	D	D	D	D	D	D	D	D	D	
CTRL	5A000002607	E	D	D	D	D	D	D	D	D	D	D	D	E	D
CTRL	5A000002612	D	D	E	D	D	E	E	D	D	P	E	E	D	D
CTRL	5A000002617		P	E	D	D	D	D	E	D	D	D	P	E	D
CTRL	5A000002623		D		D	D	D	E	E	D	D	D	E	E	D
CTRL	5A000002633	D	D	P	D	D	D	E	E	D	D	P	E	E	E
CTRL	5A000002639		D	D	D	P	E	E	D	D	D	E	D	D	D
CTRL	5A000002645	E	E	D	D	D	P	E	D	D	P	E	E	D	D
CTRL	5A000002650	D	D	P	E	E	D	D	E	E	E	E	E	D	E
CTRL	5A000002655	D	D	D	P	D	D	D	D	P	E	D	D	D	P
CTRL	5A000002660	E	D	D	P	E	D	D	D	D	D	D	D	D	D
CTRL	5A000002665		E	E	D	D	E	E	E	D	D	D	E	E	D
CTRL	5A000002671	D	D	D	D	P	E	D	D	E	E	E	D	D	D
CTRL	5A000002676	D	D	E	E	D	D	D	E	D	D	D	D	E	D
CTRL	5A000002682	D	D	D	D	D	D	D	D	D	D	D	D	D	E
CTRL	5A000002687		P	E	D	D	D	E	D	P	E	D	D	P	E
CTRL	5A000002694	E	D	D	D	P	E	D	D	D	E	E	D	D	D

Figure 1b. Oxybenzone 3000 ppm Daily Vaginal Cytology *(UIN by Sample Day)*

Treatment	UIN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
OXY 3,000	5A000002563	E	D	D	D	E	E	D	D	D	E	E	D	D	D
OXY 3,000	5A000002568		E	D	D	P	P	E	E	D	D	D	P	E	D
OXY 3,000	5A000002573	D	D	D	D	D	D	D	D	E	D	D	D	E	D
OXY 3,000	5A000002578	D	E	D	D	D	E	E	D	D	D	E	E	D	D
OXY 3,000	5A000002583	E	D	D	D	D	E	E	D	D	D	D	D	D	D
OXY 3,000	5A000002588	E	E	D	D	D	P	E	D	D	P	E	E	D	D
OXY 3,000	5A000002593	D	D	P	E	D	D	P	E	E	D	D	D	E	E
OXY 3,000	5A000002598	D	D	D	D	D	D	D	D	D	D	D	D		D
OXY 3,000	5A000002603	D	E	E	E	D	D	P	E	D	D	P	E	D	D
OXY 3,000	5A000002608	D	D	D	D	D	D	D	D	D	D	D	D	E	D
OXY 3,000	5A000002613	P	E	E	D	D	D	E	E	E	D	D	D	E	E
OXY 3,000	5A000002618	E	D	D	P	E	E	D	D	D	D	D	D	D	D
OXY 3,000	5A000002624	P	E	D	D		E	D	D	P	E	E	D	D	E
OXY 3,000	5A000002632	D	E	D	D	D	E	E	D	D	D	D	D	D	D
OXY 3,000	5A000002637	D	D	D	D	P	E	E	D	D	D	E	E	D	D
OXY 3,000	5A000002644	D	D	D	D	D	D	P	D	D	P	E	D	D	D
OXY 3,000	5A000002649	D	D	D	D	E	D	D	D	E	E	D	D	D	E
OXY 3,000	5A000002654	D	D	D	D	D	D	D	E	D	D	D	E	E	D
OXY 3,000	5A000002659	D	D	D	E	D	D	P	E	E	D	D	D	E	D
OXY 3,000	5A000002664	E	D	D	D	P	E	D	D	P	E	E	D	D	D
OXY 3,000	5A000002670		D	D	D	D	P	E	D	D	D	D	D	D	D
OXY 3,000	5A000002675		D	D	D	D	P	E	D	D	D	E	E	D	D
OXY 3,000	5A000002680	D	D	D	D	D	D	D	D	E	D	D	P	E	D
OXY 3,000	5A000002686	E	D	D	D	E	E	D	D	E	E	E	D	D	P
OXY 3,000	5A000002692	D	D	D	E	D	D	D	D	D	D	D		P	P

***Figure 1c. Oxybenzone 10,000 ppm Daily Vaginal Cytology** (UIN by Sample Day)*

Treatment	UIN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
OXY 10,000	5A000002564	D	D	P	E	D	D	D	E	E	D	D	D	P	E
OXY 10,000	5A000002569	D	D	D	D	D	D	E	D	D	E	E	D	D	P
OXY 10,000	5A000002574	D	D	E	E	D	D	D	P	E	D	D	D	E	E
OXY 10,000	5A000002579	P	E	D	D	D	D	P	E	D	D	D	D	D	D
OXY 10,000	5A000002584	D	P	E	D	D	P	E	D	D	E	E	E	D	D
OXY 10,000	5A000002589	P	E	D	D	D	E	E	D	D	D	D	D	D	D
OXY 10,000	5A000002594	P	E	D	D	D	E	E	D	D	D	P	E	D	D
OXY 10,000	5A000002599	D	E	D	D	D	P	E	D	D	E	E	E	D	D
OXY 10,000	5A000002604	D	P	E	D	D	D	P	E	D	D	D	D	E	D
OXY 10,000	5A000002609	D	D	D	D	P	E	D	D	D	D	D	D	D	D
OXY 10,000	5A000002614	E	E	D	D	D	E	E	D	D	P	E	E	D	D
OXY 10,000	5A000002619	E	E	D	D	D	P	E	D	D	D	D	E	D	D
OXY 10,000	5A000002625	E	D	D	D	D	D	P	D	D	E	E	E	D	D
OXY 10,000	5A000002630	D	D	P	E	D	D	P	E	E	D	D	D	P	E
OXY 10,000	5A000002636	D	D	E	D	D	D	D	D	D	D	D	D	P	E
OXY 10,000	5A000002643	E	D	D	D	D	P	E	D	D	D	D	D	D	D
OXY 10,000	5A000002648	D	D	D	D	E	D	D	E	E	D	D	P	E	E
OXY 10,000	5A000002653	P	E	D	D		P	E	D	D	D	D	D	D	D
OXY 10,000	5A000002658	E	D	D	D	P	E	D	D	D	E	E	D	D	D
OXY 10,000	5A000002663	E	D	D	D	E	E	D	D	D	D	E	D	D	
OXY 10,000	5A000002669		E	D	D	D	P	E	E	D	D	E	E	D	D
OXY 10,000	5A000002674	D	D	D	P	E	D	D	P	E	E	D	D	E	D
OXY 10,000	5A000002679	P	E	D	D	P	E	E	D	D	D	D	D	D	D
OXY 10,000	5A000002685	D	D	D	D	E	D	D	D	E	D	D	E	E	D
OXY 10,000	5A000002691	D	D	D		P	E	D	D	P	E	E	D	D	P

***Figure 1d. Oxybenzone 30,000 ppm Daily Vaginal Cytology** (UIN by Sample Day)*

Treatment	UIN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
OXY 30,000	5A000002565	D	D	P	E	E	D	D	D	E	E	D	D	D	P
OXY 30,000	5A000002570	D	D	P	E	E	E	D	D	D	E	E	D	D	D
OXY 30,000	5A000002575	D	D	E	D	D	D	E	E	D	D	D	D	E	E
OXY 30,000	5A000002580	D	D	D		E	D	D	D	E	E	D	D	D	D
OXY 30,000	5A000002585	E	E	D	D	D	E	D	D	D	P	E	D	D	D
OXY 30,000	5A000002590	D	E	D	D	D	D	D	D	D	D	D	D	E	E
OXY 30,000	5A000002595	E	D	D	D	D	E	D	D	D	E	E	D	D	D
OXY 30,000	5A000002600	D	D	D	D	D	P	E	D	D	P	E	E	D	D
OXY 30,000	5A000002605	E	E	D	D	D	P	E	D	D	D	D	D	D	D
OXY 30,000	5A000002610		D	D	D	D	D	D	D	D	D	D	D	D	E
OXY 30,000	5A000002615	E	E	D	D	D	D	E	E	D	D	D	E	E	D
OXY 30,000	5A000002620	D	D	D	D	P	E	D	D	D	P	E	D	D	D
OXY 30,000	5A000002626	D	D	D	D	D	D	D	D	D	E	E	D	D	P
OXY 30,000	5A000002629	D	D	D	D	D	D	D	E	E	D	D	D	D	D
OXY 30,000	5A000002635	D	D	D	D	D	D	E	D	D	E	E	D	D	D
OXY 30,000	5A000002642	P	E	D	D	D	P	E	D	D	D	P	E	D	D
OXY 30,000	5A000002647	D	D		P	D	D	D	E	E	D	D	D	P	E
OXY 30,000	5A000002652	D	D	D	D	P	E	D	D	D	P	E	D	D	D
OXY 30,000	5A000002657	D	D	D	D	D	D	D	D	E	E	D	D	D	P
OXY 30,000	5A000002662	P	E	D	D	D		E	D	D	E	E	E	D	D
OXY 30,000	5A000002668	P	E	D	D	D	D	P	E	D	D	D	D	D	D
OXY 30,000	5A000002673	D	D	D	D	D	E	E	D	D	D	P	E	D	D
OXY 30,000	5A000002678	D	D	D	D		E	E	D	D	D	E	E	D	D
OXY 30,000	5A000002684	P	E	D	D	D	D	P	E	D	D	D	P	E	E
OXY 30,000	5A000002690	D	D	D	D	D	D	D		E	D	D	D	D	E

***Figure 1e. EE_2 0.05 ppm Daily Vaginal Cytology** (UIN by Sample Day)*

Treatment	UIN	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EE2 0.05	5A000002566	D	E	D	D	D	P	E	D	D	D	D	D	D	D
EE2 0.05	5A000002571	D	P	E	D	D	D	D	D	D	D	D	D	D	D
EE2 0.05	5A000002576	D	D	D	D	D	P	E	D	D	D	D	D	D	D
EE2 0.05	5A000002581	D	P	E	E	D	D	D		P	E	D	D	D	D
EE2 0.05	5A000002586	E	D	D	P	E	D	D	E	E	E	D	D	D	P
EE2 0.05	5A000002591	D	P	E	D	D	D	D	D	D	D	D	D	D	D
EE2 0.05	5A000002596	P	E	E	D	D	D	E	D	D	D	D	P	E	D
EE2 0.05	5A000002601	D	D	P	E	D	D	D	P	E	E	D	D	E	E
EE2 0.05	5A000002606	D	D	D	D	D	P	E	D	D	D	D	D	D	D
EE2 0.05	5A000002611		E	D	D	D	E	E	D	D	D	D	D	E	E
EE2 0.05	5A000002616	D	D	D	D	P	P	D	D	D	D	D	D	D	D
EE2 0.05	5A000002622	P	E	E	D	E	E	D	D	D	D	D	D	D	D
EE2 0.05	5A000002627	D	D	P	E	E	D	D	D	E	E	D	D	D	E
EE2 0.05	5A000002628	D		D		D	D	E	E	D	D	D	D	E	E
EE2 0.05	5A000002634	E	D	D	D	P	E	D	D	D	D	D	D	D	D
EE2 0.05	5A000002640	P	E	D	D	D	D	E	D	D	D	D	D	D	D
EE2 0.05	5A000002646	P	E	E	D	D	D	P	E	D	D	D	E	E	D
EE2 0.05	5A000002651	D	D	E	E	D	D	D	E	E	D	D	D	P	E
EE2 0.05	5A000002656	D	E	D	D	D	D	P	E	D	D	D	D	D	D
EE2 0.05	5A000002661	E	D	D	D	P	E	D	D	D	P	E	E	D	D
EE2 0.05	5A000002667	D	D	D	E	D	D	E	E	E	D	D	D	P	E
EE2 0.05	5A000002672	D	D	P	D	E	D	D	P	E	E	D	D	D	P
EE2 0.05	5A000002677	D	D	D	D	D	P	E	D	D	D	P	E	D	D
EE2 0.05	5A000002683	E	E	D	D	D	P	E	D	D	D	D	D	E	D
EE2 0.05	5A000002688	D	D	P	D	D	D	D	D	P	E	D	D	D	D

A3. Data

Vaginal cytology data for statistical analysis were extracted on from the Genesis database using SAS Proc SQL, utilizing the Vortex ODBC driver.

Statistical Analysis of Vaginal Cytology 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, and by independent verification of the results.

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 given 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 independent verification of the numerical results.

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.