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TWO YEAR CHRONIC TOXICOLOGY STUDY OF BISPHENOL A (BPA) [CAS # 80-05-7] ADMINISTERED BY GAVAGE TO SPRAGUE-DAWLEY RATS (NCTR) FROM GESTATIONAL DAY 6 UNTIL BIRTH AND DIRECTLY TO F_1 PUPS FROM POSTNATAL DAY (PND) 1; CONTINUOUS AND STOP DOSE (PND 21) EXPOSURES

STATISTICAL REPORT

ANALYSIS OF TIME TO ABERRANT CYCLING DATA

PREPARED
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Statistical Analysis of Time to Aberrant Cycling Data

1. Objectives

1.1 Project Objectives

The goal of this two year chronic study is to characterize the long term toxicity of orally administered BPA, including developmental exposure, in the NCTR Sprague-Dawley (CD) rat over a broad dose range.

1.2 Analysis Objectives

The goal of this analysis is to evaluate the effects of exposure to BPA in Sprague-Dawley rats on vaginal cytology data over two years.

2. Experimental Design

The study design consisted of first generation female and male rats (F_0) for up to 600 mating pairs randomized to treatment groups in 5 loads. The goal of the F_0 matings was to obtain 352 study litters, 50 per dose group for vehicle controls and five BPA dose groups, 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 26 for each of two EE_2 dose groups, 0.05 and 0.5 µg/kg bw/day. Dams were dosed daily from gestation day (GD) 6 until parturition. Dosing was by gavage for F_0 dams and F_1 pups, the second study generation. Litters were culled to 10 pups on PND 1.There were two study dosing arms of F_1 animals, daily continuous dosing to termination, and daily dose stopped at post-natal day (PND) 21. There was a vehicle control group and five BPA groups for each study dosing arm, and EE_2 daily dose groups for the continuous dosing arm only. From the F_1 litters, pups were allocated at weaning, PND 21, to the interim (1 year) and terminal (2 year) sacrifices for the core study. For vehicle and BPA terminal sacrifice groups, there were 50 pups each; for the interim sacrifice and the EE2 terminal sacrifice groups, there were 20-26 pups each. Pups within litter and sex were assigned to different dosing arms and sacrifice times.

Vaginal Cytology Data

Vaginal opening and vaginal cytology data were collected from females in 13 cages randomly selected from each 2 year terminal sacrifice treatment group. Beginning at 16 ± 2 weeks of age, vaginal smears were collected for 14 consecutive days. One month after the 14 day vaginal swab data collection, each animal was monitored monthly for estrus status in five consecutive daily swabs until aberrant cycling, removal for death or morbidity, or terminal sacrifice. The criteria for an aberrant monthly cycle included three or more consecutive days of estrus (E, E/D or P/E) or five consecutive days that did not include an E. Aberrant cycling was defined as two consecutive months of aberrant estrus data.

3. Statistical Methods

Statistical analyses were performed separately for the BPA study arms, stop dose and continuous dose, and for the EE_2 continuous dose. Because pups within litter and sex were assigned to different dosing arms and sacrifice times, litter correlation is not a consideration for this study. Pairwise comparison tests were two-sided and all tests were conducted at the 0.05 significance level.

An accelerated failure time model assuming a lognormal distribution was used for onset of aberrant cycling, defined as occurring at first swab date of two consecutive months of aberrant estrus data. Accelerated failure time models data that is left, interval, or right censored. Multiple comparisons were adjusted using Holm's (step-down Bonferroni) method for treatment comparisons to the control. Kaplan-Meier survival curves are presented.

For the accelerated failure time model, a sensitivity analysis was also performed. During initial preweaning of animals, 93 core study females, later randomized to vaginal cytology data collection (16 in vehicle control, 61 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 16 in EE_2 µg/kg bw/day dose groups), were held in the same rooms as a special BPA 250,000 µg/kg bw/day high dose requested by an academic laboratory. In consultation with the Principal Investigator, to address the possibility of inadvertent exposure of the core study animals, a sensitivity analysis excluding these females was also performed to test the robustness of the results. Additional statistically significant pairwise comparisons from the sensitivity analysis are reported in the text.

4. Results

Results of analyses are presented in Tables (Appendix A) and in Figures (Appendix B).

4.1 BPA Treatments Stop-Dose Arm

Summary statistics of censoring for the accelerated failure time analysis are presented in Table 1 for the BPA stop dose arm. Estimates of median post-natal weeks to onset of aberrant cycling using the Kaplan-Meier Product Limit method are presented in Table 2.

Results of the accelerated failure time model are presented in Table 3 for the BPA stop dose. BPA stop dose 2500 µg/kg bw/day differed significantly from the control group (p=0.028) with longer time to aberrant cycling onset for the BPA stop dose group compared to control (56.9 compared to 41.9 weeks). In the sensitivity analysis for the BPA stop dose arm, there were no additional statistically significant results.

4.2 BPA Treatments Continuous Dose Arm

Data collection was terminated erroneously for one animal in the BPA 2500 µg/kg bw/day group (UIN=23000530792) due a protocol deviation; the animal was analyzed as right censored in the accelerated failure time model.

Summary statistics of censoring for the accelerated failure time analysis are presented in Table 4 for the BPA continuous dose arm. Estimates of median post-natal weeks to onset of aberrant cycling using the Kaplan-Meier Product Limit method are presented in Table 5.

Results of the accelerated failure time model are presented in Table 6 for the BPA continuous dose. There were no statistically significant differences for any treatment group compared to control. In the sensitivity analysis for the BPA continuous dose arm, there were no statistically significant results.

4.3 EE_2 Treatments Continuous Dose

Summary statistics of censoring for the accelerated failure time analysis are presented in Table 7 for the EE_2 continuous dose. Estimates of median post-natal weeks to onset of aberrant cycling using the Kaplan-Meier Product Limit method are presented in Table 8.

Results of the accelerated failure time model are presented in Table 9 for the EE_2 dose. EE_2 0.5 µg/kg bw/day differed significantly from the control group ($p<0.001$) with shorter time to aberrant cycling onset for the EE_2 dose group compared to control (21.9 compared to 56.8 weeks). In the sensitivity analysis for the EE_2 continuous dose, there were no additional statistically significant results.

5. Conclusions

5.1 BPA Treatments Stop-Dose Arm

BPA stop dose 2500 µg/kg bw/day differed significantly from the control group with longer time to aberrant cycling onset for the BPA stop dose group compared to control.

5.2 BPA Treatments Continuous Dose Arm

There were no statistically significant differences for treatments in the BPA continuous dose arm compared to the vehicle control.

5.3 EE_2 Treatments Continuous Dose

EE_2 0.5 µg/kg bw/day differed significantly from the control group with shorter time to aberrant cycling onset for the BPA stop dose group compared to control.

Appendices

A. Statistical Tables

a) BPA Treatments Stop Dose Arm

Table 1. Censoring of Animals for Bisphenol-A Stop-Dose Arm (µg/kg'BW'/day)			
Dose	***Censoring***	***Frequency***	***% Censored[1]***
0	Right-censored	3	11.5
	Uncensored	23	88.5
2.5	Left-censored	2	7.7
	Right-censored	4	15.4
	Uncensored	20	76.9
25	Right-censored	1	3.8
	Uncensored	25	96.2
250	Left-censored	2	7.7
	Right-censored	1	3.8
	Uncensored	23	88.5
2500	Left-censored	1	4.0
	Right-censored	4	16.0
	Uncensored	20	80.0
25000	Right-censored	2	7.7
	Uncensored	24	92.3

[1] Uncensored animals include those with aberrant cycling; left censored animals were aberrant at the start of data collection; right censored animals had normal cycling at removal.

Table 2. Time to Aberrant Cycling Median Estimates for Bisphenol-A Stop Dose (µg/kg'BW'/day)[1]			
Dose	***Post-Natal Weeks***	***Lower 95% CL***	***Upper 95% CL***
0	41.9	41.3	51.7
2.5	51.7	36.9	57.0
25	46.8	41.9	56.9
250	51.9	41.9	56.9
2500	56.9	51.7	66.6
25000	52.1	41.9	61.9

[1] Median was estimated using the Kaplan-Meier product limit method.

Table 3. Accelerated Failure Time Model for Bisphenol-A Stop-Dose (µg/kg'BW'/day)[1]					
	Dose				
	2.5	***25***	***250***	***2500***	***25000***
P-value	1.000	0.827	1.000	**0.028**	0.524

[1] P-values were adjusted for multiple comparisons to the control group using Holm's method.

b) BPA Treatments Continuous Dose Arm

Table 4. Censoring of Animals for Bisphenol-A Continuous Dose Arm (µg/kg'BW'/day)			
Dose	***Censoring***	***Frequency***	***% Censored[1]***
0	Left-censored	1	3.8
	Right-censored	2	7.7
	Uncensored	23	88.5
2.5	Right-censored	1	4.0
	Uncensored	24	96.0
25	Uncensored	25	100.0
250	Left-censored	2	8.0
	Right-censored	2	8.0
	Uncensored	21	84.0
2500	Left-censored	1	3.8
	Right-censored	4	15.4
	Uncensored	21	80.8
25000	Left-censored	1	4.0
	Uncensored	24	96.0

[1] Uncensored animals include those with aberrant cycling; left censored animals were aberrant at the start of data collection; right censored animals had normal cycling at removal.

Table 5. Time to Aberrant Cycling Median Estimates for Bisphenol-A Continuous Dose (µg/kg'BW'/day)[1]			
Dose	***Post-Natal Weeks***	***Lower 95% CL***	***Upper 95% CL***
0	56.8	42.0	66.9
2.5	47.0	36.9	52.0
25	51.9	42.1	56.9
250	56.9	46.9	61.9
2500	52.0	46.9	56.7
25000	46.9	41.7	56.9

[1] Median was estimated using the Kaplan-Meier product limit method.

Table 6. Accelerated Failure Time Model for Bisphenol-A Continuous Dose (µg/kg'BW'/day)[1]					
	Dose				
	2.5	***25***	***250***	***2500***	***25000***
P-value	0.739	0.796	0.794	0.796	0.794

[1] P-values were adjusted for multiple comparisons to the control group using Holm's method.

c) EE_2 Treatments Continuous Dose

Table 7. Censoring of Animals for Ethinyl Estradiol Dose (µg/kg'BW'/day)

Dose	*Censoring*	*Frequency*	*% Censored*[1]
0	Left-censored	1	3.8
	Right-censored	2	7.7
	Uncensored	23	88.5
0.05	Left-censored	1	3.8
	Right-censored	2	7.7
	Uncensored	23	88.5
0.5	Left-censored	20	76.9
	Uncensored	6	23.1

[1] Uncensored animals include those with aberrant cycling; left censored animals were aberrant at the start of data collection; right censored animals had normal cycling at removal.

Table 8. Time to Aberrant Cycling Median Estimates for Ethinyl Estradiol Dose (µg/kg'BW'/day)[1]

Dose	*Post-Natal Weeks*	*Lower 95% CL*	*Upper 95% CL*
0	56.8	42.0	66.9
0.05	51.8	37.0	62.1
0.5	21.9	21.7	22.0

[1] Median was estimated using the Kaplan-Meier product limit method.

Table 9. Accelerated Failure Time Model for Ethinyl Estradiol Dose (µg/kg'BW'/day)[1]

	Dose	
	0.05	*0.5*
P-value	0.356	**<.001**

[1] P-values were adjusted for multiple comparisons to the control group using Holm's method.

B. Figures

Figure 1. Kaplan-Meier Curve Time to Aberrant Cycling for BPA Stop Dose µg/kg$_{bw}$/day

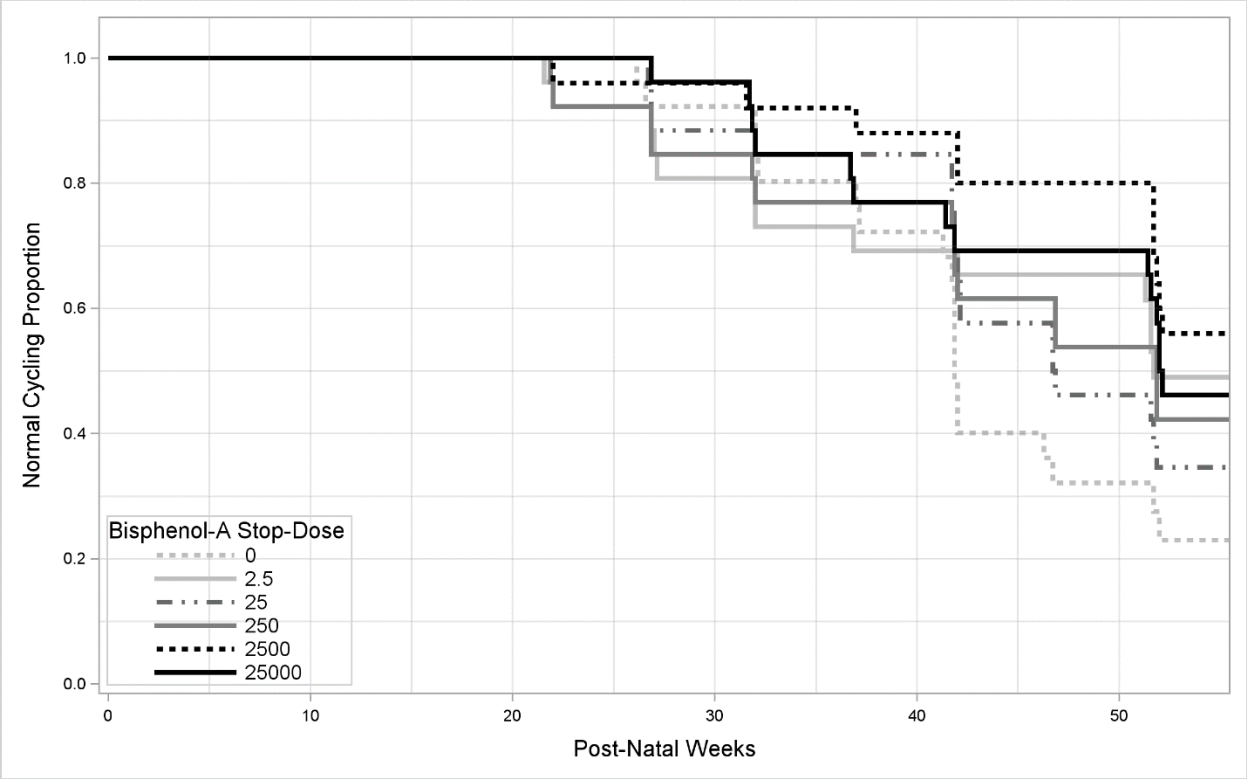


Figure 2. Kaplan-Meier Curve Time to Aberrant Cycling for BPA Continuous Dose µg/kg$_{bw}$/day

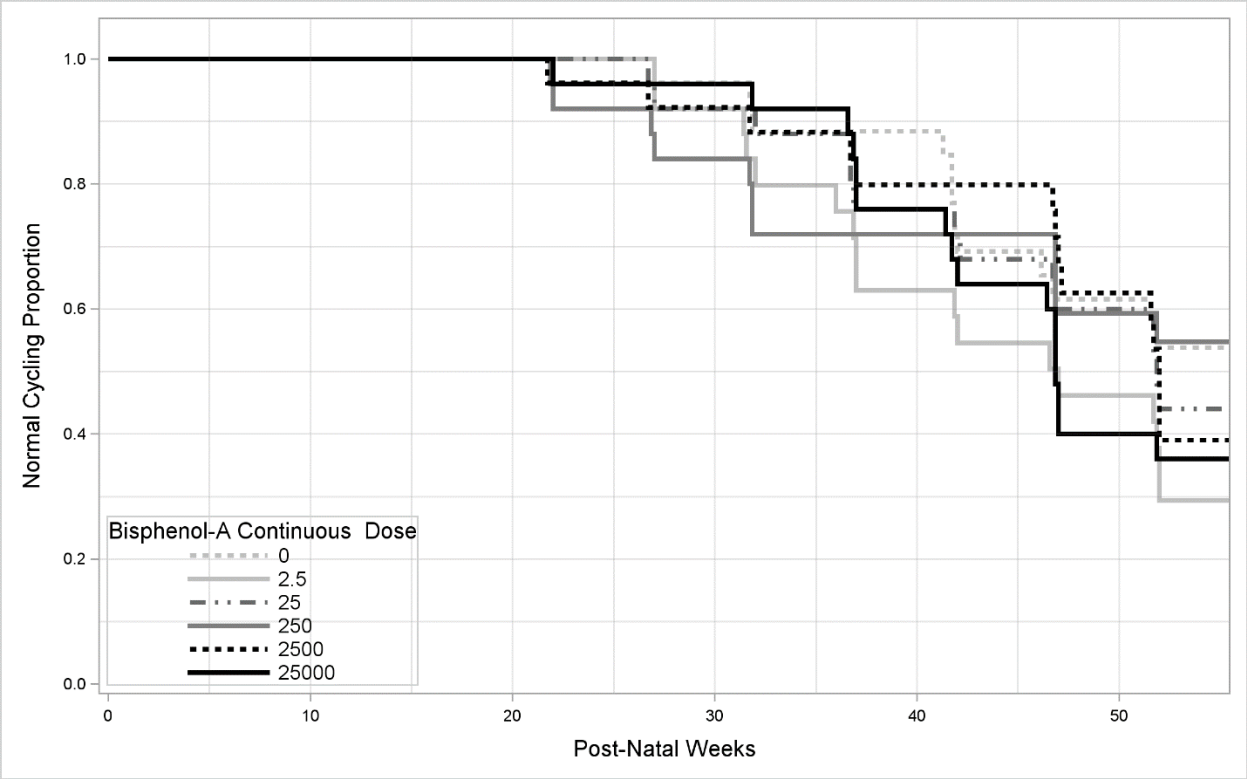
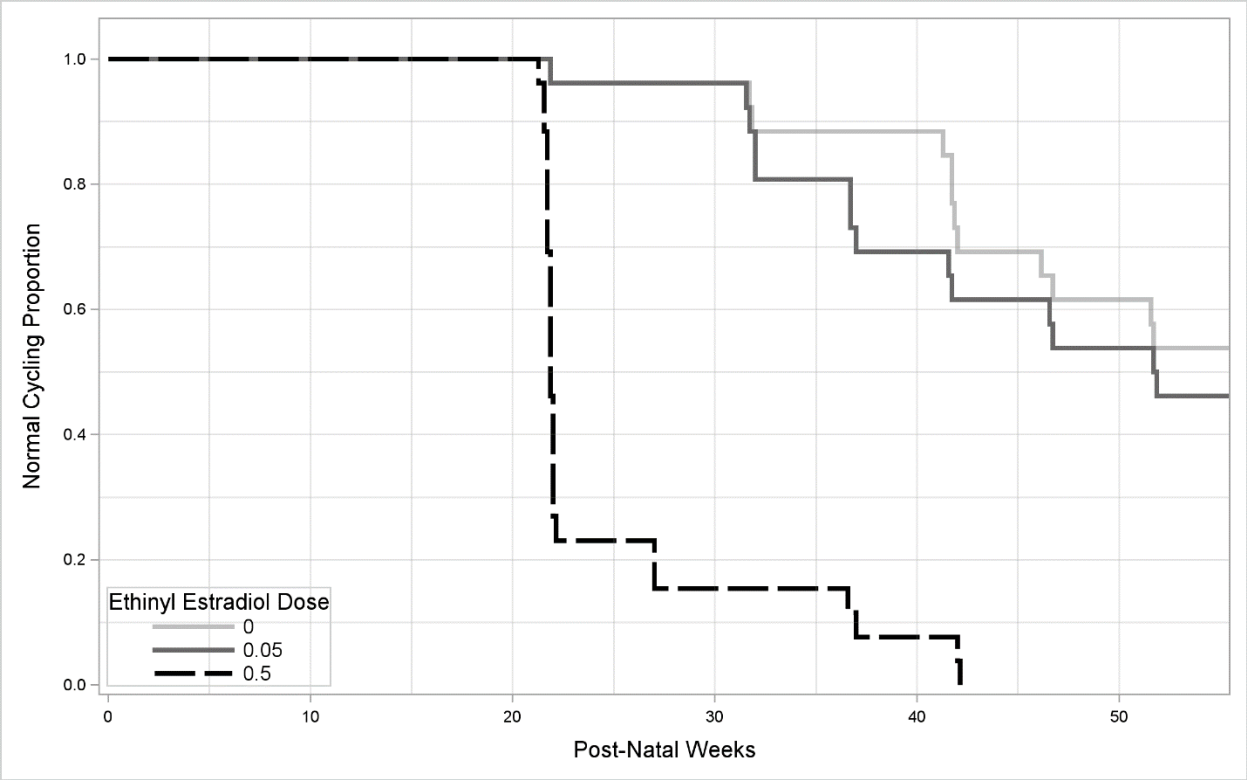


Figure 3. Kaplan-Meier Curve Time to Aberrant Cycling for EE_2 µg/kg_{bw}/day



C. Data

Time to aberrant cycling data were extracted from the Genesis database using SAS Proc SQL, utilizing the Vortex ODBC driver. The Principal Investigator provided the Procedural Statement for Evaluation and Reporting Aberrant Estrus Cycle Data and documentation of deviations relevant to aberrant cycling data.

Quality Control

1. Data Verification

The extraction of the data into SAS was verified by the statistical reviewer 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 in the tables.

3.2 Table Verification

Analysis results were output from SAS to .rtf files 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.

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.