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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 TERMINAL SACRIFICE DEVELOPMENTAL MEASURES DATA

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Statistical Analysis of Terminal Sacrifice Developmental Measures 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 terminal sacrifice developmental measure data.

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 EE_2 terminal sacrifice and all the interim sacrifice groups, there were 20-26 pups each. Pups within litter and sex were assigned to different dosing arms and sacrifice times.

Developmental Measure Data

Vaginal opening and vaginal cytology data were collected from 13 cages randomly selected from each treatment group in the 2 year terminal sacrifice arm. The selected females were monitored from PND 22 until occurrence of vaginal opening for PND endpoint in the BPA stop dose arm, and for body weight and PND endpoints in the BPA continuous dose arm and EE_2 dose.

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. Tests of trend, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups.

Developmental endpoints were age at vaginal opening for the BPA stop dose study arm, and age and body weight at vaginal opening for the BPA and EE_2 continuous dose arm. Body weight was not analyzed for the stop dose arm because body weight was not collected at vaginal opening for 31 animals. The missing values in the stop dose arm and other sporadic missing values in the continuous dose have been accounted for in protocol deviations.

Analyses of age and body weight at occurrence of vaginal opening were performed using contrasts within a one-way analysis of variance (ANOVA) to test for treatment effect. Comparisons of dosed groups to vehicle control for age and body weight were performed with Dunnett's method for adjusted contrasts.

For analysis of each endpoint, a sensitivity analysis was also performed. Of animals with PND at vaginal opening data, 91 core study animals (16 in vehicle control, 62 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 13 in EE_2 µg/kg bw/day dose groups) were held during initial preweaning 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 91 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

Due to protocol deviations, there were twelve animals in six cages excluded from the analysis of age and body weight at vaginal opening. There was delayed monitoring of vaginal opening for UIN=23000529417, 23000529592, 23000531129, and 23000531379 in the EE_2 dose, UIN=23000529337 and 23000529402 in the vehicle continuous control, and UIN=23000529338 and 23000529403 in the vehicle stop dose control. Because load number was mistaken, data was erroneously collected for UIN=23000535056 and 23000535043 in the vehicle continuous control, and UIN=23000534608 and 23000534959 in the BPA stop dose (these animals were replaced by animals in the correct load).

Results of core study analyses are presented in Tables in Appendix A.

4.1 BPA Treatments Stop-Dose Arm

Summary statistics for the BPA stop dose arm are presented in Table 1.

The ANOVA omnibus test results are given in Table 2 for the null hypothesis that all of the control and BPA stop dose treatment means for developmental measures are equal. There was no significant treatment effect for PND at vaginal opening.

Pairwise comparisons for the BPA stop dose arm are presented in Table 3. There was no significant trend or difference for any BPA stop dose treatment compared to the vehicle control for PND at vaginal opening.

In the sensitivity analysis for the BPA stop dose arm, there were no statistically significant pairwise comparisons of treatments to control.

4.2 BPA Treatments Continuous Dose Arm

Summary statistics for the BPA continuous dose arm are presented in Table 4.

The ANOVA omnibus test results are given in Table 5 for the null hypothesis that all of the control and BPA stop dose treatment means for developmental measures are equal. There was a significant treatment effect for body weight at vaginal opening ($p=0.016$).

Pairwise comparisons for the BPA continuous dose arm are presented in Table 6. There was no significant trend or difference for any BPA continuous treatment compared to the vehicle control for PND at vaginal opening.

In the sensitivity analysis for the BPA continuous dose arm, there were no statistically significant pairwise comparisons of treatments to control.

4.3 EE_2 Treatments Continuous Dose

Summary statistics for the EE_2 continuous dose are presented in Table 7.

The ANOVA omnibus test results are given in Table 8 for the null hypothesis that all of the control and EE_2 continuous dose treatment means for developmental measures are equal. There was no significant treatment effect for either body weight or PND at vaginal opening. Pairwise comparisons for the EE_2 continuous dose are presented in Table 9. There were no significant differences for EE_2 treatments compared to vehicle control for either body weight or PND at vaginal opening.

In the sensitivity analysis for EE_2 continuous dose, there were no statistically significant pairwise comparisons of treatments to control.

5. Conclusions

5.1 BPA Treatments Stop-Dose Arm

There were no statistically significant differences for the BPA stop dose arm in pairwise comparisons of treatments to control for PND at vaginal opening.

5.2 BPA Treatments Continuous Dose Arm

For pairwise comparisons of BPA continuous dose treatments to the vehicle control, there were no significant differences for body weight and PND at vaginal opening.

5.3 EE_2 Treatments Continuous Dose

There were no significant differences for EE_2 treatments compared to the vehicle control for either body weight or PND at vaginal opening.

Appendices

A. Statistical Tables

a) BPA Treatments Stop Dose Arm

Table 1. Summary Statistics at Vaginal Opening for Bisphenol-A Stop-Dose (µg/kg'BW'/day)

		PND	
Dose	***N***	***Mean***	***SE***
0	26	41.1	1.8
2.5	26	42.1	2.5
25	25	40.0	1.5
250	26	39.6	1.2
2500	26	42.4	1.2
25000	26	38.0	1.3

Table 2. ANOVA for Developmental Measures at Vaginal Opening for Bisphenol-A Stop-Dose (µg/kg'BW'/day)

Endpoint	***NumDF***	***DenDF***	***Fvalue***	***P value***
PND	5	149	1.006	0.416

Table 3. Comparison of Least Squares Mean Developmental Measures at Vaginal Opening for Bisphenol-A Stop-Dose (µg/kg$_{BW}$/day)[1]

	Dose																					
	0			***2.5***				***25***				***250***				***2500***				***25000***			
Endpoint	***Mean***	***SE***	***Pval[2]***	***Mean***	***SE***	***Pct***	***Pval***	***Mean***	***SE***	***Pct***	***Pval***	***Mean***	***SE***	***Pct***	***Pval***	***Mean***	***SE***	***Pct***	***Pval***	***Mean***	***SE***	***Pct***	***Pval***
PND	41.12	1.66	0.277	42.08	1.66	102.3	0.993	39.96	1.70	97.2	0.985	39.62	1.66	96.4	0.953	42.38	1.66	103.1	0.977	37.96	1.66	92.3	0.532

[1] Animal was the experimental unit for analysis; pairwise p-values and % are relative to control.
[2] Dose trend is shown below the control group.

b) BPA Treatments Continuous Dose Arm

Table 4. Summary Statistics at Vaginal Opening for Bisphenol-A Continuous Dose (µg/kg$_{BW}$/day)

	PND			BW (g)	
Dose	**N**	**Mean**	**SE**	**N**	**Mean**	**SE**
0	26	35.9	1.1	26	120.5	4.6
2.5	25	35.2	0.7	25	117.1	5.0
25	24	36.5	0.8	24	128.6	4.6
250	25	37.8	1.4	25	131.1	5.8
2500	25	34.1	0.5	25	109.6	2.6
25000	24	35.4	0.6	24	121.0	4.3

Table 5. ANOVA for Developmental Measures at Vaginal Opening for Bisphenol-A Continuous Dose (µg/kg$_{BW}$/day)

Endpoint	**NumDF**	**DenDF**	**Fvalue**	**P value**
Body Weight (g)	5	143	2.872	**0.016**
PND	5	143	1.852	0.106

Table 6. Comparison of Least Squares Mean Developmental Measures at Vaginal Opening for Bisphenol-A Continuous Dose (µg/kg·BW·/day)[1]

	Dose																					
	0			2.5				25				250				2500				25000			
Endpoint	Mean	SE	Pval[2]	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval	Mean	SE	Pct	Pval
Body Weight (g)	120.47	4.49	0.651	117.14	4.58	97.2	0.981	128.55	4.67	106.7	0.604	131.07	4.58	108.8	0.335	109.60	4.58	91.0	0.312	121.01	4.67	100.5	1.000
PND	35.88	0.90	0.565	35.16	0.91	98.0	0.972	36.50	0.93	101.7	0.987	37.76	0.91	105.2	0.451	34.12	0.91	95.1	0.510	35.38	0.93	98.6	0.994

[1] Animal was the experimental unit for analysis; pairwise p-values and % are relative to control.
[2] Dose trend is shown below the control group.

c) EE_2 Treatments Continuous Dose

Table 7. Summary Statistics at Vaginal Opening for Ethinyl Estradiol Dose (µg/kg'BW'/day)

	PND			BW (g)		
Dose	**N**	**Mean**	**SE**	**N**	**Mean**	**SE**
0	26	35.9	1.1	26	120.5	4.6
0.05	25	35.5	0.7	25	123.0	4.8
0.5	21	34.8	2.8	21	117.1	11.8

Table 8. ANOVA for Developmental Measures at Vaginal Opening for Ethinyl Estradiol Dose (µg/kg'BW'/day)

Endpoint	**NumDF**	**DenDF**	**Fvalue**	**P value**
Body Weight (g)	2	69	0.156	0.855
PND	2	69	0.115	0.891

Table 9. Comparison of Least Squares Mean Developmental Measures at Vaginal Opening for Ethinyl Estradiol Dose (µg/kg'BW'/day)[1]

	Dose									
	0		0.05				0.5			
Endpoint	**Mean**	**SE**	**Mean**	**SE**	**Pct**	**Pval**	**Mean**	**SE**	**Pct**	**Pval**
Body Weight (g)	120.47	6.94	122.96	7.07	102.1	0.956	117.11	7.72	97.2	0.928
PND	35.88	1.58	35.52	1.61	99.0	0.981	34.76	1.76	96.9	0.853

[1] Animal was the experimental unit for analysis; pairwise p-values and % are relative to control.

B. Data

Developmental measures data were extracted from the Genesis database using SAS Proc SQL, utilizing the Vortex ODBC driver.

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.

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.