NCTR PROTOCOL E0219001

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

STATISTICAL ANALYSIS OF GESTATIONAL WEIGHT DATA

PREPARED
BY

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FOR

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Statistical Analysis of Gestational Weight 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 test the treatment effect of exposure to BPA in Sprague-Dawley rats based on gestational weight 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. 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. Pups within litter and sex were assigned to different dosing arms and sacrifice times. Additional pups were assigned to other protocols that provided animals and tissues to academic investigators.

Gestational Weight Data

Gestational weight data were collected from dams with litters allocated to the core study or used for the academic investigator study, including any litters produced over the core study goals.

3. Statistical Methods

Analyses were performed separately for the BPA and EE_2 treatments. Gestational weight at parturition was analyzed using analysis of covariance (ANOCOVA) with terms for treatment group, dam weight at baseline as a covariate, litter size as a covariate, and the interaction between treatment and litter size. Data was collected at baseline on GD 0 or GD 1 prior to dosing and daily from GD 6 to parturition. Gestational weight at parturition was defined as the last dam body weight prior to delivery.

Pairwise comparisons of treatment means to the control group were performed using contrasts with Dunnett's method of adjustment for multiple comparisons. Tests of trend, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. All tests were performed as two-sided tests.

For gestational weight endpoint, a sensitivity analysis was also performed. For a portion of the gestational period, 85 dams (16 in vehicle control, 50 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 19 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, a sensitivity analysis

excluding these 85 dams 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

Tables are included in Appendix A and figures are included in Appendix B. Three dams were not included in the analysis of gestation weight because baseline weight was not collected at GD 0 or GD 1 (one each for vehicle control, BPA 250, and BPA 2500 µg/kg bw/day dose groups).

4.1 BPA Treatments

Summary statistics are presented for the BPA treatments in Table 1.

In the analysis for the BPA treatments, the covariates littersize and baseline weight were statistically significant (both $p<0.001$). There was no significant treatment effect and the interaction between littersize and treatment was not significant. Pairwise comparisons of dosed groups to control are shown in Table 2. There was no statistically significant trend in the analysis of the BPA and vehicle control groups. There were no statistically significant differences for any BPA treatment compared to the vehicle control.

In the sensitivity analyses for BPA dose groups, there were no additional statistically significant results.

4.2 EE_2 Treatments

Summary statistics are presented for the EE_2 treatments in Table 3.

In the analysis for the EE_2 treatments, the covariates littersize and baseline weight were statistically significant (both $p<0.001$). There was no significant treatment effect and the interaction between littersize and treatment was not significant. Pairwise comparisons of dosed groups to control are shown in Table 4. There were no statistically significant differences for any EE_2 treatment compared to the vehicle control.

In the sensitivity analyses for EE_2 dose groups, there were no additional statistically significant results.

5. Conclusions

There were no statistically significant differences for any dosed treatment compared to the vehicle control in the analyses of either BPA or EE2 treatments.

Appendices

A. Statistical Tables

a) BPA Treatments

Table 1. Summary Statistics for Gestational Weights for Bisphenol-A Dose (µg/kg$_{BW}$/day)[1]

	Treatments (µg/kg)																	
	Control			***BPA 2.5***			***BPA 25***			***BPA 250***			***BPA 2500***			***BPA 25000***		
Weight (g)	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***
Baseline	72	244.5	3.0	65	248.1	3.3	61	247.6	3.7	63	243.8	3.2	63	246.1	3.1	64	252.0	3.7
GD 6	72	275.4	3.2	65	280.7	3.6	59	278.1	4.0	63	274.5	3.5	63	277.8	3.4	64	282.6	3.9
Parturition	72	393.2	4.5	65	406.3	5.3	61	397.3	5.7	63	393.9	5.1	63	401.5	5.0	64	395.7	4.7

[1] Baseline for analysis of gestational weight at parturition was defined as weight at GD 0 or GD 1; parturition ranged from GD 21 to GD 23.

Table 2. Comparisons of Least Squares Mean Gestational Weights Across Bisphenol-A Dose (µg/kg$_{BW}$/day)[1]

Control			***BPA 2.5***				***BPA 25***				***BPA 250***				***BPA 2500***				***BPA 25000***			
Mean	***SE***	***P***	***Mean***	***SE***	***Pct***	***P***	***Mean***	***SE***	***Pct***	***P***	***Mean***	***SE***	***Pct***	***P***	***Mean***	***SE***	***Pct***	***P***	***Mean***	***SE***	***Pct***	***P***
396.3	2.2	0.639	402.7	2.4	101.6	0.187	396.8	2.4	100.1	1.000	398.8	2.4	100.6	0.909	401.6	2.4	101.3	0.353	391.5	2.4	98.8	0.446

[1] All p-values and % are relative to the control group, except p-value for trend shown below control; analysis was performed with covariates baseline, littersize, and the interaction between treatment and littersize.

b) EE_2 Treatments

Table 3. Summary Statistics for Gestational Weights for Ethinyl Estradiol Dose (µg/kg$_{BW}$/day)[1]

	Control			*EE2 0.05*			*EE2 0.5*		
Weight (g)	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***	***N***	***Mean***	***SE***
Baseline	72	244.	3.0	41	247.	4.1	51	253.	3.8
GD 6	72	275.	3.2	41	278.	4.6	51	284.	4.2
Parturition	72	393.	4.5	41	398.	6.5	51	402.	5.7

[1] Baseline for analysis of gestational weight at parturition was defined as weight at GD 0 or GD 1; parturition ranged from GD 21 to GD 23.

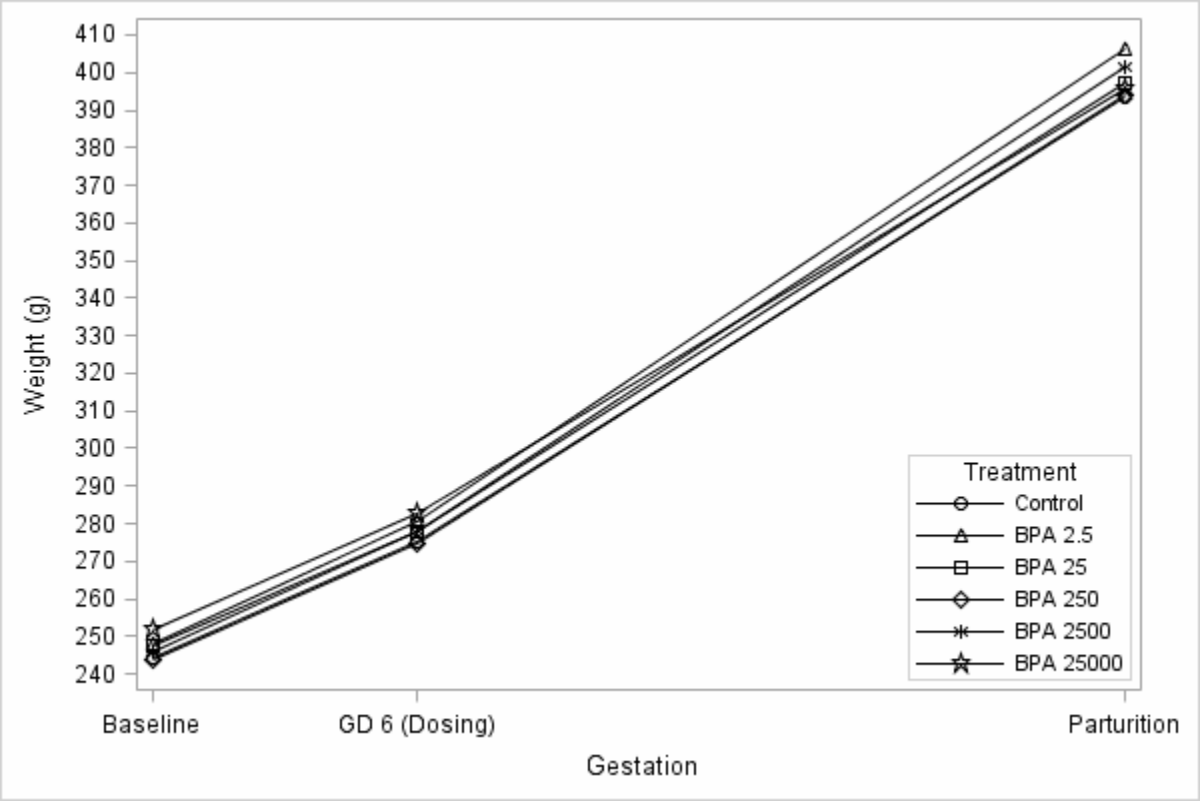
Table 4. Comparisons of Least Squares Mean Gestational Weights for Ethinyl Estradiol Dose (µg/kg$_{BW}$/day)

Control		*EE2 0.05*				*EE2 0.5*			
Mean	***SE***	***Mean***	***SE***	***Pct***	***Pval***	***Mean***	***SE***	***Pct***	***Pval***
397.7	2.2	399.6	3.0	100.5	0.836	394.7	2.7	99.3	0.619

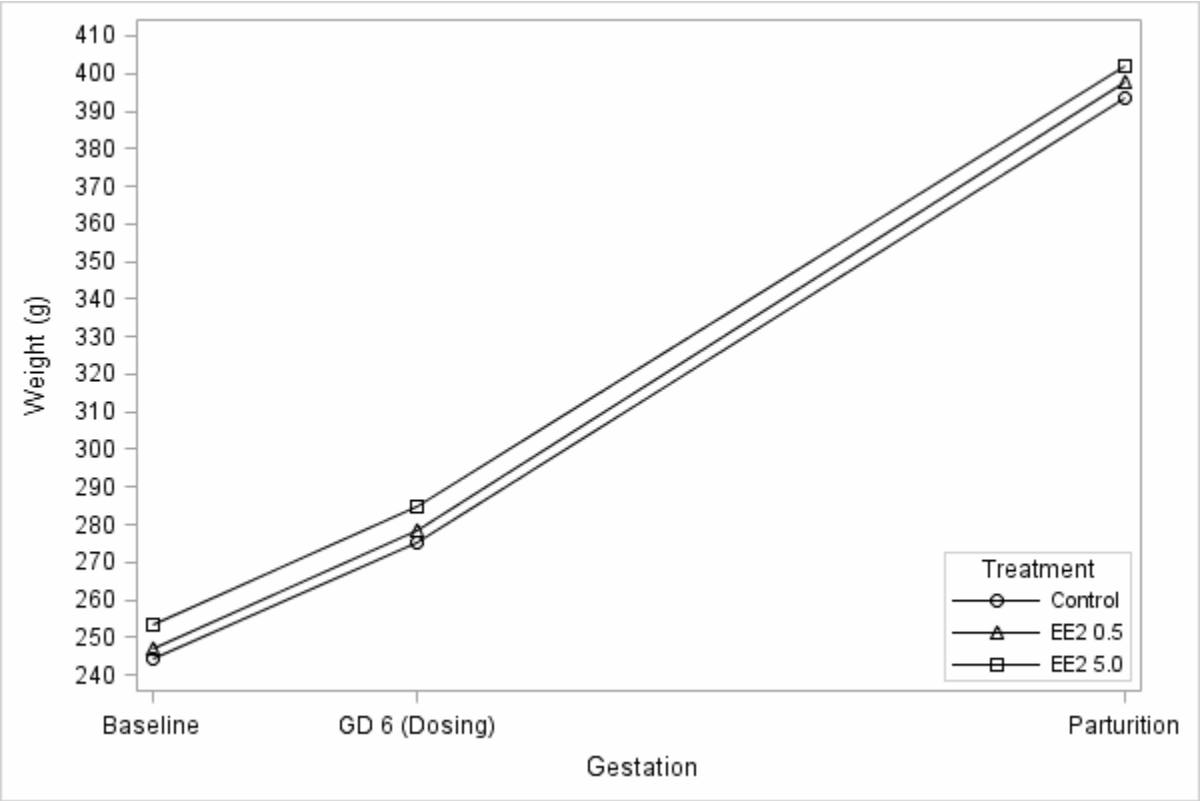
[1] All p-values and % are relative to the control group; analysis was performed with covariates baseline, littersize, and the interaction between treatment and littersize.

B. Figures

a) Figure 1. Gestational Weights from Baseline to Parturition for BPA Treatments



b) Figure 2. Gestational Weights from Baseline to Parturition for EE_2 Treatments



C. Data

Gestational weight 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 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 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.

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.