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 PREWEANING BODY WEIGHT DATA

PREPARED BY

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FOR

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Statistical Analysis of Preweaning Body 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 preweaning body 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. 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. 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.

Preweaning Body Weight Data

Preweaning body weight data were collected from pups that were allocated to either the core study or used for the academic investigator study, which included any litters produced over the core study goals.

3. Statistical Methods

Analyses and adjustments for multiple comparisons were performed separately for the BPA and EE_2 treatments.

For preweaning pup body weight data, analysis was performed using contrasts within sex and PND stratified one-way repeated measures, mixed model analysis of variance (ANOVA) to test for treatment effect accounting for litter correlation assuming a compound symmetric correlation structure. The experimental unit was the litter. The cross-sectional analysis was performed on selected PND of biological relevance, PND 1, 4, 7, 14, and 21, so that the intra-litter correlation could be accounted for accurately.

Pairwise comparisons of treatment means to the vehicle control group mean 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 control groups. All tests were performed as two-sided tests at the 0.05 significance level.

For each endpoint, a sensitivity analysis was also performed. During initial preweaning of animals, 802 pups (150 in vehicle control, 480 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 172 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 802 animals 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 presented in Appendix A and Figures are presented in Appendix B.

4.1 BPA Treatments

Summary statistics for the BPA treatments are presented in Table 1 for females and in Table 2 for males. The number of pups by dose and sex ranged from 1 to 8, generally n=5 (32% to 56%).

Results of ANOVA for BPA preweaning female pup body weights are given in Table 3. The ANOVA omnibus test results are given for the null hypothesis that all of the control and BPA treatment means for weight are equal. There were no significant treatment effects. Comparisons of BPA treatments to the vehicle control for preweaning female body weights are given in Table 4. There were no significant trends for females and no significant differences between any of the treatment groups and the vehicle control group.

Results of ANOVA for BPA preweaning male pup body weights are given in Table 5. The ANOVA omnibus test results are given for the null hypothesis that all of the control and BPA treatment means for weight are equal. There was a significant treatment effect at PND 4 (p=0.041). Comparisons of BPA treatments to the vehicle control group for preweaning male body weights are given in Table 6. There were no significant trends for males and no significant differences between any of the treatment groups and vehicle control.

There were no additional statistically significant results in the sensitivity analysis of BPA dose for either females or males.

4.2 EE_2 Treatments

Summary statistics for the EE_2 treatments are presented in Table 7 for females in Table 8 for males. The number of pups by dose and sex ranged from 1 to 7, generally n=5 (42% to 56%).

Results of ANOVA for EE_2 preweaning female pup body weights are given in Table 9. The ANOVA omnibus test results are given for the null hypothesis that all of the control and EE_2 treatment means for weight are equal. There were significant treatment effects at PND 4 and PND 7 (p=0.006 and =0.021, respectively). Comparisons of EE_2 treatments to the vehicle group for preweaning female body weights are given in Table 10. There were no significant trends for females. There were significant differences for EE_2 0.05 µg/kg bw/day compared to the vehicle control at PND 4 and PND 7 (p=0.015 and =0.025, respectively). Mean weights were lower in the dosed group relative to control (6.7% and 6.4% lower than control, respectively).

Results of ANOVA for EE_2 preweaning male pup body weights are given in Table 11. The ANOVA omnibus test results are given for the null hypothesis that all of the control and EE_2 treatment means for weight are equal. There were no significant treatment effects. Comparisons of EE_2 treatments to vehicle control for preweaning male body weights are given in Table 12. There

were no significant trends for males and no significant differences between any of the treatment groups and the vehicle control group.

There were no additional statistically significant results in the sensitivity analysis of EE_2 dose for either females or males.

5. Conclusions

In comparisons of BPA dosed groups to the control group, there were no significant differences for preweaning body weights for females or males. There were significant differences for females in the EE_2 0.05 µg/kg bw/day group compared to the vehicle control at PND 4 and PND 7, with lower mean weights in the dosed group relative to control. In comparisons of EE_2 dosed groups to the control group for males, there were no significant differences for preweaning body weights.

Appendices

A. Statistical Tables

a) BPA Treatments

Table 1. Summary Statistics for Preweaning Female Body Weight Bisphenol-A[1]

	Dose (µg/kg$_{BW}$/day)																	
	0			2.5			25			250			2500			25000		
PND	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
1	71	6.8	0.1	60	6.7	0.1	57	6.8	0.1	58	6.8	0.1	59	6.7	0.1	59	6.8	0.1
4	71	10.6	0.1	59	10.1	0.2	57	10.5	0.2	58	10.4	0.1	58	10.3	0.2	59	10.4	0.2
7	71	15.8	0.2	59	15.2	0.2	57	15.6	0.2	58	15.3	0.2	58	15.1	0.3	59	15.4	0.2
14	71	30.6	0.4	59	30.1	0.4	57	30.9	0.4	58	30.0	0.4	58	29.6	0.4	59	29.9	0.4
21	70	50.1	0.6	56	49.5	0.7	55	50.6	0.6	56	49.2	0.7	56	48.6	0.7	57	49.1	0.6

[1] Summary statistics were calculated using mean pup weight within litter weighted by litter size (N equals the number of litters).

Table 2. Summary Statistics for Preweaning Male Body Weight Bisphenol-A[1]

	Dose (µg/kg$_{BW}$/day)																	
	0			2.5			25			250			2500			25000		
PND	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
1	71	7.2	0.1	60	7.1	0.1	57	7.3	0.1	59	7.2	0.1	60	7.1	0.1	60	7.3	0.1
4	71	11.4	0.2	59	11.0	0.1	57	11.4	0.2	58	11.2	0.2	59	10.9	0.2	60	11.4	0.2
7	71	16.9	0.2	59	16.4	0.2	57	16.8	0.3	58	16.4	0.2	59	16.2	0.3	60	16.7	0.2
14	71	32.3	0.4	59	31.7	0.4	57	32.4	0.4	58	31.5	0.4	59	31.3	0.4	60	31.7	0.4
21	70	53.0	0.6	58	52.2	0.6	56	53.6	0.6	58	51.8	0.7	59	51.5	0.7	59	52.4	0.7

[1] Summary statistics were calculated using mean pup weight within litter weighted by litter size (N refers to the number of litters).

Table 3. ANOVA Results for Female Body Weight Bisphenol-A				
PND[1]	***NumDF***	***DenDF***	***Fvalue***	***P value***
1	5	358	0.606	0.695
4	5	356	1.672	0.140
7	5	356	1.465	0.200
14	5	356	1.519	0.183
21	5	344	1.698	0.134

[1] Analysis was conducted separately at each PND with litter as the unit of analysis.

Table 4. Comparisons of Least Squares Means for Female Body Weight Bisphenol-A[1]																						
Dose (µg/kg·BW/day)																						
	0			***2.5***				***25***				***250***				***2500***				***25000***			
PND	***Mean***	***SE***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***
1	6.8	0.1	0.994	6.7	0.1	98.1	0.814	6.9	0.1	100.7	0.996	6.8	0.1	99.2	0.994	6.7	0.1	98.1	0.813	6.9	0.1	100.3	1.000
4	10.7	0.2	0.472	10.2	0.2	94.8	0.068	10.6	0.2	99.0	0.990	10.4	0.2	97.3	0.611	10.2	0.2	95.6	0.154	10.5	0.2	98.0	0.847
7	15.9	0.2	0.324	15.3	0.2	96.4	0.303	15.7	0.2	99.2	0.997	15.3	0.2	96.6	0.367	15.1	0.2	95.4	0.118	15.6	0.2	98.6	0.955
14	30.8	0.4	0.084	30.2	0.4	98.1	0.763	31.1	0.4	100.9	0.989	30.1	0.4	97.9	0.679	29.7	0.4	96.4	0.171	30.1	0.4	97.9	0.678
21	50.3	0.6	0.083	49.6	0.7	98.6	0.913	50.9	0.7	101.2	0.945	49.1	0.7	97.6	0.546	48.5	0.7	96.4	0.174	49.5	0.7	98.3	0.831

[1] All p-values and % are relative to the 0 dose control group, except p-value for dose trend shown below control.

Table 5. ANOVA Results for Male Body Weight Bisphenol-A				
PND[1]	***NumDF***	***DenDF***	***Fvalue***	***P value***
1	5	361	1.394	0.225
4	5	358	2.332	**0.041**
7	5	358	1.672	0.140
14	5	358	1.274	0.274
21	5	354	1.151	0.332

[1] Analysis was conducted separately at each PND with litter as the unit of analysis.

Table 6. Comparisons of Least Squares Means for Male Body Weight Bisphenol-A[1]																					
Dose (µg/kg·BW/day)																					
	0			***2.5***				***25***				***250***				***2500***				***25000***			
PND	***Mean***	***SE***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***
1	7.3	0.1	0.674	7.1	0.1	97.5	0.605	7.4	0.1	101.6	0.897	7.2	0.1	98.8	0.965	7.1	0.1	97.9	0.754	7.4	0.1	101.3	0.951
4	11.5	0.2	0.751	11.0	0.2	95.3	0.102	11.5	0.2	100.0	1.000	11.1	0.2	97.0	0.475	11.0	0.2	95.3	0.101	11.5	0.2	99.8	1.000
7	17.0	0.2	0.438	16.3	0.2	96.3	0.243	17.0	0.3	100.0	1.000	16.4	0.3	96.8	0.369	16.3	0.2	96.0	0.167	16.8	0.2	99.0	0.986
14	32.4	0.4	0.171	31.7	0.4	97.9	0.639	32.6	0.4	100.4	1.000	31.6	0.4	97.3	0.426	31.4	0.4	96.9	0.260	31.9	0.4	98.4	0.824
21	53.1	0.6	0.451	52.3	0.7	98.6	0.904	53.8	0.7	101.4	0.910	52.0	0.7	97.9	0.665	51.9	0.7	97.7	0.578	52.9	0.7	99.6	1.000

[1] All p-values and % are relative to the 0 dose control group, except p-value for dose trend shown below control.

b) EE_2 Treatments

Table 7. Summary Statistics for Preweaning Female Body Weight Ethinyl Estradiol[1]

	Dose (µg/kg·BW·/day)								
	0			0.05			0.5		
PND	N	Mean	SE	N	Mean	SE	N	Mean	SE
1	71	6.8	0.1	34	6.6	0.1	47	6.9	0.1
4	71	10.6	0.1	34	10.1	0.2	47	10.9	0.2
7	71	15.8	0.2	34	15.0	0.3	47	16.0	0.3
14	71	30.6	0.4	34	29.9	0.6	47	31.4	0.4
21	70	50.1	0.6	33	50.2	0.9	47	51.9	0.7

[1] Summary statistics were calculated using mean pup weight within litter weighted by litter size (N equals the number of litters).

Table 8. Summary Statistics for Preweaning Male Body Weight Ethinyl Estradiol[1]

	Dose (µg/kg·BW·/day)								
	0			0.05			0.5		
PND	N	Mean	SE	N	Mean	SE	N	Mean	SE
1	71	7.2	0.1	32	7.0	0.1	49	7.2	0.1
4	71	11.4	0.2	32	10.8	0.2	48	11.3	0.2
7	71	16.9	0.2	32	16.1	0.4	48	16.6	0.3
14	71	32.3	0.4	32	31.8	0.6	48	32.5	0.5
21	70	53.0	0.6	32	53.0	1.0	47	54.0	0.8

[1] Summary statistics were calculated using mean pup weight within litter weighted by litter size (N refers to the number of litters).

Table 9. ANOVA Results for Female Body Weight Ethinyl Estradiol				
PND[1]	***NumDF***	***DenDF***	***Fvalue***	***P value***
1	2	149	1.923	0.149
4	2	149	5.194	**0.006**
7	2	149	3.930	**0.021**
14	2	149	2.338	0.100
21	2	147	1.306	0.273

[1] Analysis was conducted separately at each PND with litter as the unit of analysis.

Table 10. Comparisons of Least Squares Means for Female Body Weight Ethinyl Estradiol[1]										
	Dose (µg/kg'BW'/day)									
	0		***0.05***				***0.5***			
PND	***Mean***	***SE***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***
1	6.8	0.1	6.6	0.1	96.5	0.244	6.9	0.1	101.4	0.755
4	10.7	0.2	10.0	0.2	93.3	**0.015**	10.9	0.2	101.6	0.726
7	15.9	0.2	14.9	0.3	93.6	**0.025**	16.0	0.3	100.6	0.954
14	30.8	0.4	29.8	0.6	96.7	0.238	31.3	0.5	101.8	0.579
21	50.3	0.6	50.1	0.9	99.6	0.971	51.7	0.8	102.7	0.275

[1] All p-values and % are relative to the 0 dose control group.

Table 11. ANOVA Results for Male Body Weight Ethinyl Estradiol				
PND[1]	***NumDF***	***DenDF***	***Fvalue***	***P value***
1	2	149	0.767	0.466
4	2	148	2.170	0.117
7	2	148	1.668	0.192
14	2	148	0.457	0.634
21	2	146	0.443	0.642

[1] Analysis was conducted separately at each PND with litter as the unit of analysis.

Table 12. Comparisons of Least Squares Means for Male Body Weight Ethinyl Estradiol[1]										
	Dose (µg/kg'BW'/day)									
	0		***0.05***				***0.5***			
PND	***Mean***	***SE***	***Mean***	***SE***	***Pct***	***P value***	***Mean***	***SE***	***Pct***	***P value***
1	7.3	0.1	7.1	0.1	97.2	0.410	7.3	0.1	99.9	0.998
4	11.5	0.2	10.9	0.2	94.7	0.078	11.4	0.2	99.0	0.883
7	17.0	0.2	16.2	0.4	95.4	0.130	16.7	0.3	98.4	0.696
14	32.4	0.4	31.9	0.6	98.4	0.690	32.6	0.5	100.6	0.943
21	53.1	0.7	53.5	1.0	100.9	0.908	54.1	0.8	101.9	0.560

[1] All p-values and % are relative to the 0 dose control group.

B. Figures

Figure 1. Body Weight (g) for Bisphenol-A Females

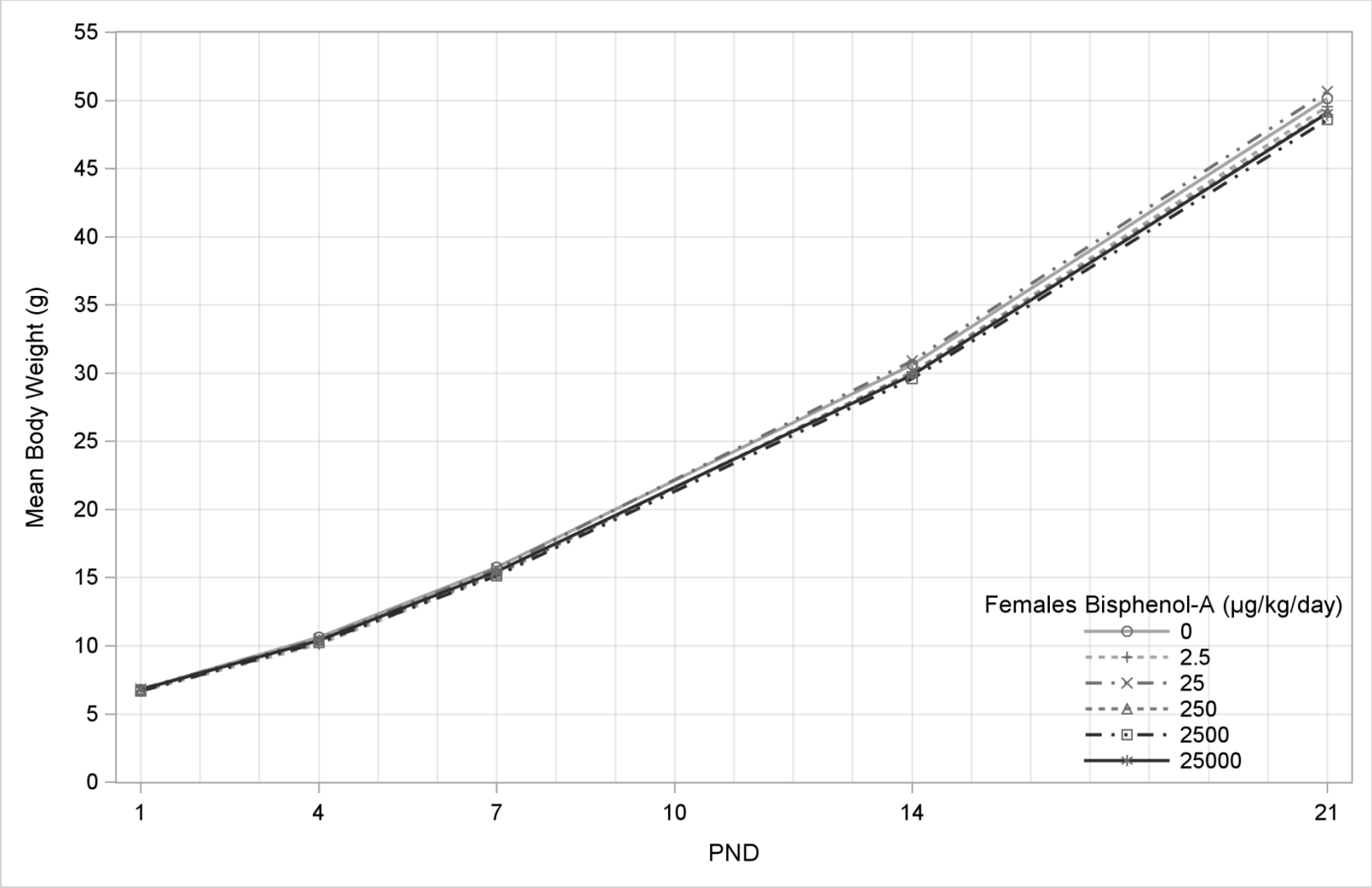


Figure 2. Body Weight (g) for Bisphenol-A Males

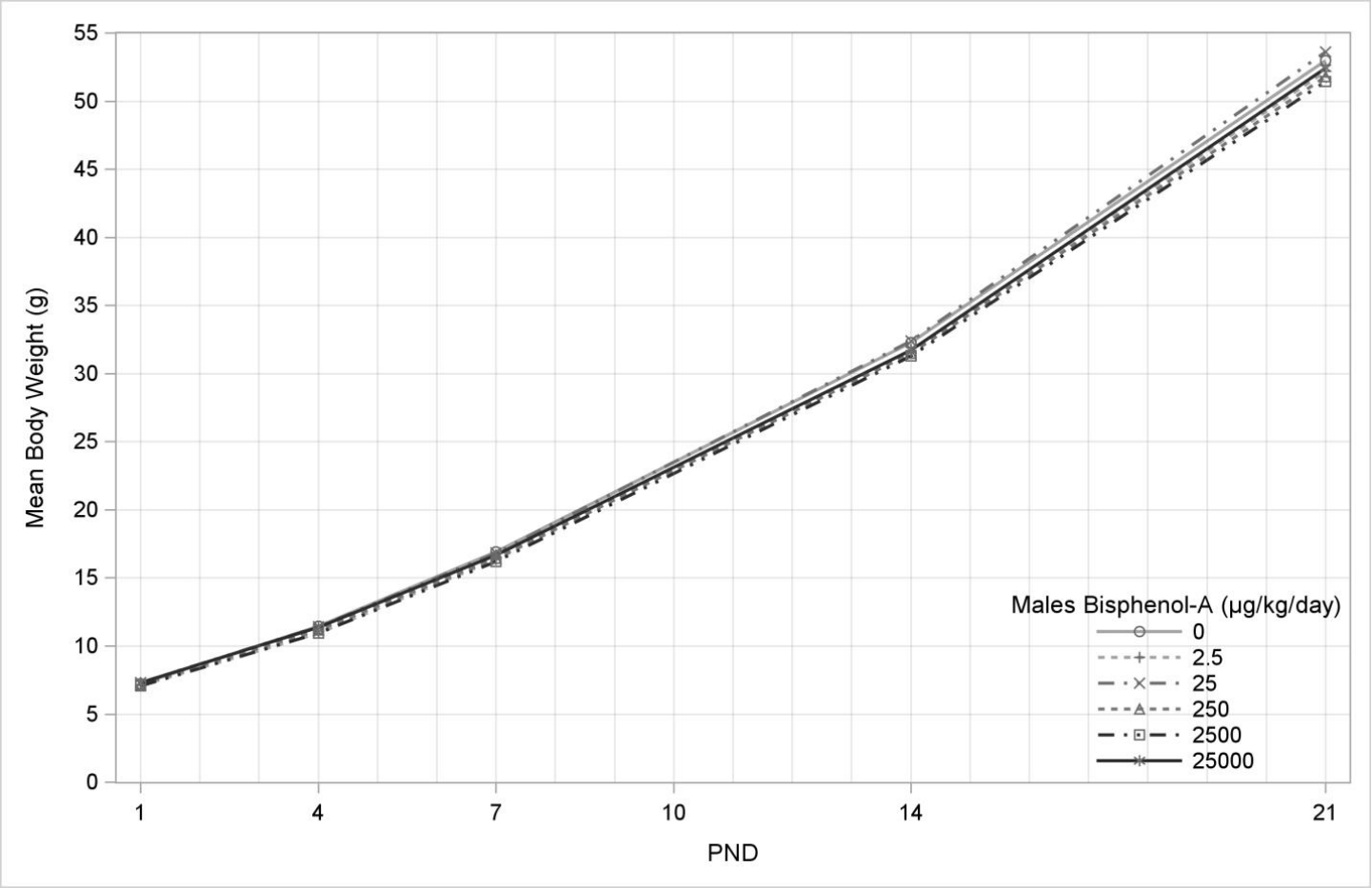


Figure 3. Body Weight (g) for Ethinyl Estradiol Females

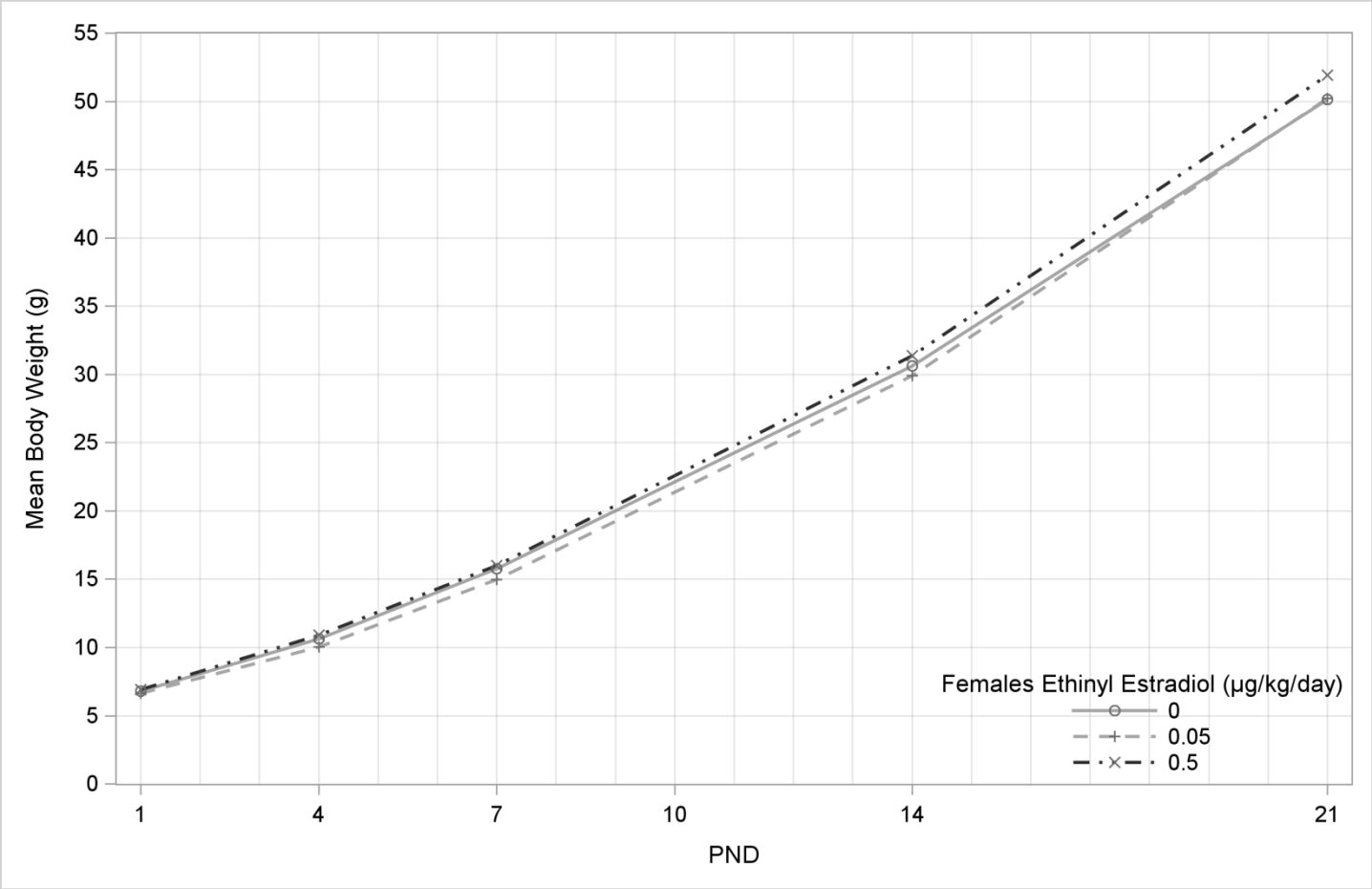
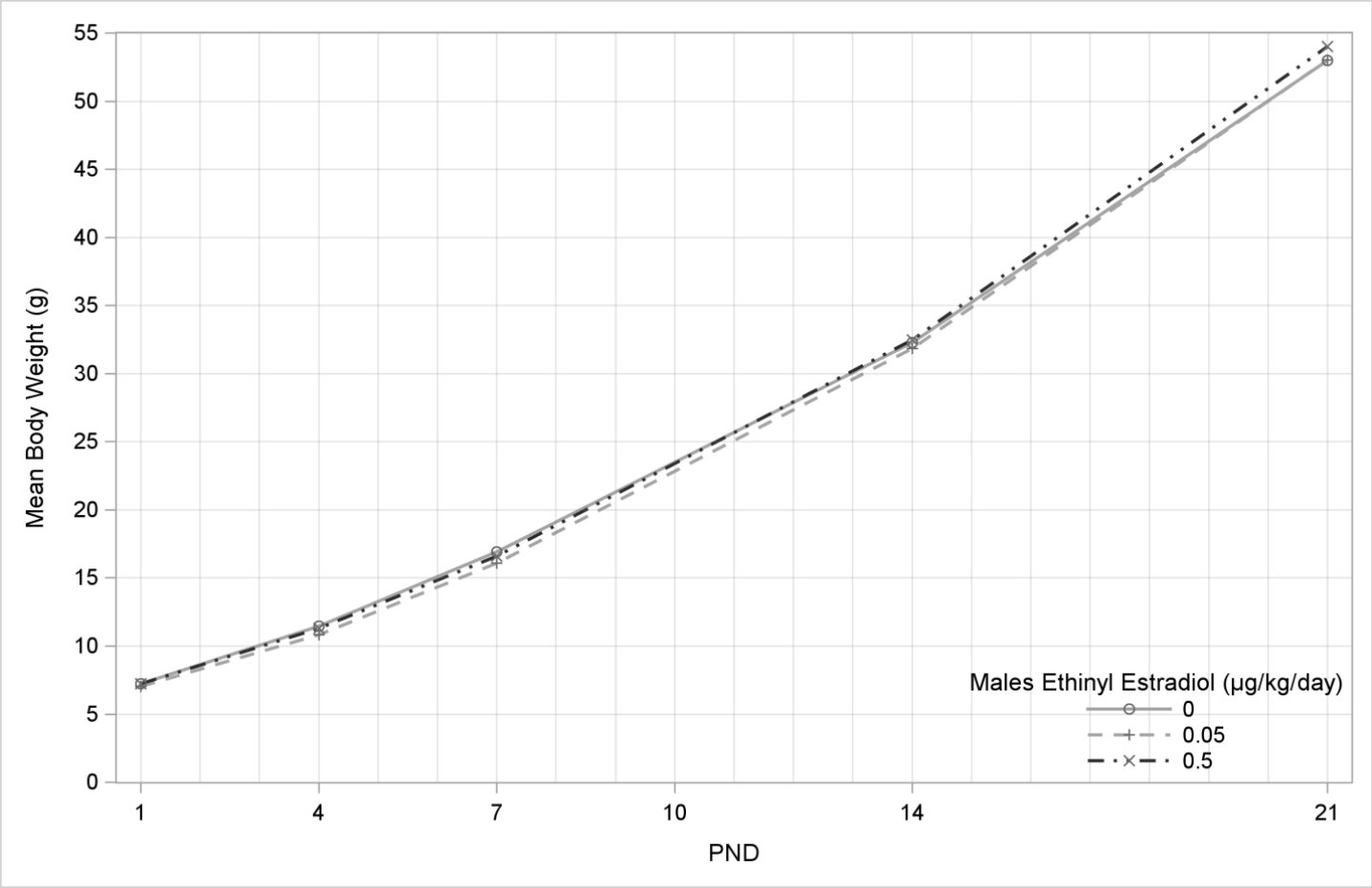


Figure 4. Body Weight (g) for Ethinyl Estradiol Males



C. Data

Preweaning body 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.