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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

STATISTICAL ANALYSIS OF INTERIM SACRIFICE SURVIVAL DATA

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FOR

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Statistical Analysis of Interim Sacrifice Survival 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 regarding survival for the 1 year interim sacrifice.

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.

Survival Data

For this analysis, survival is followed from weaning to 1 year for the animals allocated to 1 year interim sacrifice.

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. Animals with a disposition observed as dead or moribund were treated as uncensored observations, while those observed as reaching terminal sacrifice were considered censored.

To compare survival of treatment groups to the control group, Cox proportional hazards regression analysis was performed. For analyses with 100% survival in one or more groups, a modified Cox proportional hazards regression analysis was performed by adjusting the number of uncensored observations by adding one for each treatment group and sex. This adjustment allows estimability, resulting in a conservative analysis. The survival time of each member of a population is assumed to follow its own hazard function. In Cox regression, the hazard functions of any two groups are assumed to be proportional at any particular time. Multiple comparisons of treatments to the vehicle control group were adjusted using Holm's (step-down Bonferroni) method, and all tests were performed as two-sided. Test of dose trend, increasing treatment effect with increasing dose, was performed for the BPA groups.

For analysis of each endpoint, a sensitivity analysis was also performed. During initial preweaning of animals, 134 core study 1 year interim sacrifice animals (22 in vehicle control, 84 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 28 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 134 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

Results of analyses using all study animals are presented in Appendix A for Tables and in Appendix B for Figures.

4.1 BPA Treatments Stop Dose Arm

Disposition counts and proportions for the BPA stop dose arm are presented in Table 1 for females and in Table 2 for males.

The results of the proportional hazards model analysis for the BPA stop dose arm are presented in Table 3 for females and in Table 4 for males. Dose trend and hazard ratios of treatment groups to the vehicle control were not significant for females or males.

In the sensitivity analyses for the BPA stop dose arm, there were no statistically significant results for females or males.

4.2 BPA Treatments Continuous Dose Arm

Disposition counts and proportions for the BPA continuous dose arm are presented in Table 5 for females and in Table 6 for males.

The results of the proportional hazards model analysis for the BPA continuous dose arm are presented in Table 7 for females and in Table 8 for males. Dose trend and hazard ratios of treatment groups to the vehicle control were not significant for females or males.

In the sensitivity analyses for the BPA continuous dose arm, there were no statistically significant results for females or males.

4.3 EE_2 Treatments Continuous Dose

Disposition counts and proportions for the EE_2 continuous dose are presented in Table 9 for females and in Table 10 for males.

The results of the proportional hazards model analysis for the EE_2 continuous dose are presented in Table 11 for females and in Table 12 for males. Hazard ratios of treatment groups to the vehicle control were not significant for females or males.

In the sensitivity analyses for the EE_2 continuous dose, there were no statistically significant results for females or males.

5. Conclusions

5.1 BPA Treatments Stop Dose Arm

There were no significant differences in survival for the BPA stop dose treatments compared to vehicle control for females or males.

5.2 BPA Treatments Continuous Dose Arm

There were no significant differences in survival for the BPA continuous dose treatments compared to vehicle control for females or males.

5.3 EE_2 Treatments Continuous Dose

There were no significant differences in survival for the EE_2 continuous dose treatments compared to vehicle control for females or males.

Appendices

A. Statistical Tables

a) BPA Treatments Stop Dose Arm

Table 1. Disposition and Censoring of Animals for Interim Sacrifice
Female Bisphenol-A Stop Dose Arm

Dose (µg/kg·BW/day)	*N*	*Interval Sacrifice*	*Moribund*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	20	20	0	20	0	1.000
2.5	22	22	0	22	0	1.000
25	20	20	0	20	0	1.000
250	22	22	0	22	0	1.000
2500	20	20	0	20	0	1.000
25000	22	20	2	20	2	0.909

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 2. Disposition and Censoring of Animals for Interim Sacrifice
Male Bisphenol-A Stop Dose Arm

Dose (µg/kg·BW/day)	*N*	*Dead*	*Interval Sacrifice*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	20	0	20	20	0	1.000
2.5	20	0	20	20	0	1.000
25	20	1	19	19	1	0.950
250	19	0	19	19	0	1.000
2500	20	0	20	20	0	1.000
25000	22	0	22	22	0	1.000

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 3. Cox Proportional Hazards Analysis for Interim Sacrifice Female Bisphenol-A Stop Dose Arm

Dose (µg/kg·BW/day)[1]	*Hazard Ratio[2]*	*P-value[3]*
0	-	0.455
2.5	0.978	1.000
25	1.000	1.000
250	0.978	1.000
2500	1.000	1.000
25000	2.949	1.000

[2] Hazard ratios are relative to vehicle control.
[3] P-values for dose comparisons to control are adjusted using Holm's method.
[1] P-value for dose trend is shown for vehicle control.

Table 4. Cox Proportional Hazards Analysis for Interim Sacrifice Male Bisphenol-A Stop Dose Arm

Dose (µg/kg·BW/day)[1]	*Hazard Ratio[2]*	*P-value[3]*
0	-	0.927
2.5	1.000	1.000
25	2.000	1.000
250	1.007	1.000
2500	1.000	1.000
25000	0.987	1.000

[2] Hazard ratios are relative to vehicle control.
[3] P-values for dose comparisons to control are adjusted using Holm's method.
[1] P-value for dose trend is shown for vehicle control.

b) BPA Treatments Continuous Dose Arm

Table 5. Disposition and Censoring of Animals for Interim Sacrifice
Female Bisphenol-A Continuous Dose Arm

Dose (µg/kg·BW/day)	*N*	*Dead*	*Interval Sacrifice*	*Moribund*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	23	1	21	1	21	2	0.913
2.5	22	0	22	0	22	0	1.000
25	22	1	21	0	21	1	0.955
250	24	0	22	2	22	2	0.917
2500	20	0	20	0	20	0	1.000
25000	24	0	24	0	24	0	1.000

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 6. Disposition and Censoring of Animals for Interim Sacrifice
Male Bisphenol-A Continuous Dose Arm

Dose (µg/kg·BW/day)	*N*	*Dead*	*Interval Sacrifice*	*Moribund*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	22	0	18	4	18	4	0.818
2.5	22	0	22	0	22	0	1.000
25	20	1	18	1	18	2	0.900
250	24	0	24	0	24	0	1.000
2500	20	2	18	0	18	2	0.900
25000	22	1	21	0	21	1	0.955

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 7. Cox Proportional Hazards Analysis for Interim Sacrifice Female Bisphenol-A Continuous Dose Arm

Dose (µg/kg·BW/day)[1]	*Hazard Ratio*[2]	*P-value*[3]
0	-	0.470
2.5	0.332	1.000
25	0.667	1.000
250	0.969	1.000
2500	0.346	1.000
25000	0.319	1.000

[2] Hazard ratios are relative to vehicle control.
[3] P-values for dose comparisons to control are adjusted using Holm's method.
[1] P-value for dose trend is shown for vehicle control.

Table 8. Cox Proportional Hazards Analysis for Interim Sacrifice Male Bisphenol-A Continuous Dose Arm

Dose (µg/kg·BW/day)[1]	*Hazard Ratio*[2]	*P-value*[3]
0	-	0.666
2.5	0.192	0.597
25	0.623	1.000
250	0.182	0.597
2500	0.621	1.000
25000	0.392	0.789

[2] Hazard ratios are relative to vehicle control.
[3] P-values for dose comparisons to control are adjusted using Holm's method.
[1] P-value for dose trend is shown for vehicle control.

c) EE_2 Treatments Continuous Dose

Table 9. Disposition and Censoring of Animals for Interim Sacrifice Female Ethinyl Estradiol Dose

Dose (µg/kg·BW/day)	*N*	*Dead*	*Interval Sacrifice*	*Moribund*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	23	1	21	1	21	2	0.913
0.05	26	1	24	1	24	2	0.923
0.5	26	0	26	0	26	0	1.000

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 10. Disposition and Censoring of Animals for Interim Sacrifice Male Ethinyl Estradiol Dose

Dose (µg/kg·BW/day)	*N*	*Dead*	*Interval Sacrifice*	*Moribund*	*Censored*	*Uncensored*	*Proportion Censored*[1]
0	22	0	18	4	18	4	0.818
0.05	26	2	22	2	22	4	0.846
0.5	26	3	23	0	23	3	0.885

[1] Uncensored animals include those that were moribund or dead; censored animals include those that reached terminal sacrifice.

Table 11. Cox Proportional Hazards Analysis for Interim Sacrifice Female Ethinyl Estradiol Dose

Dose (µg/kg·BW/day)	*Hazard Ratio[1]*	*P-value[2]*
0.05	0.923	0.921
0.5	0.304	0.605

[1] Hazard ratios are relative to vehicle control.
[2] P-values for dose comparisons to control are adjusted using Holm's method.

Table 12. Cox Proportional Hazards Analysis for Interim Sacrifice Male Ethinyl Estradiol Dose

Dose (µg/kg·BW/day)	*Hazard Ratio[1]*	*P-value[2]*
0.05	0.815	1.000
0.5	0.607	1.000

[1] Hazard ratios are relative to vehicle control.
[2] P-values for dose comparisons to control are adjusted using Holm's method.

B. Figures

Figure 1. Kaplan-Meier Survival Curve for Interim Sacrifice Female BPA Stop Dose Arm (Weeks 4-52)

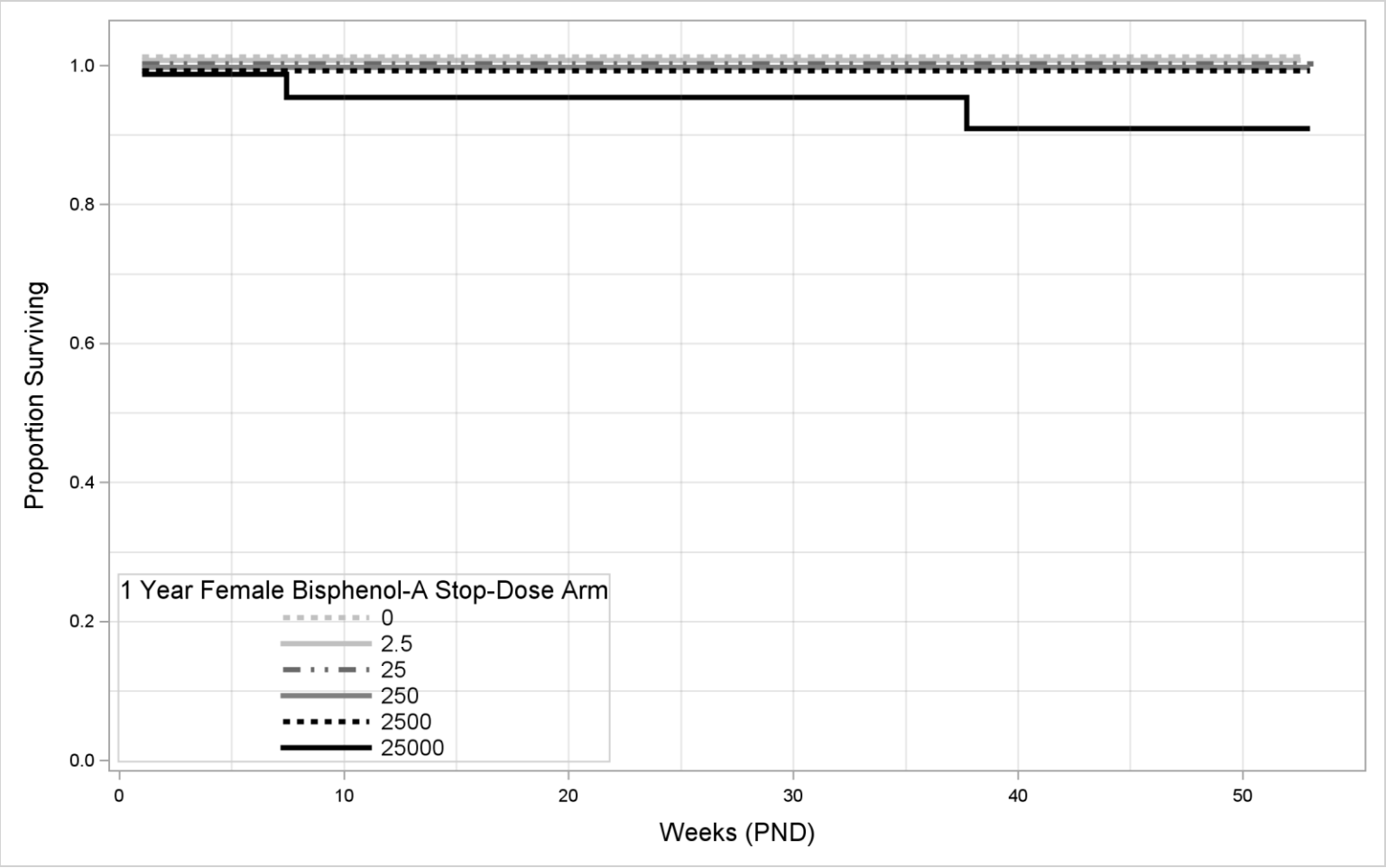


Figure 2. Kaplan-Meier Survival Curve for Interim Sacrifice Male BPA Stop Dose Arm (Weeks 4-52)

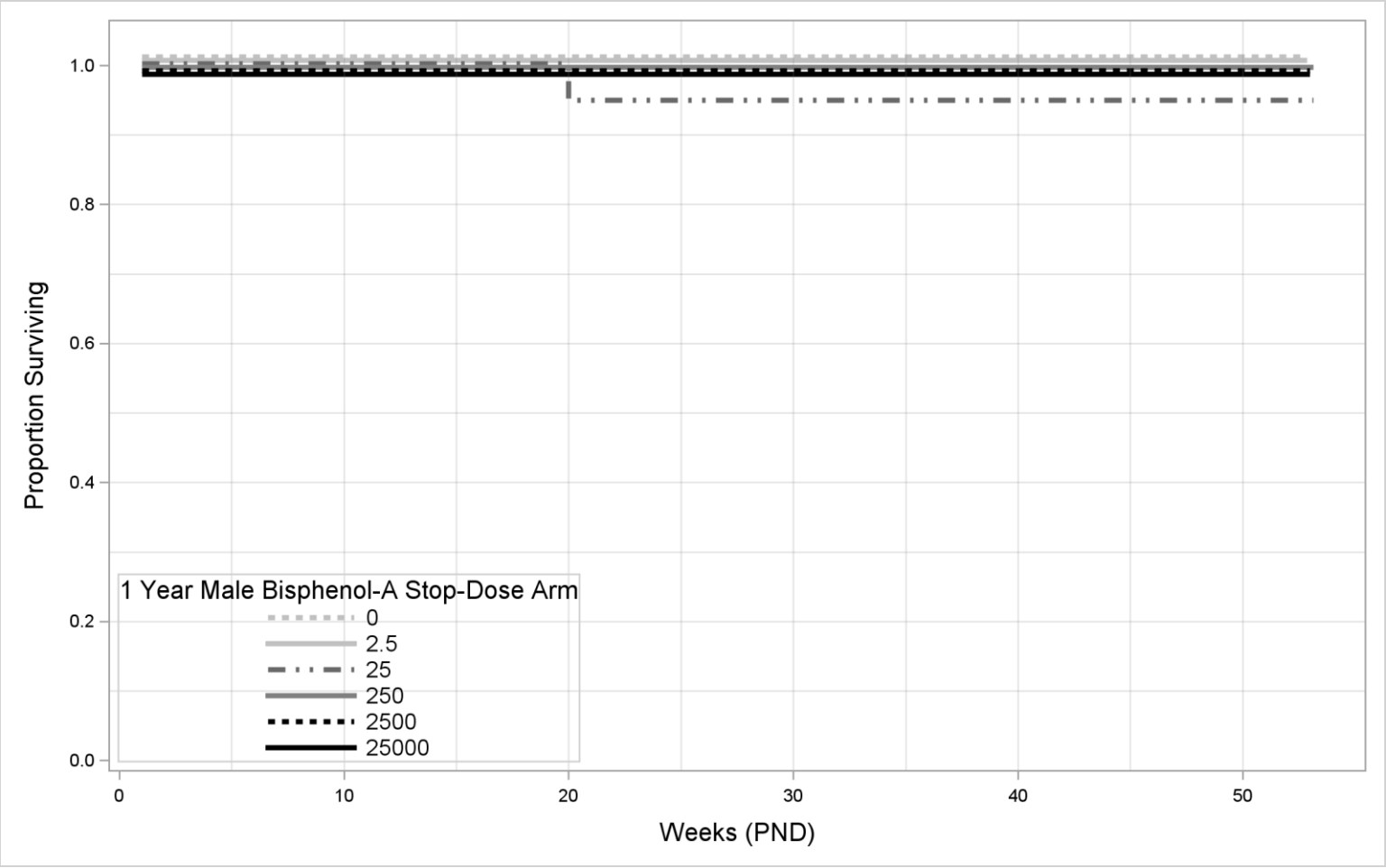


Figure 3. Kaplan-Meier Survival Curve for Interim Sacrifice Female BPA Continuous Dose Arm (Weeks 4-52)

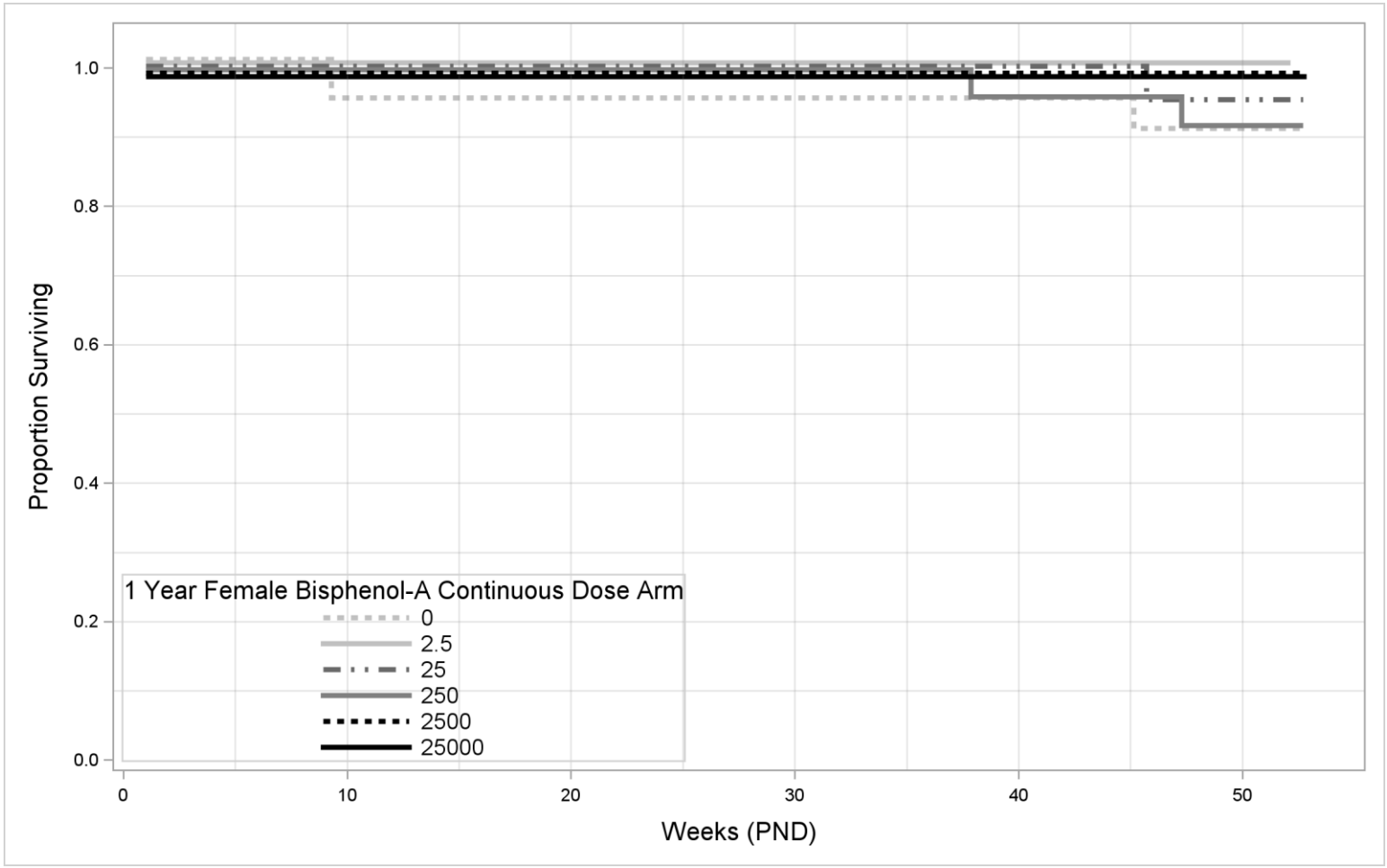


Figure 4. Kaplan-Meier Survival Curve for Interim Sacrifice Male BPA Continuous Dose Arm (Weeks 4-52)

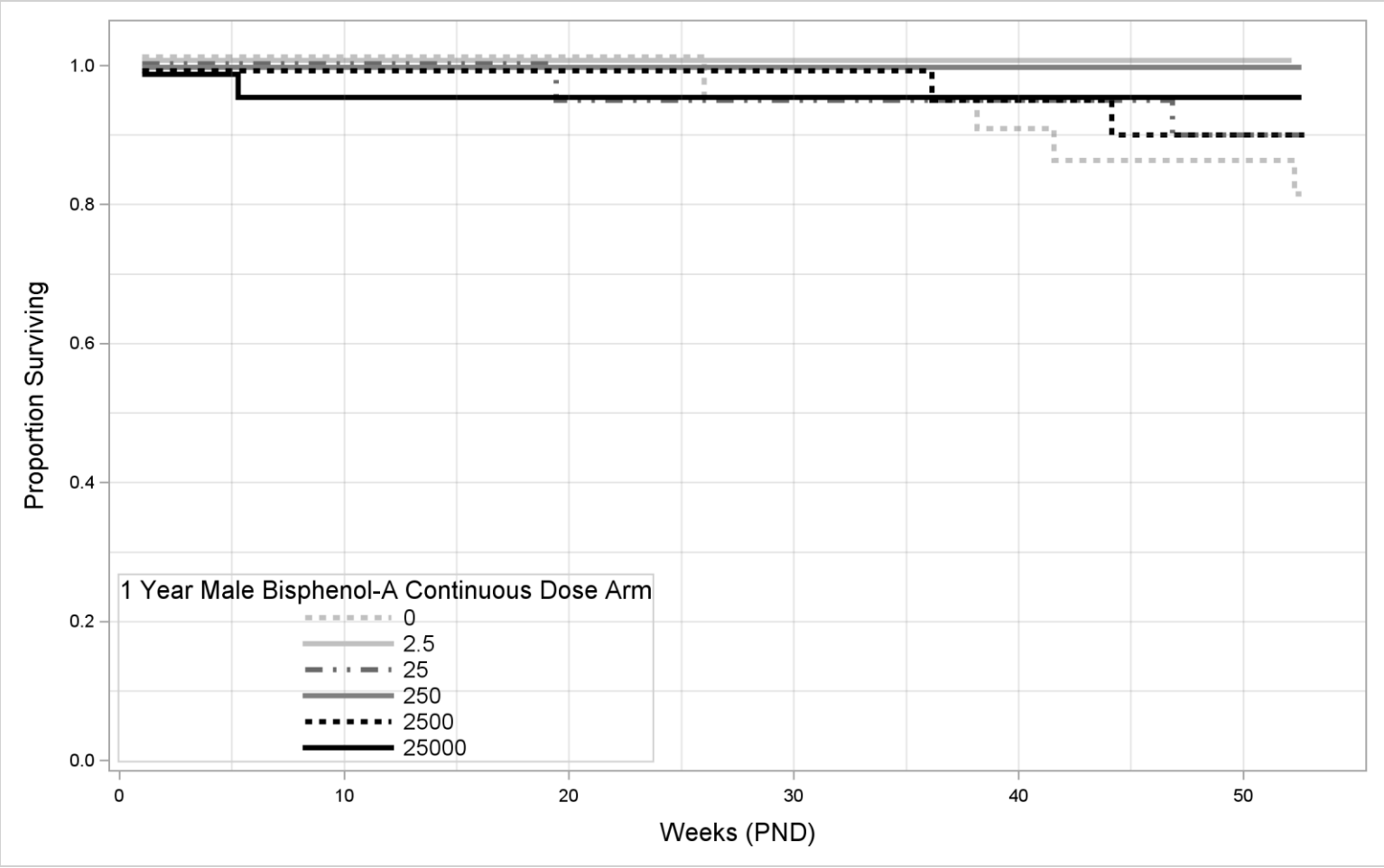


Figure 5. Kaplan-Meier Survival Curve for Interim Sacrifice Female EE_2 Continuous Dose (Weeks 4-52)

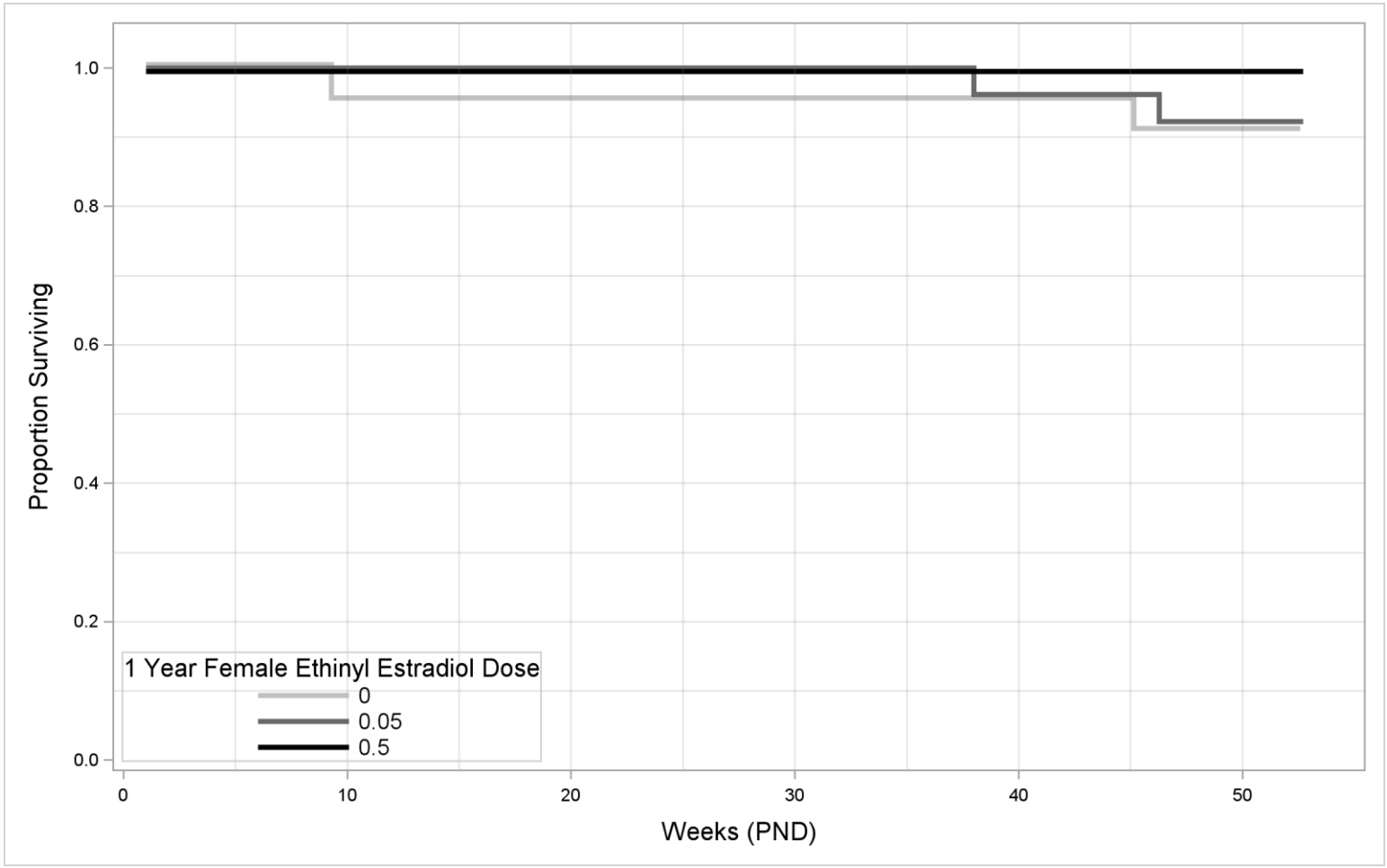
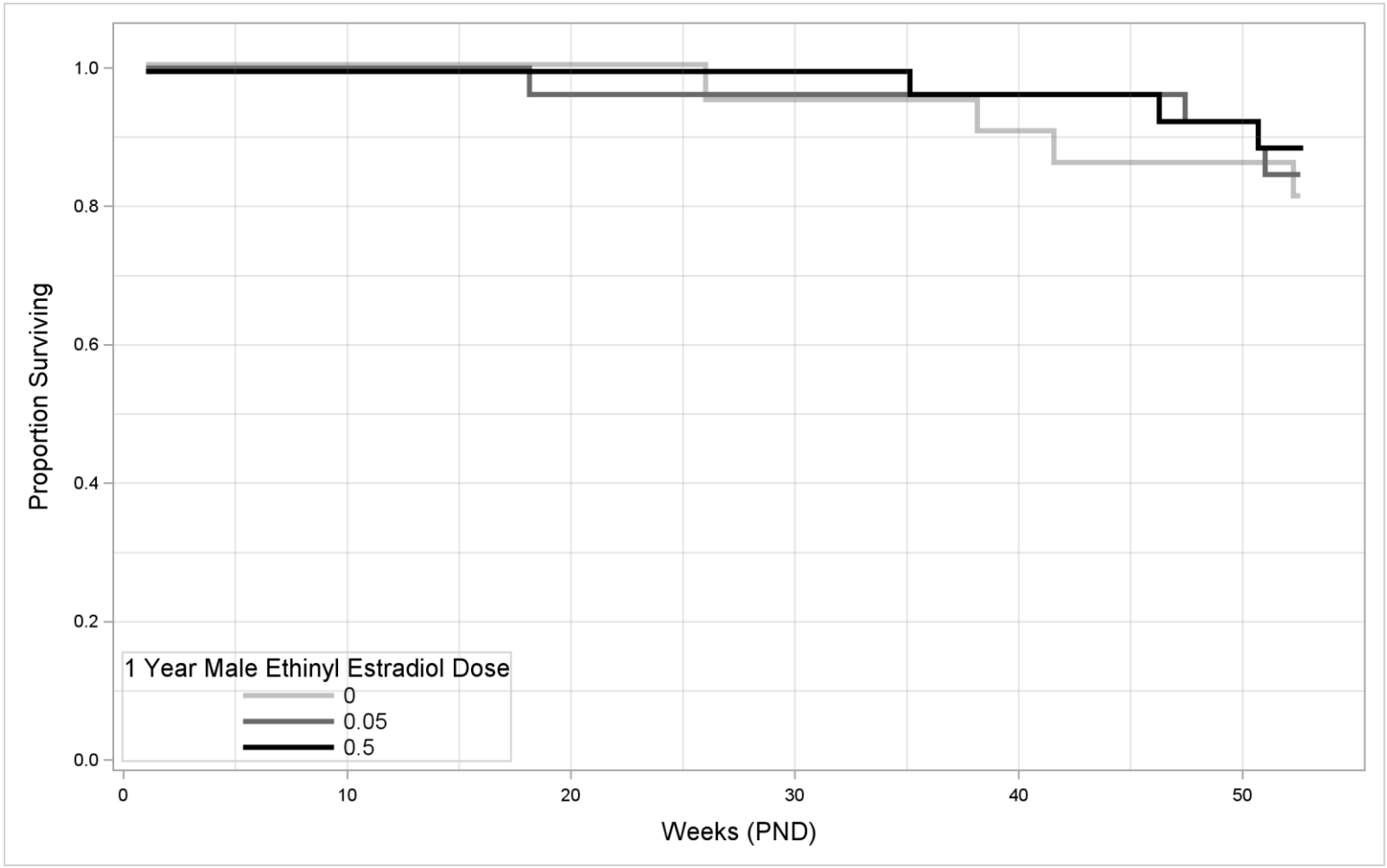


Figure 6. Kaplan-Meier Survival Curve for Interim Sacrifice Male EE_2 Continuous Dose (Weeks 4-52)



C. Data

Survival 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 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.