**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 SURVIVAL DATA

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**FOR**

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# Signatures

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# Statistical Analysis of Preweaning 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 preweaning survival.

## 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.

***Survival Data***

For this analysis, survival of all pups in the study after culling on PND 1 is followed until weaning at PND 21.

## 3. Statistical Methods

Statistical analyses were performed separately for the BPA dose groups and for the $EE_2$ dose groups. Animals with a disposition observed as dead or moribund were treated as uncensored observations, while those observed as reaching PND 21 were considered censored.

To compare survival of treatment groups to the control group, Cox proportional hazards regression analysis was performed. 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 and vehicle control groups.

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

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

### 4.1 BPA Treatments

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

The results of the proportional hazards model analysis for BPA dose groups 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 BPA dose groups, there were no statistically significant results for females or males.

### 4.2 $EE_2$ Treatments

Disposition counts and proportions for $EE_2$ dose groups are presented in Table 5 for females and in Table 6 for males.

The results of the proportional hazards model analysis for $EE_2$ dose groups are presented in Table 7 for females and in Table 8 for males. The hazard ratio was significant for the $EE_2$ 0.05 µg/kg bw/day dose group for females (hazard ratio=3.540, p=0.005). Hazard ratios of treatment groups to the vehicle control were not significant for males.

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

## 5. Conclusions

### 5.1 BPA Treatments

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

### 5.2 $EE_2$ Treatments

The hazard ratio was significant for the $EE_2$ 0.05 µg/kg bw/day dose group for females, with greater hazard for the dosed group relative to control. There were no significant differences in survival for $EE_2$ dose groups compared to vehicle control for males.

# Appendices

## A. Statistical Tables

***a) BPA Treatments***

***Table 1. Disposition and Censoring of Preweaning Females Bisphenol-A***

| *Dose (µg/kg$_{BW}$/day)* | *N* | *Dead* | *Missing* | *Moribund* | *PND 21* | *Reallocate* | *Censored* | *Uncensored* | *Proportion Censored*[1] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 311 | 5 | 3 | 1 | 294 | 8 | 302 | 9 | 0.971 |
| 2.5 | 266 | 11 | 5 | 1 | 241 | 8 | 249 | 17 | 0.936 |
| 25 | 259 | 7 | 2 | 5 | 237 | 8 | 245 | 14 | 0.946 |
| 250 | 250 | 6 | 5 | 4 | 227 | 8 | 235 | 15 | 0.940 |
| 2500 | 260 | 2 | 13 | 1 | 236 | 8 | 244 | 16 | 0.938 |
| 25000 | 244 | 3 | 6 | 2 | 225 | 8 | 233 | 11 | 0.955 |

[1] Uncensored animals include those that were dead, missing, or moribund; animals that reached PND 21 and reallocated animals are considered censored (reallocates were planned reassignments to an academic laboratory study at PND 15).

***Table 2. Disposition and Censoring of Preweaning Males Bisphenol-A***

| *Dose (µg/kg$_{BW}$/day)* | *N* | *Dead* | *Missing* | *Moribund* | *PND 21* | *Reallocate* | *Censored* | *Uncensored* | *Proportion Censored*[1] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 338 | 5 | 9 | 1 | 315 | 8 | 323 | 15 | 0.956 |
| 2.5 | 300 | 1 | 14 | 3 | 274 | 8 | 282 | 18 | 0.940 |
| 25 | 281 | 4 | 5 | 1 | 263 | 8 | 271 | 10 | 0.964 |
| 250 | 292 | 8 | 9 | 0 | 267 | 8 | 275 | 17 | 0.942 |
| 2500 | 292 | 4 | 6 | 1 | 273 | 8 | 281 | 11 | 0.962 |
| 25000 | 275 | 2 | 1 | 4 | 260 | 8 | 268 | 7 | 0.975 |

[1] Uncensored animals include those that were dead, missing, or moribund; animals that reached PND 21 and reallocated animals are considered censored (reallocates were planned reassignments to an academic laboratory study at PND 15).

***Table 3. Cox Proportional Hazards Analysis for Female Bisphenol-A Dose (µg/kg$_{BW}$/day)[1]***

| *Dose* | *Hazard Ratio[2]* | *P-value[3]* |
|---|---|---|
| 0 | - | 0.361 |
| 2.5 | 2.251 | 0.245 |
| 25 | 1.878 | 0.280 |
| 250 | 2.104 | 0.245 |
| 2500 | 2.185 | 0.245 |
| 25000 | 1.574 | 0.313 |

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

***Table 4. Cox Proportional Hazards Analysis for Male Bisphenol-A Dose (µg/kg$_{BW}$/day)[1]***

| *Dose* | *Hazard Ratio[2]* | *P-value[3]* |
|---|---|---|
| 0 | - | 0.143 |
| 2.5 | 1.367 | 1.000 |
| 25 | 0.795 | 1.000 |
| 250 | 1.320 | 1.000 |
| 2500 | 0.842 | 1.000 |
| 25000 | 0.565 | 1.000 |

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

***b) $EE_2$ Treatments***

*Table 5. Disposition and Censoring of Preweaning Females Ethinyl Estradiol*

| Dose ($\mu g/kg_{BW}/day$) | N | Dead | Missing | Moribund | PND 21 | Reallocate | Censored | Uncensored | Proportion Censored[1] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 311 | 5 | 3 | 1 | 294 | 8 | 302 | 9 | 0.971 |
| 0.05 | 153 | 8 | 5 | 2 | 130 | 8 | 138 | 15 | 0.902 |
| 0.5 | 180 | 5 | 3 | 0 | 164 | 8 | 172 | 8 | 0.956 |

[1] Uncensored animals include those that were dead, missing, or moribund; animals that reached PND 21 and reallocated animals are considered censored (reallocates were planned reassignments to an academic laboratory study at PND 15).

*Table 6. Disposition and Censoring of Preweaning Males Ethinyl Estradiol*

| Dose ($\mu g/kg_{BW}/day$) | N | Dead | Missing | Moribund | PND 21 | Reallocate | Censored | Uncensored | Proportion Censored[1] |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 338 | 5 | 9 | 1 | 315 | 8 | 323 | 15 | 0.956 |
| 0.05 | 156 | 8 | 5 | 2 | 133 | 8 | 141 | 15 | 0.904 |
| 0.5 | 208 | 5 | 6 | 0 | 189 | 8 | 197 | 11 | 0.947 |

[1] Uncensored animals include those that were dead, missing, or moribund; animals that reached PND 21 and reallocated animals are considered censored (reallocates were planned reassignments to an academic laboratory study at PND 15).

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***Table 7. Cox Proportional Hazards Analysis for Female Ethinyl Estradiol Dose (µg/kg·BW/day)***

| *Dose* | *Hazard Ratio*[1] | *P-value*[2] |
|---|---|---|
| 0.05 | 3.540 | 0.005* |
| 0.5 | 1.547 | 0.369 |

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

***Table 8. Cox Proportional Hazards Analysis for Male Ethinyl Estradiol Dose (µg/kg·BW/day)***

| *Dose* | *Hazard Ratio*[1] | *P-value*[2] |
|---|---|---|
| 0.05 | 2.196 | 0.062 |
| 0.5 | 1.193 | 0.656 |

[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 Female BPA Treatments***

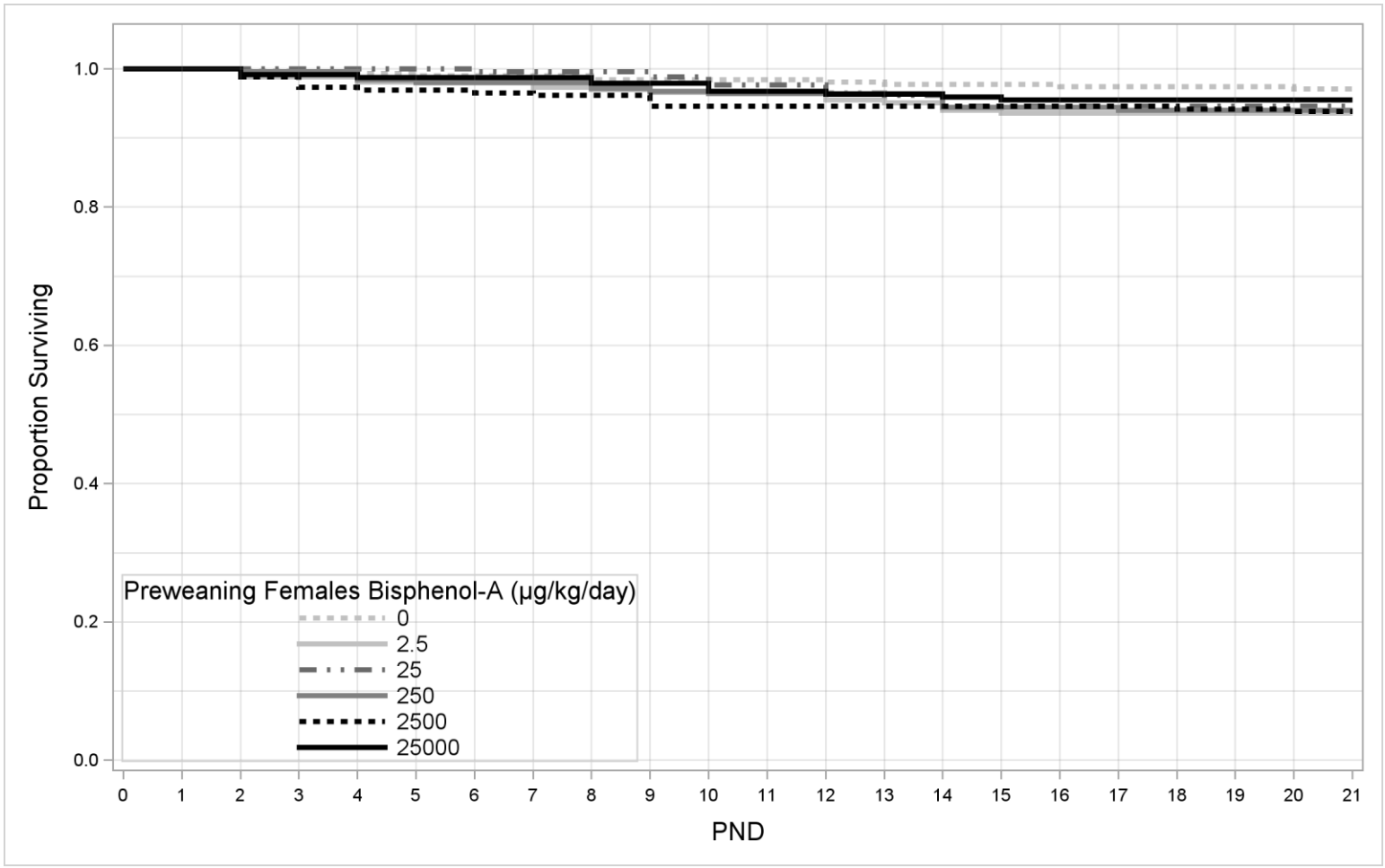


***Figure 2. Kaplan-Meier Survival Curve for Male BPA Treatments***

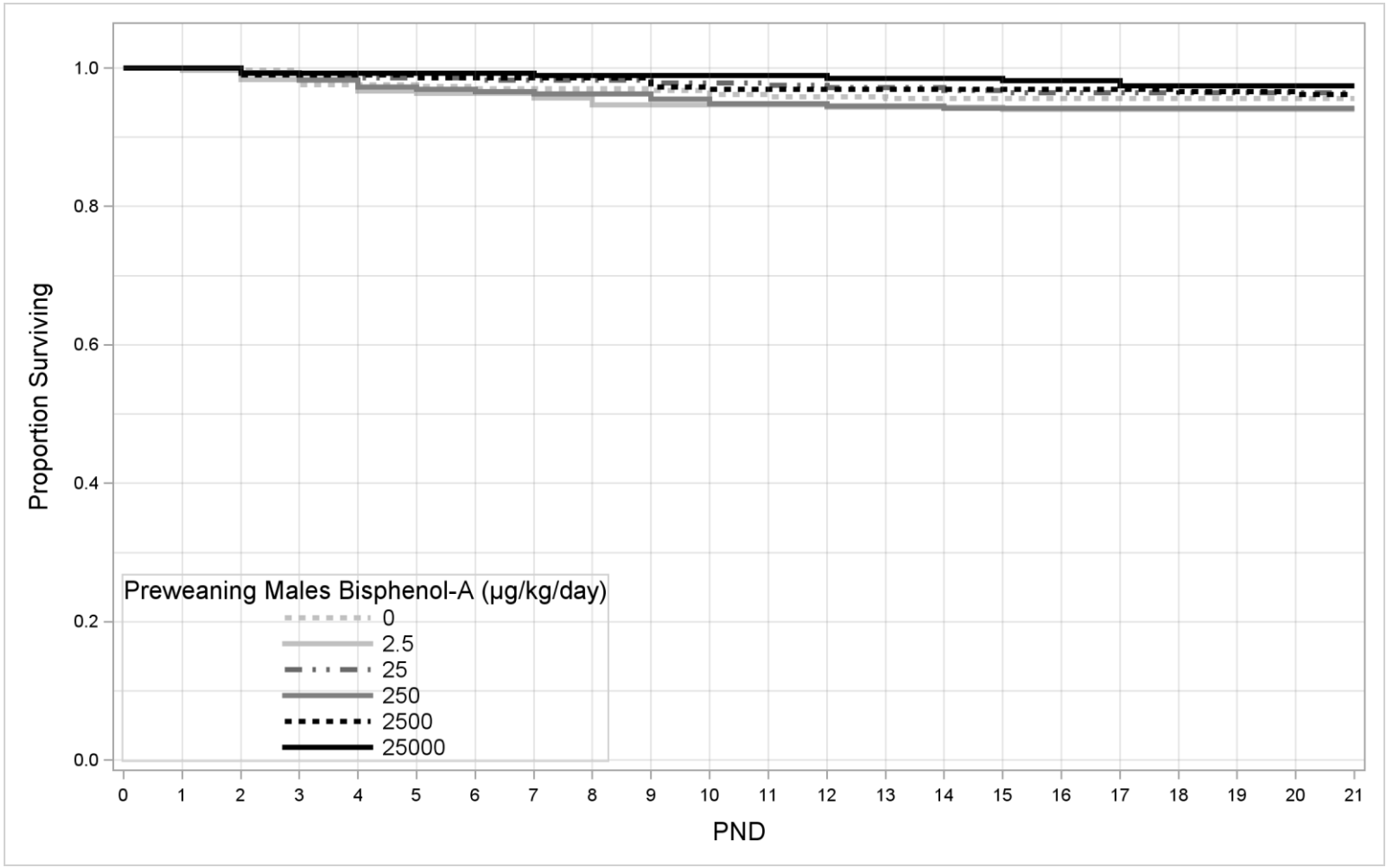


***Figure 3. Kaplan-Meier Survival Curve for Female $EE_2$ Treatments***

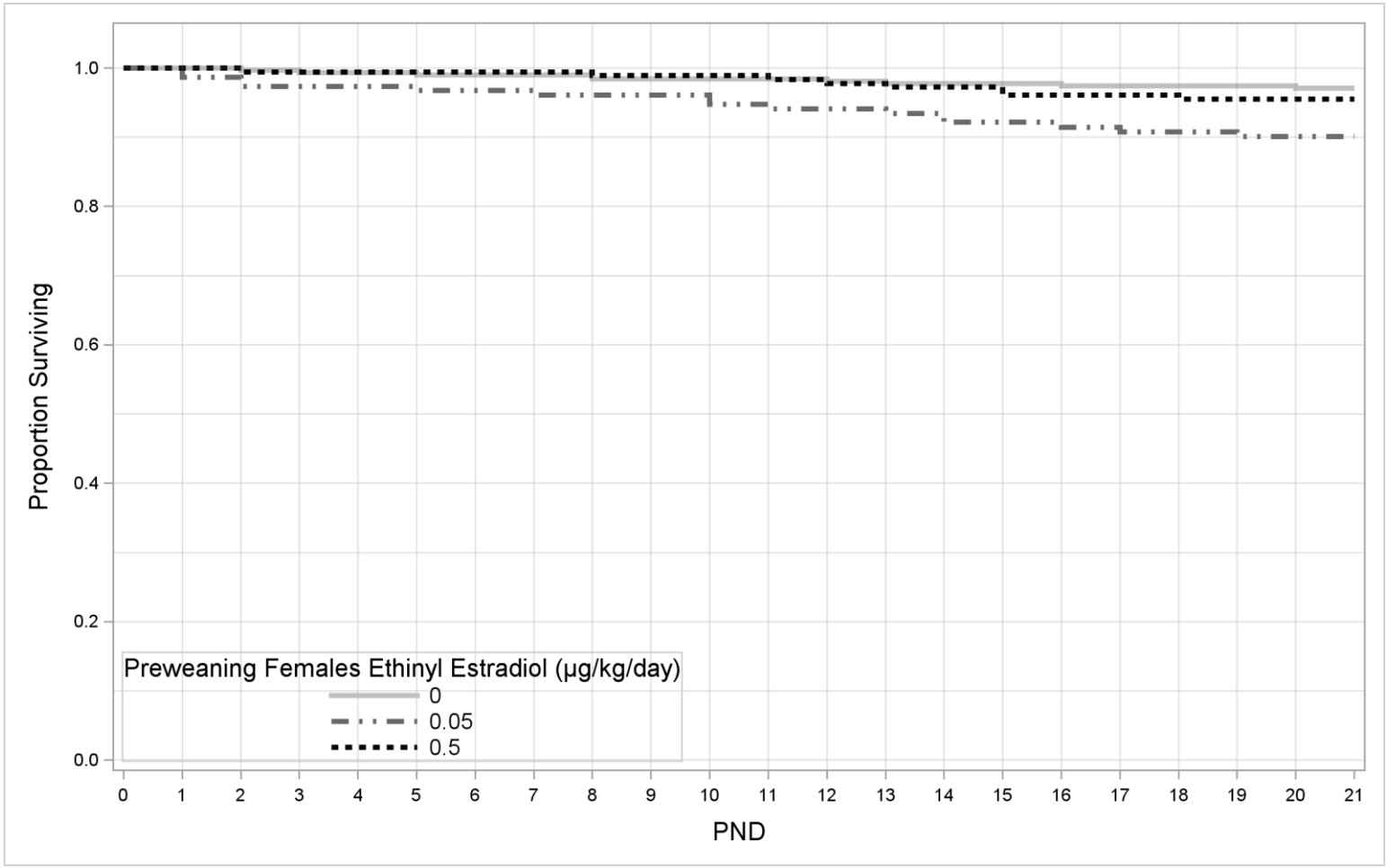
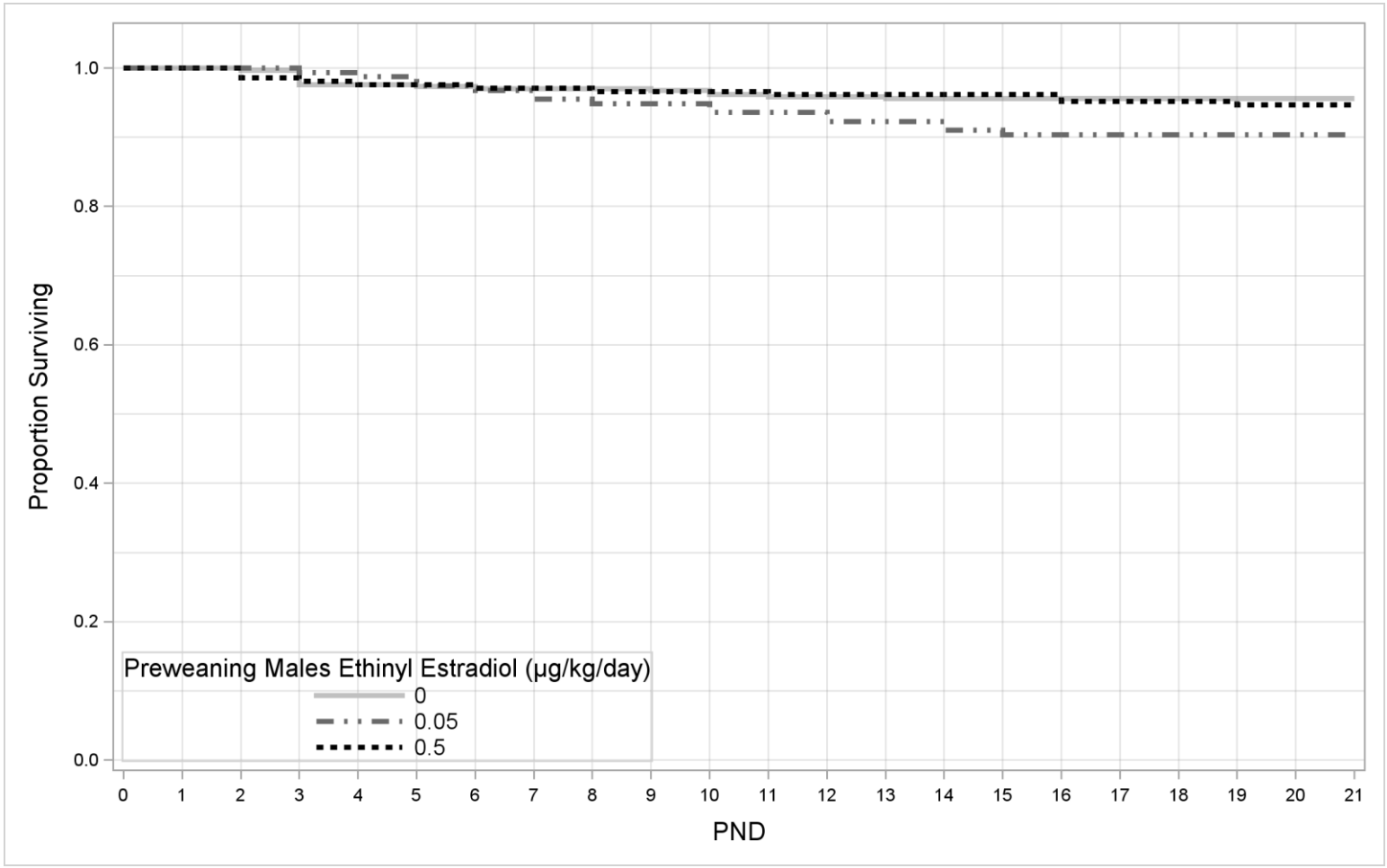


***Figure 4. Kaplan-Meier Survival Curve for Male EE2 Treatments***



## 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 .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.