**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 INTERIM SACRIFICE SPERM PARAMETER DATA

**PREPARED BY**

**BETH E. JULIAR**
**DIVISION OF BIOINFORMATICS AND BIOSTATISTICS**

**FOR**

**NATIONAL CENTER FOR TOXICOLOGICAL RESEARCH**
**3900 NCTR ROAD**
**JEFFERSON, ARKANSAS 72079**

# Signatures

*Report prepared by Statistician:* *Date*

---

**Beth E. Juliar**, M.A., M.S., Division of Bioinformatics and Biostatistics/NCTR/FDA

*Report reviewed by Statistician:* *Date*

---

**Paul Felton**, M.S., Division of Bioinformatics and Biostatistics /NCTR/FDA

*Statistical Team Leader:* *Date*

---

**Paul Felton**, M.S., Division of Bioinformatics and Biostatistics /NCTR/FDA

# Table of Contents

# Statistical Analysis of Interim Sacrifice Sperm Parameter 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 based on sperm parameters from the one year interim sacrifice animals.

## 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.

***Sperm Parameters Data***

For the one year interim sacrifice males, the left testis was to be used for evaluation of testicular spermatid head counts, and the left epididymis was to be used for epididymal sperm counts, morphology, and motility evaluations.

## 3. Statistical Methods

Statistical analyses and comparisons were conducted separately by compound and dosing regimen: vehicle control and BPA treatments in the stop dose arm; vehicle control and BPA treatments in the continuous dose arm; and vehicle control and $EE_2$ treatments in the continuous dose arm. Analysis of sperm morphology data was performed using a generalized linear model with a Poisson distribution and a log link function. Each treatment was compared to the vehicle control group, and adjustment for multiple comparisons was performed using Dunnett's method. Percent sperm motility, testes sperm counts, and cauda sperm counts were analyzed using an ANOVA model with Kenward-Roger estimated degrees of freedom (Kenward and Roger, 1997). Each treatment was compared to the control group, and adjustment for multiple comparisons was performed using Dunnett's method. Tests of trend, increasing treatment effect with increasing dose, were performed for each compound and dosing regimen. Tests were conducted as two-sided at the 0.05 significance level.

For each sperm endpoint, a sensitivity analysis was also performed. During initial preweaning of animals, 60 core study 1 year interim sacrifice males with sperm data (8 in vehicle control, 40 in BPA 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 12 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 60 males was also performed to test the robustness of the results. Additional statistically significant pairwise comparisons from the sensitivity analysis are reported in the text.

***References***

Kenward, M. G. and Roger, J. H. (1997), "Small Sample Inference for Fixed Effects from Restricted Maximum Likelihood," *Biometrics* **53**:983–997.

## *4. Results*

Tables are included in Appendix A.

### *4.1 BPA Treatments in the Stop Dose Arm*

Counts and percentages for sperm morphology are given in Table 1 for BPA treatments in the stop dose arm. Analysis results for sperm morphology are given in Table 3. Only five animals total in BPA 25, 250 and 2500 µg/kg bw/day treatment groups had sperm head abnormalities, and there were no significant results for comparisons of treatments to the vehicle control. For sperm tail abnormalities and total abnormalities, there were no statistically significant differences for any BPA treatment compared to the vehicle control.

Summary statistics for percent sperm motility, cauda sperm counts, and testes sperm counts are presented in Table 2. ANOVA results from analysis of treatment effect for percent motility, cauda counts, and testes counts are presented in Table 4. There were no statistically significant trends or differences for treatment groups compared to the control. Because of unequal variance across treatment groups for sperm testes counts identified using Levene's test, a nonparametric analysis was also conducted, but there was no difference in conclusions.

There were no statistically significant results in the sensitivity analyses for any sperm endpoint in the stop dose BPA arm.

### *4.2 BPA Treatments in the Continuous Dose Arm*

Counts and percentages for sperm morphology are given in Table 5 for BPA treatments in the continuous dose arm. Analysis results for sperm morphology are given in Table 7. There were no significant results for comparisons of treatments to the vehicle control for sperm head, tail, or total abnormalities.

Summary statistics for percent sperm motility, cauda sperm counts, and testes sperm counts are presented in Table 6. ANOVA results from analysis of treatment effect for percent motility, cauda counts, and testes counts are presented in Table 8. There were no statistically significant trends or differences for treatment groups compared to the vehicle control.

There were no statistically significant results in the sensitivity analyses for any sperm endpoint in the continuous dose BPA arm.

### *4.3 $EE_2$ Treatments in the Continuous Dose Arm*

Counts and percentages for sperm morphology are given in Table 9 for $EE_2$ treatments in the continuous dose arm. Analysis results for sperm morphology are given in Table 11. There were no significant results for comparisons of treatments to the vehicle control for sperm head, tail, or total abnormalities.

Summary statistics for percent sperm motility, cauda sperm counts, and testes sperm counts are presented in Table 10. ANOVA results from analysis of treatment effect for percent motility, cauda counts, and testes counts are presented in Table 12. There were no statistically significant trends or differences for treatment groups compared to the vehicle control.

There were no statistically significant results in the sensitivity analyses for any sperm endpoint in the continuous dose $EE_2$ arm.

## *5. Conclusions*

In both the stop dose and the continuous dose arms, there were no significant results in analysis of BPA or $EE_2$ treatments compared to the vehicle control for sperm head, tail, or total abnormalities. In analyses of percent sperm motility, cauda sperm counts and testes sperm counts, there were no statistically significant differences for BPA or $EE_2$ treatment groups compared to the vehicle control in the stop dose or continuous dose arm.

# Appendices

## *A. Statistical Tables*

***a) BPA Treatments in the Stop Dose Arm***

| *Table 1. Counts of Sperm Morphology Abnormality for Bisphenol-A Stop Dose (µg/kg·BW/day)* | | | | |
|---|---|---|---|---|
| ***Dose*** | ***Type*** | ***Abnormal Count*** | ***N (Animals)*** | ***Percent*** |
| 0 | Head | 0 | 20 | 100.0 |
| | Tail | 0 | 17 | 85.0 |
| | | 1 | 3 | 15.0 |
| | Total | 0 | 17 | 85.0 |
| | | 1 | 3 | 15.0 |
| 2.5 | Head | 0 | 20 | 100.0 |
| | Tail | 0 | 17 | 85.0 |
| | | 1 | 3 | 15.0 |
| | Total | 0 | 17 | 85.0 |
| | | 1 | 3 | 15.0 |
| 25 | Head | 0 | 17 | 89.5 |
| | | 1 | 2 | 10.5 |
| | Tail | 0 | 16 | 84.2 |
| | | 1 | 2 | 10.5 |
| | | 2 | 1 | 5.3 |
| | Total | 0 | 15 | 78.9 |
| | | 1 | 2 | 10.5 |
| | | 2 | 2 | 10.5 |
| 250 | Head | 0 | 18 | 94.7 |
| | | 1 | 1 | 5.3 |
| | Tail | 0 | 17 | 89.5 |
| | | 1 | 2 | 10.5 |
| | Total | 0 | 16 | 84.2 |
| | | 1 | 3 | 15.8 |
| 2500 | Head | 0 | 18 | 90.0 |
| | | 1 | 2 | 10.0 |
| | Tail | 0 | 16 | 80.0 |
| | | 1 | 4 | 20.0 |
| | Total | 0 | 14 | 70.0 |
| | | 1 | 6 | 30.0 |
| 25000 | Head | 0 | 22 | 100.0 |
| | Tail | 0 | 22 | 100.0 |
| | Total | 0 | 22 | 100.0 |

| *Table 2. Summary Statistics of Sperm Outcomes for Bisphenol-A Stop-Dose (µg/kg·BW/day)* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***0*** | | | ***2.5*** | | | ***25*** | | | ***250*** | | | ***2500*** | | | ***25000*** | | |
| ***Outcome*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** |
| Cauda Counts | 20 | 1059.1 | 86.6 | 20 | 1185.5 | 83.3 | 19 | 1016.7 | 65.5 | 19 | 1111.3 | 66.1 | 20 | 1020.3 | 89.1 | 22 | 1015.6 | 81.1 |
| Percent Motility | 20 | 74.8 | 4.3 | 20 | 75.8 | 2.3 | 19 | 72.4 | 3.4 | 19 | 70.2 | 4.2 | 20 | 67.9 | 5.6 | 22 | 77.7 | 2.2 |
| Testes Counts | 20 | 76.5 | 9.4 | 20 | 77.7 | 4.8 | 19 | 72.2 | 5.3 | 19 | 72.5 | 6.3 | 20 | 73.6 | 9.0 | 22 | 75.8 | 4.7 |

| *Table 3. Poisson Regression of Sperm Morphology Abnormality Counts Per Animal for Bisphenol-A Stop Dose (µg/kg·BW/day)* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***0*** | | | ***2.5*** | | | ***25*** | | | ***250*** | | | ***2500*** | | | ***25000*** | | |
| ***Abnormality*** | ***Mean[2]*** | ***SE*** | ***Trend*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** |
| Total | 0.15 | 0.09 | 0.970 | 0.15 | 0.09 | 1.000 | 0.32 | 0.13 | 0.748 | 0.16 | 0.09 | 1.000 | 0.30 | 0.12 | 0.795 | 0.00 | 0.00 | 1.000 |
| Head | 0.00 | 0.00 | 0.993 | 0.00 | 0.00 | 1.000 | 0.11 | 0.07 | 1.000 | 0.05 | 0.05 | 1.000 | 0.10 | 0.07 | 1.000 | 0.00 | 0.00 | 1.000 |
| Tail | 0.15 | 0.09 | 0.969 | 0.15 | 0.09 | 1.000 | 0.21 | 0.11 | 0.992 | 0.11 | 0.07 | 0.996 | 0.20 | 0.10 | 0.996 | 0.00 | 0.00 | 1.000 |

[1] All p-values for dose comparisons are relative to control and were adjusted using Dunnett's method.
[2] Mean counts and standard errors were estimated using Poisson analysis.

| *Table 4. ANOVA Comparison of Least Squares Mean Sperm Outcomes for Bisphenol-A Stop Dose (µg/kg·BW/day)[1]* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***0*** | | | ***2.5*** | | | ***25*** | | | ***250*** | | | ***2500*** | | | ***25000*** | | |
| ***Outcome*** | ***Mean*** | ***SE*** | ***Trend*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** |
| Percent Sperm Motility | 74.8 | 3.8 | 0.725 | 75.8 | 3.8 | 1.000 | 72.4 | 3.9 | 0.991 | 70.2 | 3.9 | 0.872 | 67.9 | 3.8 | 0.581 | 77.7 | 3.7 | 0.974 |
| Cauda Sperm Counts | 1059.1 | 79.7 | 0.348 | 1185.5 | 79.7 | 0.690 | 1016.7 | 81.8 | 0.996 | 1111.3 | 81.8 | 0.989 | 1020.3 | 79.7 | 0.997 | 1015.6 | 76.0 | 0.994 |
| Testes Sperm Counts | 76.5 | 6.8 | 0.786 | 77.7 | 6.8 | 1.000 | 72.2 | 7.0 | 0.990 | 72.5 | 7.0 | 0.993 | 73.6 | 6.8 | 0.998 | 75.8 | 6.5 | 1.000 |

[1] All p-values for dose comparisons are relative to the control group and were adjusted using Dunnett's method.

***b) BPA Treatments in the Continuous Dose Arm***

| *Table 5. Counts of Sperm Morphology Abnormality for Bisphenol-A Continuous Dose (µg/kg'BW/day)* | | | | |
|---|---|---|---|---|
| ***Dose*** | ***Type*** | ***Abnormal Count*** | ***N (Animals)*** | ***Percent*** |
| 0 | Head | 0 | 18 | 100.0 |
| | Tail | 0 | 15 | 83.3 |
| | | 1 | 3 | 16.7 |
| | Total | 0 | 15 | 83.3 |
| | | 1 | 3 | 16.7 |
| 2.5 | Head | 0 | 21 | 95.5 |
| | | 1 | 1 | 4.5 |
| | Tail | 0 | 15 | 68.2 |
| | | 1 | 6 | 27.3 |
| | | 2 | 1 | 4.5 |
| | Total | 0 | 14 | 63.6 |
| | | 1 | 7 | 31.8 |
| | | 2 | 1 | 4.5 |
| 25 | Head | 0 | 17 | 94.4 |
| | | 1 | 1 | 5.6 |
| | Tail | 0 | 15 | 83.3 |
| | | 1 | 3 | 16.7 |
| | Total | 0 | 15 | 83.3 |
| | | 1 | 2 | 11.1 |
| | | 2 | 1 | 5.6 |
| 250 | Head | 0 | 22 | 91.7 |
| | | 1 | 2 | 8.3 |
| | Tail | 0 | 22 | 91.7 |
| | | 1 | 2 | 8.3 |
| | Total | 0 | 20 | 83.3 |
| | | 1 | 4 | 16.7 |
| 2500 | Head | 0 | 16 | 88.9 |
| | | 1 | 2 | 11.1 |
| | Tail | 0 | 16 | 88.9 |
| | | 1 | 2 | 11.1 |
| | Total | 0 | 14 | 77.8 |
| | | 1 | 4 | 22.2 |
| 25000 | Head | 0 | 21 | 100.0 |
| | Tail | 0 | 18 | 85.7 |
| | | 1 | 3 | 14.3 |
| | Total | 0 | 18 | 85.7 |
| | | 1 | 3 | 14.3 |

| *Table 6. Summary Statistics of Sperm Outcomes for Bisphenol-A Continuous Dose (µg/kg'BW/day)* | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***0*** | | | ***2.5*** | | | ***25*** | | | ***250*** | | | ***2500*** | | | ***25000*** | | |
| ***Outcome*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** | ***N*** | ***Mean*** | ***SE*** |
| Cauda Counts | 18 | 990.8 | 78.0 | 22 | 999.3 | 87.7 | 18 | 1075.5 | 68.5 | 24 | 997.7 | 68.7 | 18 | 1061.9 | 51.0 | 21 | 1027.2 | 80.7 |
| Percent Motility | 18 | 65.9 | 4.6 | 22 | 64.0 | 5.1 | 18 | 72.4 | 3.1 | 24 | 66.7 | 4.5 | 18 | 69.9 | 3.4 | 21 | 69.4 | 4.2 |
| Testes Counts | 18 | 83.4 | 9.5 | 22 | 76.8 | 7.7 | 18 | 88.5 | 6.1 | 24 | 81.5 | 6.8 | 18 | 85.1 | 7.1 | 21 | 76.6 | 6.0 |

*Table 7. Poisson Regression of Sperm Morphology Abnormality Counts Per Animal for Bisphenol-A Continuous Dose (µg/kg·BW·/day)[1]*

| | 0 | | | 2.5 | | | 25 | | | 250 | | | 2500 | | | 25000 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Abnormality* | *Mean[2]* | *SE* | *Trend* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* |
| Total | 0.17 | 0.10 | 0.524 | 0.41 | 0.14 | 0.491 | 0.22 | 0.11 | 0.993 | 0.17 | 0.08 | 1.000 | 0.22 | 0.11 | 0.993 | 0.14 | 0.08 | 1.000 |
| Head | 0.00 | 0.00 | 0.999 | 0.05 | 0.05 | 1.000 | 0.06 | 0.06 | 1.000 | 0.08 | 0.06 | 1.000 | 0.11 | 0.08 | 1.000 | 0.00 | 0.00 | 1.000 |
| Tail | 0.17 | 0.10 | 0.299 | 0.36 | 0.13 | 0.666 | 0.17 | 0.10 | 1.000 | 0.08 | 0.06 | 0.907 | 0.11 | 0.08 | 0.990 | 0.14 | 0.08 | 1.000 |

[1] All p-values for dose comparisons are relative to control and were adjusted using Dunnett's method.
[2] Mean counts and standard errors were estimated using Poisson analysis.

*Table 8. ANOVA Comparison of Least Squares Mean Sperm Outcomes for Bisphenol-A Continuous Dose (µg/kg·BW·/day)[1]*

| | 0 | | | 2.5 | | | 25 | | | 250 | | | 2500 | | | 25000 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Outcome* | *Mean* | *SE* | *Trend* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* | *Mean* | *SE* | *P* |
| Percent Sperm Motility | 65.9 | 4.6 | 0.425 | 64.0 | 4.2 | 0.998 | 72.4 | 4.6 | 0.769 | 66.7 | 4.0 | 1.000 | 69.9 | 4.6 | 0.956 | 69.4 | 4.3 | 0.971 |
| Cauda Sperm Counts | 990.8 | 79.2 | 0.647 | 999.3 | 71.7 | 1.000 | 1075.5 | 79.2 | 0.904 | 997.7 | 68.6 | 1.000 | 1061.9 | 79.2 | 0.950 | 1027.2 | 73.3 | 0.997 |
| Testes Sperm Counts | 83.4 | 7.7 | 0.789 | 76.8 | 7.0 | 0.950 | 88.5 | 7.7 | 0.986 | 81.5 | 6.7 | 1.000 | 85.1 | 7.7 | 1.000 | 76.6 | 7.1 | 0.944 |

[1] All p-values for dose comparisons are relative to the control group and were adjusted using Dunnett's method.

### *c) $EE_2$ Treatments in the Continuous Dose Arm*

*Table 9. Counts of Sperm Morphology Abnormality for Ethinyl Estradiol Dose (µg/kg·BW/day)*

| Dose | Type | Abnormal Count | N (Animals) | Percent |
|---|---|---|---|---|
| 0 | Head | 0 | 18 | 100.0 |
| | Tail | 0 | 15 | 83.3 |
| | | 1 | 3 | 16.7 |
| | Total | 0 | 15 | 83.3 |
| | | 1 | 3 | 16.7 |
| 0.05 | Head | 0 | 17 | 77.3 |
| | | 1 | 5 | 22.7 |
| | Tail | 0 | 17 | 77.3 |
| | | 1 | 5 | 22.7 |
| | Total | 0 | 14 | 63.6 |
| | | 1 | 6 | 27.3 |
| | | 2 | 2 | 9.1 |
| 0.5 | Head | 0 | 22 | 95.7 |
| | | 1 | 1 | 4.3 |
| | Tail | 0 | 21 | 91.3 |
| | | 1 | 1 | 4.3 |
| | | 2 | 1 | 4.3 |
| | Total | 0 | 20 | 87.0 |
| | | 1 | 2 | 8.7 |
| | | 2 | 1 | 4.3 |

*Table 10. Summary Statistics of Sperm Outcomes for Ethinyl Estradiol Dose (µg/kg·BW/day)*

| | 0 | | | 0.05 | | | 0.5 | | |
|---|---|---|---|---|---|---|---|---|---|
| Outcome | N | Mean | SE | N | Mean | SE | N | Mean | SE |
| Cauda Counts | 18 | 990.8 | 78.0 | 22 | 891.7 | 71.6 | 23 | 856.9 | 53.1 |
| Percent Motility | 18 | 65.9 | 4.6 | 22 | 67.2 | 4.8 | 23 | 70.8 | 2.7 |
| Testes Counts | 18 | 83.4 | 9.5 | 22 | 70.0 | 4.7 | 23 | 73.7 | 4.5 |

***Table 11. Poisson Regression of Sperm Morphology Abnormality Counts Per Animal for Ethinyl Estradiol Dose (µg/kg·BW/day)[1]***

| | 0 | | 0.05 | | | 0.5 | | |
|---|---|---|---|---|---|---|---|---|
| ***Abnormality*** | ***Mean[2]*** | ***SE*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** |
| Total | 0.17 | 0.10 | 0.45 | 0.14 | 0.213 | 0.17 | 0.09 | 0.997 |
| Head | 0.00 | 0.00 | 0.23 | 0.10 | 0.972 | 0.04 | 0.04 | 0.976 |
| Tail | 0.17 | 0.10 | 0.23 | 0.10 | 0.873 | 0.13 | 0.08 | 0.934 |

[1] All p-values for dose comparisons are relative to control and were adjusted using Dunnett's method.
[2] Mean counts and standard errors were estimated using Poisson analysis.

***Table 12. ANOVA Comparison of Least Squares Mean Sperm Outcomes for Ethinyl Estradiol Dose (µg/kg·BW/day)[1]***

| | 0 | | 0.05 | | | 0.5 | | |
|---|---|---|---|---|---|---|---|---|
| ***Outcome*** | ***Mean*** | ***SE*** | ***Mean*** | ***SE*** | ***P*** | ***Mean*** | ***SE*** | ***P*** |
| Percent Sperm Motility | 65.9 | 4.4 | 67.2 | 4.0 | 0.968 | 70.8 | 3.9 | 0.617 |
| Cauda Sperm Counts | 990.8 | 72.3 | 891.7 | 65.4 | 0.487 | 856.9 | 64.0 | 0.282 |
| Testes Sperm Counts | 83.4 | 6.7 | 70.0 | 6.0 | 0.239 | 73.7 | 5.9 | 0.445 |

[1] All p-values for dose comparisons are relative to the control group and were adjusted using Dunnett's method.

### *B. Data*

Sperm parameter data were provided in an Excel spreadsheet from the Principal Investigator.

# 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.

## 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.