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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 UTERINE IMPLANTATION SITES DATA

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FOR

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Statistical Analysis of Uterine Implantation Sites 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 ammonium sulfide implantation (AVIS) 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.

AVIS Data

AVIS data were collected for dams with a sperm-positive vaginal smear or an in situ vaginal copulation plug.

3. Statistical Methods

AVIS counts were analyzed using a one-way analysis of variance (ANOVA) with Dunnett's test for comparisons to the vehicle control group, adjusted for multiple comparisons.

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 animals was also performed to test the robustness of the results.

4. Results

Tables are included in Appendix A.

4.1 BPA Treatments

Summary statistics and p-values for comparisons to the vehicle control group are given in Table 1. There were no statistically significant differences from control in mean AVIS count for any BPA or EE_2 group.

4.2 Sensitivity Analysis

There were no statistically significant differences from control in mean AVIS count for any BPA or EE_2 group.

5. Conclusions

In comparisons of BPA and EE_2 dosed groups to the control group, there were no significant differences for mean AVIS count.

Appendices

A. Statistical Tables

Table 1. Comparison of AVIS Count Across Treatment Groups using Dunnett's Test

Dose ***(µg/kg·BW/day)***	N	Mean	Std Err.	Min	Median	Max	P Value
Control	78	12.8	0.5	0.0	14.0	19.0	
BPA 2.5	74	12.4	0.6	0.0	14.5	19.0	0.994
BPA 25	74	11.2	0.7	0.0	13.0	18.0	0.264
BPA 250	78	11.2	0.6	0.0	13.0	20.0	0.268
BPA 2500	74	11.6	0.6	0.0	14.0	18.0	0.540
BPA 25000	70	12.2	0.6	0.0	13.0	22.0	0.972
EE2 0.5	49	11.7	0.8	0.0	13.0	18.0	0.750
EE2 5.0	59	12.1	0.7	0.0	15.0	18.0	0.949

B. Data

AVIS data were provided in a QA audited PDF by the PI, were exported to Microsoft Excel, and imported into SAS for analysis.

Quality Control

1. Data Verification

The extraction of the data into SAS was verified by the 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.

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.