

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 F₁ PUPS FROM POSTNATAL DAY (PND) 1; CONTINUOUS AND
STOP DOSE (PND 21) EXPOSURES**

STATISTICAL REPORT

STATISTICAL ANALYSIS OF INTERIM SACRIFICE FEED CONSUMPTION DATA

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Table of Contents

1. Objectives	1
1.1 Project Objectives.....	1
1.2 Analysis Objectives	1
2. Experimental Design.....	1
3. Statistical Methods.....	1
4. Results.....	1
4.1 BPA Stop Dose Treatments	2
4.2 BPA Continuous Dose Treatments.....	2
4.3 EE ₂ Treatments	2
Appendices.....	3
A. Statistical Tables	3
a) BPA Stop Dose Treatments	4
b) BPA Continuous Dose Treatments	6
c) EE ₂ Treatments	8
B. Figures.....	10
C. Data	17
Quality Control	18

Statistical Analysis of Interim Sacrifice Feed Consumption 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 summarize the effects of exposure to BPA in Sprague-Dawley rats regarding feed consumption.

2. Experimental Design

The study design consisted of first generation female and male rats (F₀) for up to 600 mating pairs randomized to treatment groups in 5 loads. The goal of the F₀ 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 EE₂ 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 F₀ dams and F₁ pups, the second study generation. Litters were culled to 10 pups on PND 1. There were two study dosing arms of F₁ 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 EE₂ daily dose groups for the continuous dosing arm only. From the F₁ 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.

Feed Consumption Data

Feed consumption was measured weekly through approximately 13 weeks of age and approximately monthly afterwards.

3. Statistical Methods

For this analysis, feed consumption of pups was followed beginning at 4 through 48 weeks of age. Statistical analyses were performed separately for the BPA study arms, stop dose and continuous dose, and for EE₂ continuous dose. Mean daily feed consumption for each animal was estimated at the midpoint of 4 week intervals for each group.

4. Results

Results of analyses using all study animals are presented in Appendix A for Tables and in Appendix B for Figures. Animals were removed up to a full month following their last monthly feed measurement. Therefore, most animals had no measurement for their final month on study. In addition, there were a number of missing values throughout the study due to technical problems in data collection.

4.1 BPA Stop Dose Treatments

Summary statistics of feed consumption for BPA stop dose groups are presented in Table 1 for females and in Table 2 for males.

4.2 BPA Continuous Dose Treatments

Summary statistics of feed consumption for BPA continuous dose groups are presented in Table 3 for females and in Table 4 for males.

4.3 EE₂ Treatments

Summary statistics of feed consumption for EE₂ dose groups are presented in Table 5 for females and in Table 6 for males.

Appendices

A. Statistical Tables

a) *BPA Stop Dose Treatments*

Table 1. Interim Summary Statistics for Mean Daily Feed Consumption (g) per Animal¹ for Female Bisphenol-A Stop-Dose

<i>Weeks</i>	<i>Dose (µg/kg_{BW}/day)</i>																				
	<i>0</i>			<i>2.5</i>			<i>25</i>			<i>250</i>			<i>2500</i>			<i>25000</i>					
<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	
4	10	8.6	1.3	11	6.4	0.7	9	7.9	0.5	11	7.0	0.6	10	8.8	0.8	11	7.6	0.6			
5-8	10	15.6	0.6	11	15.2	0.4	10	16.3	0.4	11	16.1	0.6	10	15.9	0.4	11	16.5	0.7			
9-12	10	18.9	0.7	11	19.1	0.5	10	19.6	0.6	11	18.9	0.5	10	19.0	0.7	11	19.6	0.7			
13-16	10	18.4	0.8	11	18.2	0.4	10	17.6	0.6	11	19.0	0.7	10	18.3	0.6	11	19.0	0.9			
17-20	10	17.7	0.7	11	17.2	0.3	10	16.7	0.4	11	18.4	0.7	10	16.3	1.1	11	18.2	0.8			
21-24	10	17.3	0.6	11	19.2	1.3	9	18.3	1.0	11	16.1	1.3	10	17.4	0.5	11	18.5	1.0			
25-28	10	17.9	0.8	11	17.3	0.7	10	18.5	2.4	11	16.2	1.4	10	17.3	0.5	11	19.5	1.0			
29-32	10	21.3	1.8	8	18.2	2.0	9	17.5	1.8	11	17.9	2.9	9	14.9	1.3	9	19.9	2.8			
33-36	10	16.0	1.4	11	16.6	1.4	10	13.2	1.8	11	17.7	1.0	10	15.0	1.7	9	16.3	1.9			
37-40	9	17.4	2.3	11	15.9	1.6	8	15.2	1.9	10	15.8	1.9	10	15.5	1.8	11	18.7	2.2			
41-44	10	9.8	1.7	7	12.0	2.7	10	16.5	1.0	11	16.1	2.0	10	15.3	1.5	10	16.9	1.3			
45-48	8	13.3	1.9	11	15.0	2.1	10	16.8	1.5	11	17.4	1.5	10	15.7	1.7	10	15.6	1.6			

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

Table 2. Interim Summary Statistics for Mean Daily Feed Consumption (g) per Animal¹ for Male Bisphenol-A Stop-Dose

Weeks	Dose ($\mu\text{g}/\text{kg}_{\text{BW}}/\text{day}$)																	
	0			2.5			25			250			2500			25000		
N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	
4	10	7.1	0.5	10	8.1	0.7	10	15.6	5.4	10	8.9	0.6	10	7.9	0.8	11	8.7	0.6
5-8	10	20.3	0.7	10	21.4	0.7	10	21.2	0.7	10	21.0	0.6	10	19.5	0.7	11	20.7	0.8
9-12	10	24.4	0.9	10	26.9	0.8	10	26.3	0.3	10	25.7	0.9	10	25.1	0.5	11	25.7	0.5
13-16	10	25.4	0.8	10	26.9	0.8	10	26.3	0.4	10	24.7	0.7	10	25.6	0.7	11	25.8	0.7
17-20	10	25.4	0.7	10	26.5	0.7	10	26.3	0.5	10	25.1	0.8	10	25.4	0.6	11	26.4	0.6
21-24	10	24.8	0.8	10	26.1	1.4	10	26.6	0.7	10	22.9	1.6	10	23.7	0.7	11	25.7	0.6
25-28	10	25.2	1.0	9	24.3	2.6	7	23.3	3.1	9	25.0	4.5	10	25.4	0.8	11	22.7	1.7
29-32	10	21.1	1.8	9	23.4	2.5	10	26.5	1.9	10	20.4	2.8	10	16.7	3.1	11	18.2	2.6
33-36	10	21.1	2.2	10	21.3	2.7	10	21.4	2.2	10	24.1	3.4	10	18.9	3.0	11	21.0	3.1
37-40	10	22.9	2.4	10	18.6	2.3	9	18.7	3.6	10	18.5	2.8	9	27.5	0.8	11	23.2	1.6
41-44	10	14.2	2.8	10	22.4	3.1	9	19.6	2.6	8	23.0	2.8	10	20.5	2.5	11	19.2	2.1
45-48	9	19.3	3.0	10	25.0	2.5	10	22.1	2.2	9	25.7	1.8	10	23.4	2.2	11	20.4	2.9

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

b) BPA Continuous Dose Treatments

Table 3. Interim Summary Statistics for Mean Daily Feed Consumption (g) per Animal¹ for Female Bisphenol-A Continuous Dose

<i>Weeks</i>	<i>Dose (µg/kg_{BW}/day)</i>																	
	<i>0</i>			<i>2.5</i>			<i>25</i>			<i>250</i>			<i>2500</i>			<i>25000</i>		
	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>
4	12	7.5	0.4	11	6.9	0.3	11	7.7	0.9	12	7.4	0.3	10	7.2	0.4	12	7.4	0.2
5-8	12	15.5	0.5	11	15.3	0.3	11	15.8	0.3	12	15.0	0.5	10	14.7	0.3	12	15.3	0.4
9-12	12	19.0	0.9	11	18.8	0.6	11	19.2	0.5	12	18.2	0.5	10	17.6	0.4	12	18.3	0.5
13-16	12	18.6	0.7	11	18.4	0.5	11	18.9	0.5	12	17.6	0.5	10	16.3	0.3	12	17.9	0.5
17-20	12	17.3	0.5	11	17.5	0.6	11	17.6	0.5	12	15.0	1.0	10	16.2	0.5	12	17.0	0.5
21-24	12	17.3	0.5	11	17.2	0.7	11	17.2	0.4	12	16.5	0.6	10	16.5	0.4	12	17.8	0.6
25-28	12	16.9	0.4	11	18.0	0.8	11	17.0	1.4	12	15.4	1.7	10	17.3	0.7	12	17.4	0.6
29-32	11	17.6	2.4	11	18.2	1.6	11	16.0	1.6	12	17.1	0.6	10	18.4	1.1	11	17.7	0.8
33-36	12	14.8	1.5	11	15.9	1.3	11	16.9	1.5	12	18.0	1.2	10	13.7	2.0	12	17.4	1.3
37-40	12	17.0	1.6	11	15.6	1.3	11	19.0	1.3	10	16.8	1.4	10	17.7	0.9	11	16.7	1.4
41-44	12	17.4	0.8	10	17.1	1.5	9	15.0	2.3	10	9.5	2.6	7	15.0	2.2	12	14.6	1.8
45-48	12	16.0	1.3	9	14.4	2.6	11	12.6	2.5	9	12.5	2.7	9	16.9	1.8	12	16.5	1.8

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

Table 4. Interim Summary Statistics for Mean Daily Feed Consumption (g) per Animal¹ for Male Bisphenol-A Continuous Dose

Weeks	Dose ($\mu\text{g}/\text{kg}_{\text{BW}}/\text{day}$)																	
	0			2.5			25			250			2500			25000		
N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE	
4	11	8.3	0.6	11	7.6	0.4	10	8.0	0.4	12	8.2	0.3	10	8.8	0.3	11	7.9	0.3
5-8	11	19.1	0.7	11	19.8	0.3	10	19.5	0.7	12	19.7	0.5	10	19.4	0.7	11	19.2	0.5
9-12	11	24.7	0.6	11	24.9	0.4	10	25.2	0.8	12	25.1	0.5	10	24.4	0.7	11	23.6	0.8
13-16	11	24.5	0.7	11	25.1	0.5	10	25.0	0.7	12	25.5	0.4	10	24.4	0.8	11	23.6	0.7
17-20	11	24.2	0.7	11	25.4	1.1	10	24.7	0.8	12	24.9	0.4	10	24.2	0.7	11	24.3	0.6
21-24	11	23.9	0.6	11	24.8	0.6	10	24.4	0.7	12	24.4	0.4	10	24.1	0.8	11	23.8	0.6
25-28	11	23.7	0.6	11	23.0	1.2	10	22.9	1.5	12	18.9	2.6	10	24.2	0.8	10	22.0	1.8
29-32	11	21.2	2.1	11	21.9	1.8	10	23.1	1.6	12	21.8	1.5	10	21.7	2.5	11	21.4	1.6
33-36	11	21.6	1.2	11	23.8	1.1	10	22.2	1.7	12	22.5	1.7	10	20.4	2.0	11	23.8	0.7
37-40	11	21.7	2.0	11	23.3	2.0	10	22.5	2.8	12	21.0	2.3	9	20.7	2.5	10	22.2	0.9
41-44	11	23.9	1.4	11	20.4	2.2	10	18.5	6.8	12	15.6	2.4	10	22.0	2.9	10	18.4	2.6
45-48	11	16.6	3.0	11	17.7	3.1	9	19.5	3.1	12	16.7	2.1	9	21.5	3.0	11	19.3	2.2

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

c) *EE₂ Treatments*

Table 5. Interim Summary Statistics for Mean Daily Feed Consumption (g) per Animal¹ for Female Ethinyl Estradiol

<i>Weeks</i>	<i>Dose (µg/kg_{BW}/day)</i>								
	<i>0</i>			<i>0.05</i>			<i>0.5</i>		
	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>	<i>N</i>	<i>Mean</i>	<i>SE</i>
4	12	7.5	0.4	13	6.6	0.4	13	7.8	0.4
5-8	12	15.5	0.5	13	15.5	0.6	13	15.7	0.4
9-12	12	19.0	0.9	13	18.2	0.3	13	17.8	0.4
13-16	12	18.6	0.7	13	18.2	0.4	13	17.6	0.6
17-20	12	17.3	0.5	13	17.5	0.3	13	17.7	0.5
21-24	12	17.3	0.5	13	17.2	0.5	13	17.6	0.6
25-28	12	16.9	0.4	13	18.3	0.7	13	18.4	0.7
29-32	11	17.6	2.4	13	15.4	1.6	13	17.1	1.3
33-36	12	14.8	1.5	13	14.3	0.9	12	16.6	1.7
37-40	12	17.0	1.6	13	17.0	1.0	12	15.1	2.1
41-44	12	17.4	0.8	13	15.2	1.7	13	14.8	1.9
45-48	12	16.0	1.3	12	15.1	2.4	12	16.9	2.0

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

Analysis of Interim Sacrifice Feed Consumption Data

Table 6. Interim Summary Statistics for Mean Daily Feed Consumption(g) per Animal¹ for Male Ethinyl Estradiol

Weeks	Dose ($\mu\text{g}/\text{kg}_{\text{BW}}/\text{day}$)								
	0			0.05			0.5		
	N	Mean	SE	N	Mean	SE	N	Mean	SE
4	11	8.3	0.6	13	8.3	0.5	12	9.2	0.4
5-8	11	19.1	0.7	13	19.6	0.6	13	20.2	0.4
9-12	11	24.7	0.6	13	24.6	0.7	13	24.9	0.4
13-16	11	24.5	0.7	13	24.9	0.5	13	24.4	0.5
17-20	11	24.2	0.7	13	24.8	0.7	13	24.9	0.5
21-24	11	23.9	0.6	13	24.5	0.8	13	24.7	0.6
25-28	11	23.7	0.6	13	23.4	1.4	13	23.2	1.2
29-32	11	21.2	2.1	13	20.9	1.6	13	22.5	1.7
33-36	11	21.6	1.2	13	21.5	1.5	13	21.5	1.9
37-40	11	21.7	2.0	13	19.9	1.8	13	19.4	2.0
41-44	11	23.9	1.4	13	18.9	2.4	13	20.7	2.3
45-48	11	16.6	3.0	13	22.8	2.3	13	21.7	2.2

¹ N indicates the number of cages; animals were housed two per cage without replacement of dead or moribund cagemates.

B. Figures

Figure 1. Feed Consumption (g) for Interim Sacrifice Females in the BPA Stop Dose Arm

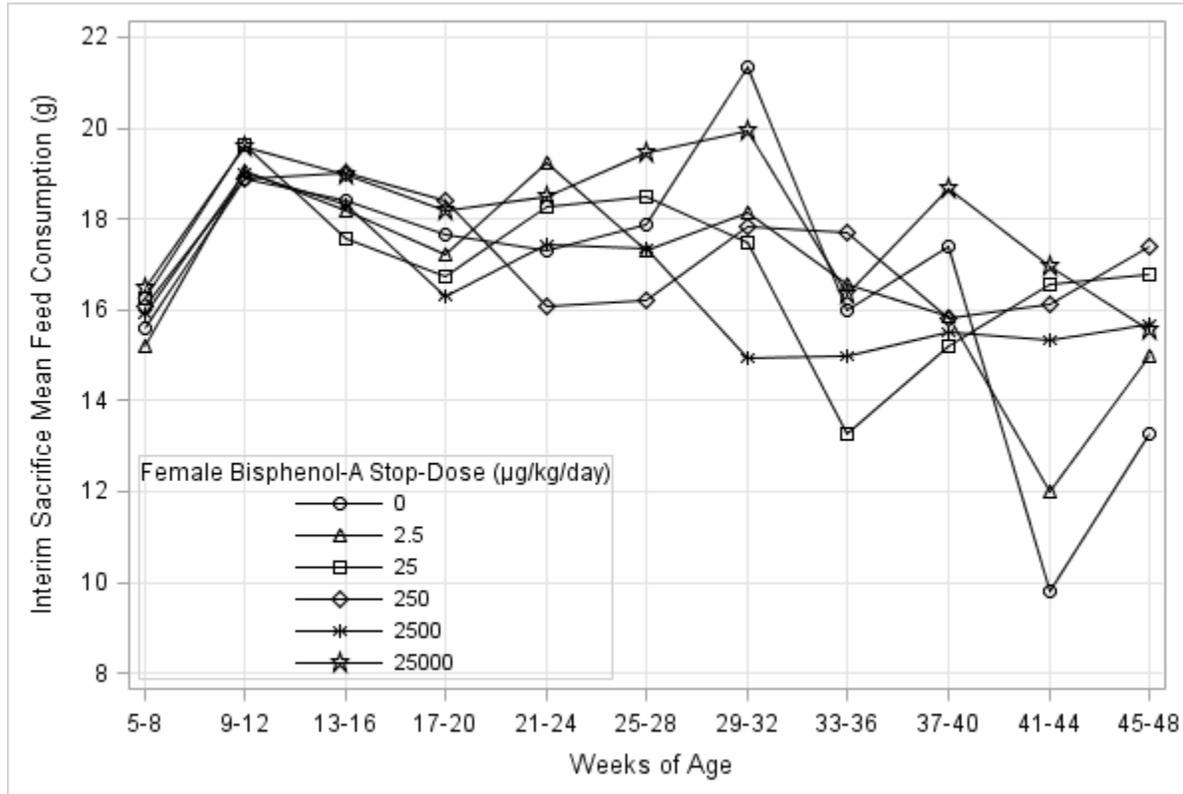


Figure 2. Feed Consumption (g) for Interim Sacrifice Males in the BPA Stop Dose Arm

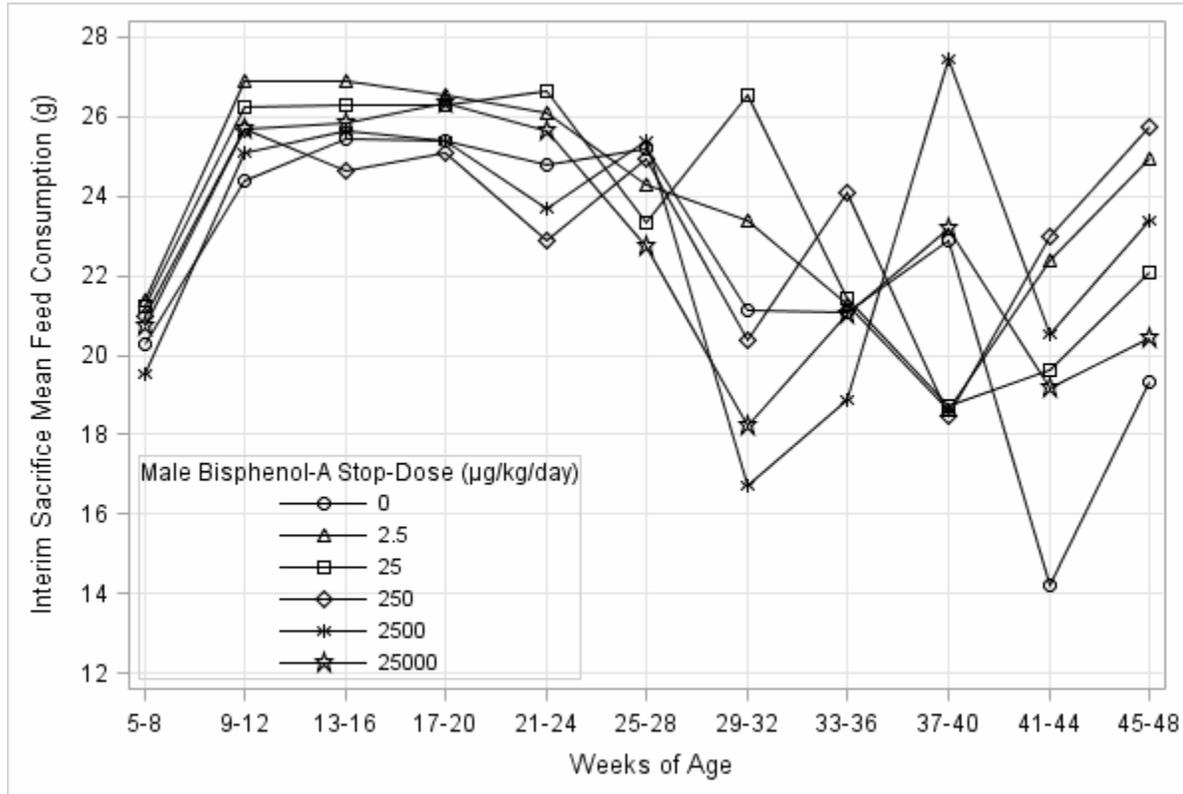


Figure 3. Feed Consumption (g) for Interim Sacrifice Females in the BPA Continuous Dose Arm

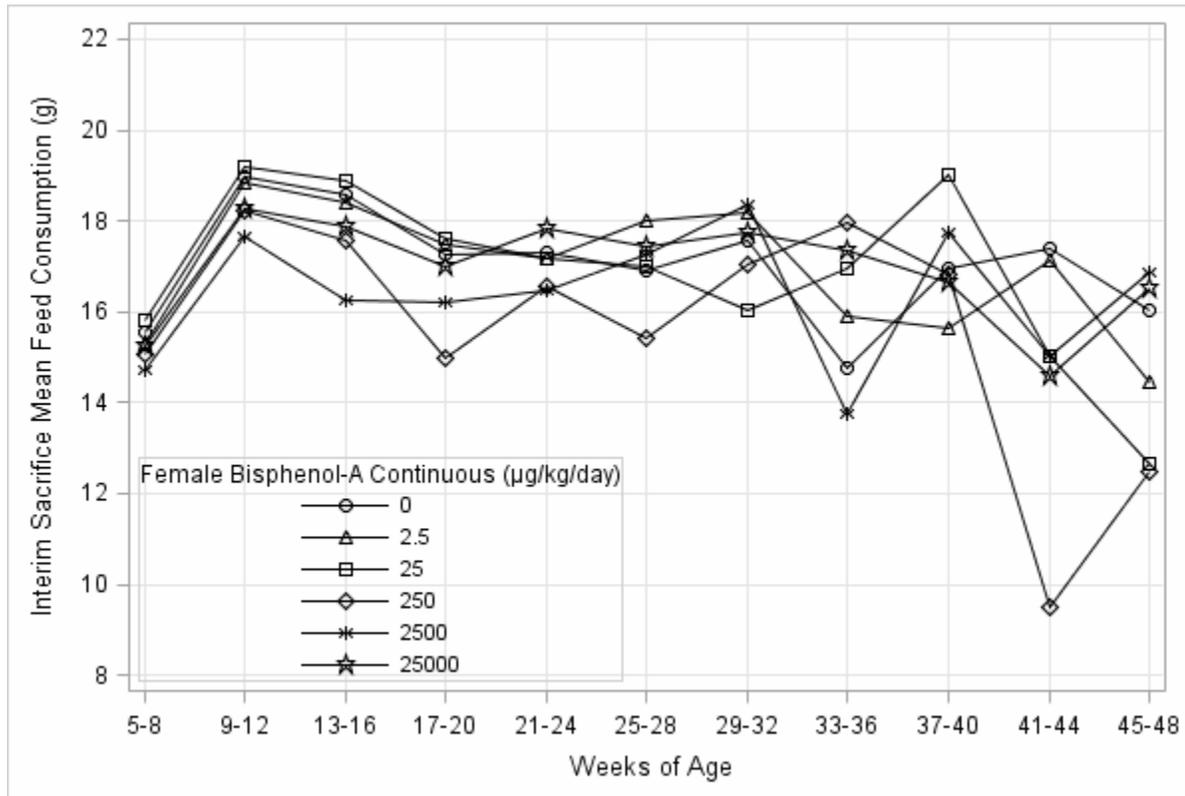


Figure 4. Feed Consumption (g) for Interim Sacrifice Males in the BPA Continuous Dose Arm

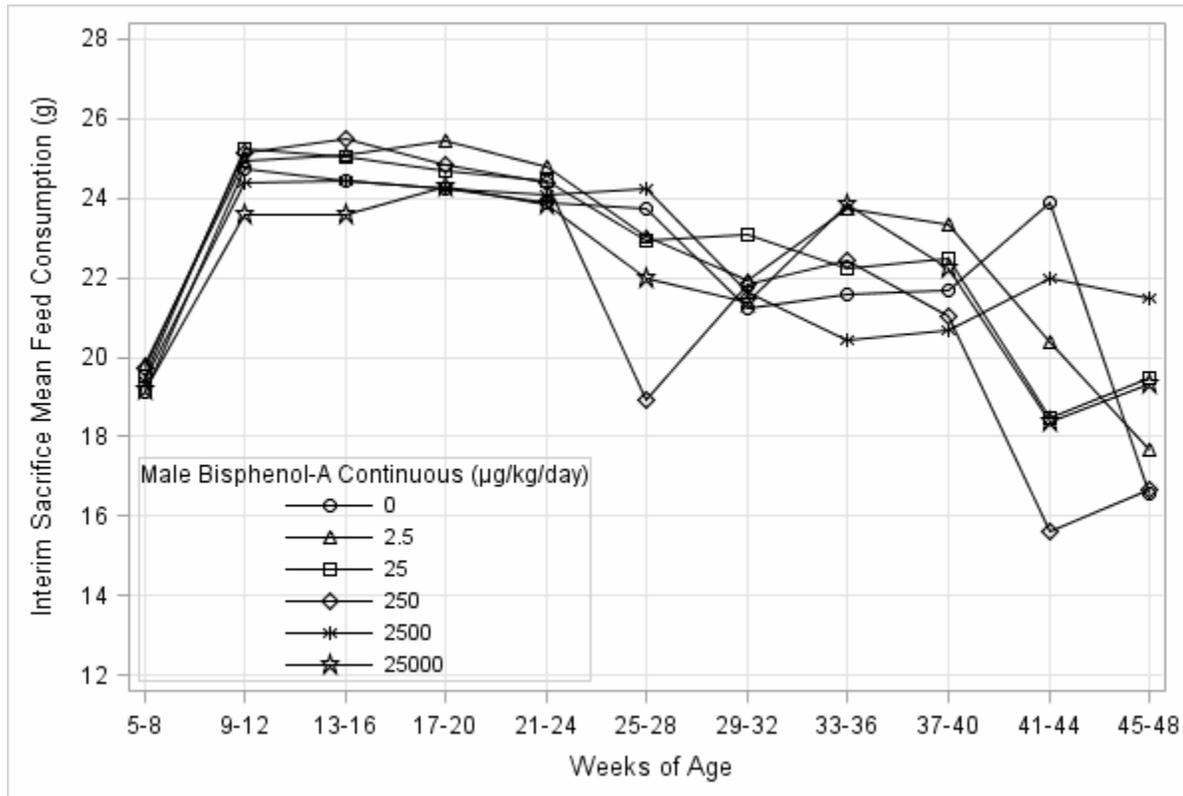


Figure 5. Feed Consumption (g) for Interim Sacrifice Females in the EE₂ Dose

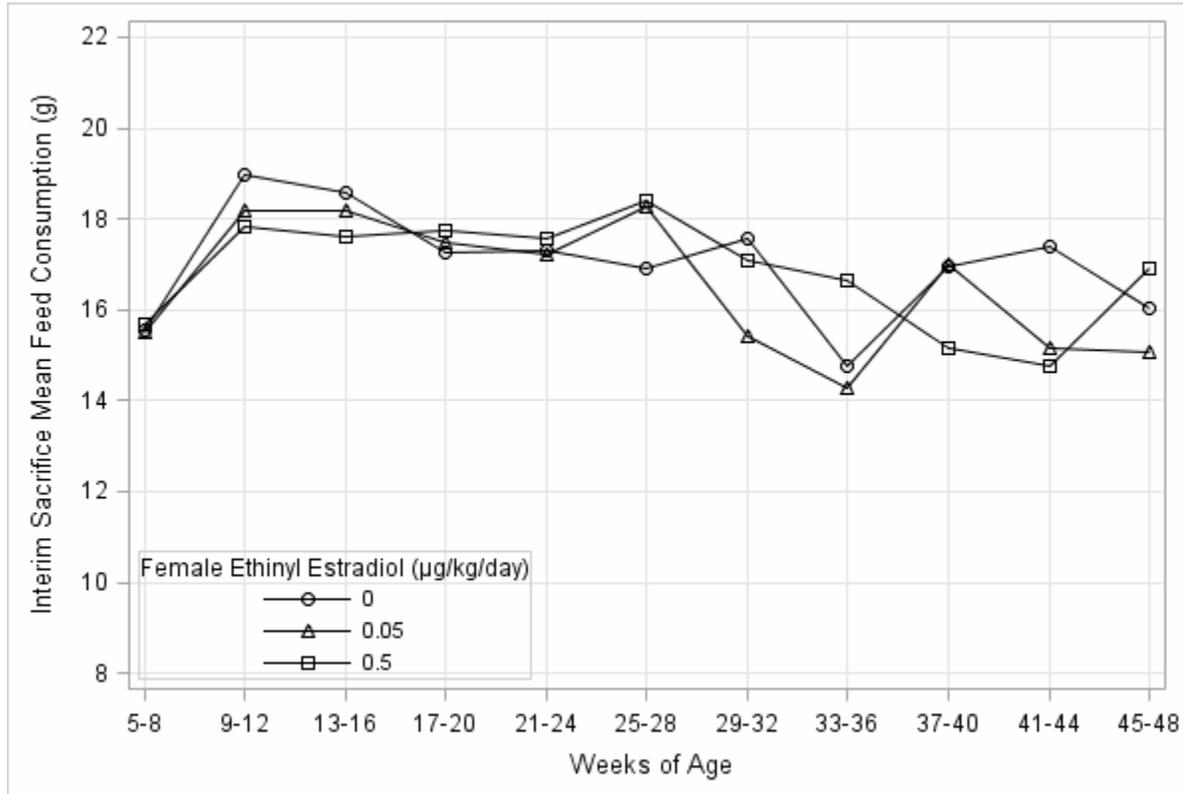
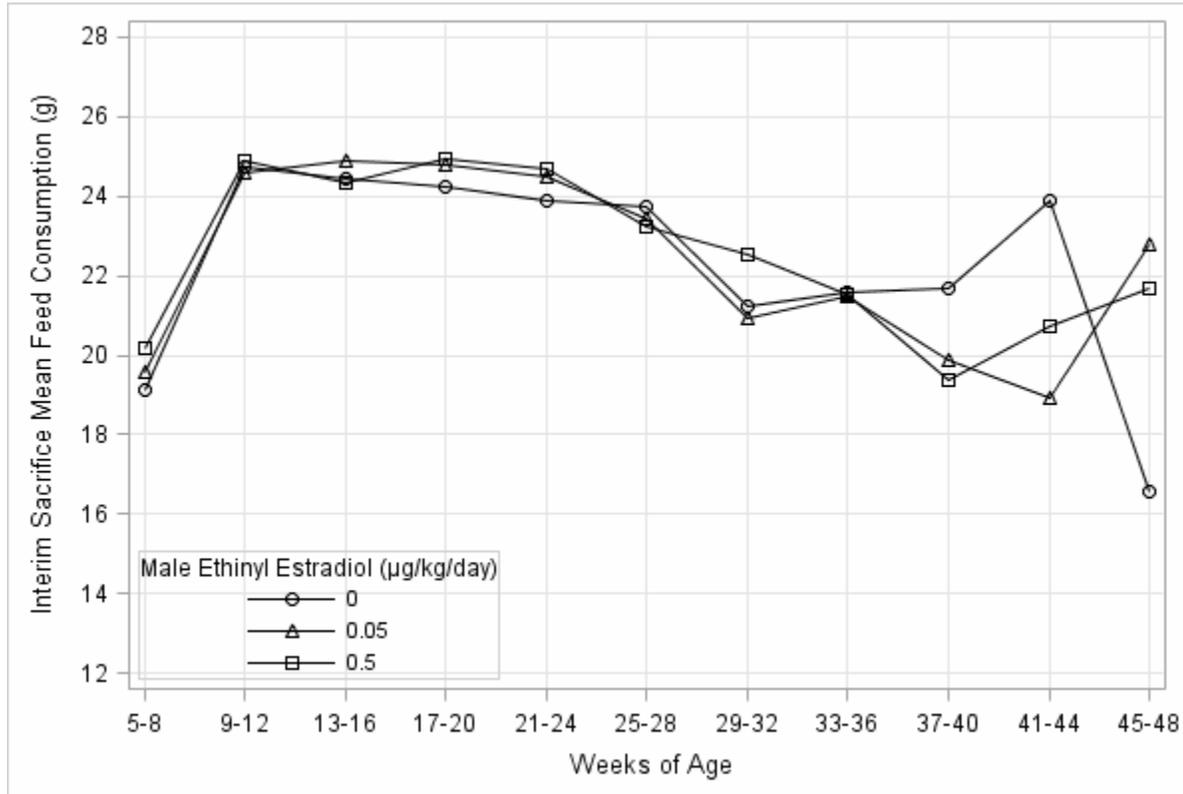


Figure 6. Feed Consumption (g) for Interim Sacrifice Males in the EE₂ Dose



C. Data

Feed consumption 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.

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.