

**Study Number:** MOG08002B  
**Test Type:** MOG  
**Route:** Dosing in Feed  
**Species/Strain:** Rat/Sprague-Dawley

**R16: Pubertal Markers Summary**  
**Test Compound:** Bisphenol AF  
**CAS Number:** 1478-61-1

**Date Report Requested:** 07/17/2020  
**Time Report Requested:** 11:04:14  
**Lab:** RTI

**Study Number:**

MOG08002B

**Study Gender:**

Both

**PWG Approval Date**

See web page for date of PWG Approval

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Generation	Cohort	Male				
		Treatment Groups (ppm)				
		0	338	1125	3750	
F1	All Males	No. Examined (litters)	54 (22)	55 (24)	53 (22)	50 (20)
		No. Removed (litters)	0 (0)	0 (0)	0 (0)	0 (0)
		No. Not Attaining BPS (litters)	0 (0)	0 (0)	0 (0)	10 (9)
		Day of BPS				
		Mean Analysis				
		Litter Mean ± SE	45.6 ± 0.3 **	45.3 ± 0.4	51.1 ± 0.7 **	80.0 ± 2.1 **
		Litter Mean of Adjusted ± SE	46.4 ± 0.2 **	46.0 ± 0.3	50.8 ± 0.7 **	78.3 ± 2.1 **
		Proportional Hazards Analysis				
		Litter-based Model	p<0.001	p=0.115	p<0.001	p<0.001
		BW at Attainment (g)	200.4 ± 1.8 **	188.0 ± 2.1 **	195.1 ± 3.5	217.9 ± 3.6 **
		BW at Weaning (g)	77.5 ± 1.3 **	77.0 ± 1.6	68.6 ± 1.5 **	55.4 ± 1.7 **

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Generation	Cohort	Female				
		Treatment Groups (ppm)				
		0	338	1125	3750	
F1	All Females	No. Examined (litters)	66 (22)	67 (24)	65 (22)	60 (22)
		No. Removed (litters)	0 (0)	0 (0)	0 (0)	2 (1)
		No. Not Attaining VO (litters)	0 (0)	0 (0)	0 (0)	0 (0)
		Day of VO				
		Mean Analysis				
		Litter Mean ± SE	34.6 ± 0.3 **	32.8 ± 0.4 *	27.8 ± 0.4 **	30.0 ± 0.8 **
		Litter Mean of Adjusted ± SE	35.8 ± 0.3 **	33.8 ± 0.3 **	27.8 ± 0.3 **	27.9 ± 0.7 **
		Proportional Hazards Analysis				
		Litter-based Model	p<0.001	p=0.006	p<0.001	p<0.001
		BW at Attainment (g)	103.2 ± 1.7 **	90.9 ± 1.6 **	63.2 ± 1.4 **	60.5 ± 2.6 **
		BW at Weaning (g)	71.4 ± 1.6 **	70.3 ± 1.3	64.9 ± 1.7 **	53.0 ± 1.4 **

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		Male			
Generation	Cohort		Treatment Groups (ppm)		
			0	338	1125
F2	All Males	No. Examined (litters)	52 (17)	70 (19)	27 (7)
		No. Removed (litters)	0 (0)	0 (0)	0 (0)
		No. Not Attaining BPS (litters)	0 (0)	0 (0)	0 (0)
		Day of BPS			
		Mean Analysis			
		Litter Mean ± SE	45.7 ± 0.7 **	44.8 ± 0.3	53.3 ± 1.3 **
		Litter Mean of Adjusted ± SE	46.5 ± 0.7 **	45.0 ± 0.4	52.1 ± 1.1 **
		Proportional Hazards Analysis			
		Litter-based Model	p<0.001	p=0.081	p<0.001
		BW at Attainment (g)	209.5 ± 4.4	195.1 ± 2.6 *	222.1 ± 7.4
BW at Weaning (g)	89.4 ± 2.4 *	86.8 ± 1.5	79.9 ± 4.2		

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		Female			
Generation	Cohort	Treatment Groups (ppm)			
		0	338	1125	
F2	All Females	No. Examined (litters)	78 (18)	77 (19)	20 (7)
		No. Removed (litters)	0 (0)	0 (0)	0 (0)
		No. Not Attaining VO (litters)	0 (0)	0 (0)	0 (0)
		Day of VO			
		Mean Analysis			
		Litter Mean ± SE	34.3 ± 0.3 **	31.6 ± 0.6 **	25.8 ± 0.5 **
		Litter Mean of Adjusted ± SE	34.7 ± 0.3 **	31.3 ± 0.6 **	25.1 ± 0.5 **
		Proportional Hazards Analysis			
		Litter-based Model	p<0.001	p<0.001	p<0.001
		BW at Attainment (g)	113.8 ± 1.8 **	94.3 ± 2.5 **	65.9 ± 2.2 **
BW at Weaning (g)	82.3 ± 1.7 *	77.8 ± 1.2	75.2 ± 1.8 *		

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#### LEGEND

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BPS = Balanopreputial separation; BW = Body weight; VO = Vaginal opening

No. Examined (litters) = the number of animals or pups examined (number of litters)

No. Removed (litters) is the number of animals (number of litters contributing) that died or were removed prior to the end of the observation period and did not attain. These animals were excluded from all analyses.

No. Not Attaining BPS (litters) and No. Not Attaining VO (litters) is the number of animals (number of litters contributing) that survived to the end of the observation period without attaining.

Summary statistics and mixed model results are presented for animals that attained during the observation period for Day of BPS and Day of VO Mean Analysis endpoint.

Means of litter means presented for Day of BPS and Day of VO Litter Mean  $\pm$  SE. Trend and pairwise tests were based on mixed models for day of attainment with dose as a covariate and a random effect for litter. The Dunnett-Hsu adjustment was used for multiple comparisons.

Mean adjusted day of attainment was calculated from the mean of the litter means of the weaning weight-adjusted attainment days for individual pups. Trend and pairwise tests were based on mixed models for day of attainment with dose and weaning weight as covariates and a random effect for litter. The Dunnett-Hsu adjustment was used for multiple comparisons.

Animals that did not attain by the end of the observation period were included in the proportional hazards analysis.

P-values for trend and pairwise comparisons for the Litter-based Model of the Proportional Hazards Analysis were calculated from a Cox proportional hazards model with dose and weaning weight as covariates and a random effect for litter, and a Hommel adjustment for multiple comparisons.

Analysis of body weight at attainment and body weight at weaning were performed using mixed effects models with dose as covariate and a random effect for litter. The Dunnett-Hsu adjustment was used for multiple comparisons. Animals that attained during the observation period were used for analysis.

Statistical significance for the control group indicates a significant trend test

Statistical significance for a treatment group indicates a significant pairwise test compared to the vehicle control group

\* Statistically significant at  $P \leq 0.05$

\*\* Statistically significant at  $P \leq 0.01$

The F1 Extra Females and PND 28 Bio-Female selections were excluded from the analysis.

The F1 3750 ppm animals were unable to produce a F2 generation, hence this group was not evaluated.

**\*\* END OF REPORT \*\***