

**Study Number:** R88007B  
**Test Type:** RACB  
**Route:** Dosing in Feed  
**Species/Strain:** Rat/Sprague-Dawley

**R16: Pubertal Markers Summary**  
**Test Compound:** Butyl Paraben  
**CAS Number:** 94-26-8

**Date Report Requested:** 03/22/2019  
**Time Report Requested:** 15:11:27  
**Lab:** RTI

**C Number:** R88007B  
**Study Gender:** Both  
**PWG Approval Date** See web page for date of PWG Approval

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Generation	Litter	Cohort	Male				
			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	All Males	No. Examined (litters)	86 (19)	74 (14)	93 (18)	32 (7)
			No. Removed (litters) <sup>a</sup>	1 (1)	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			Day of BPS				
			Mean Analysis <sup>c</sup>				
			Litter Mean $\pm$ SE <sup>d</sup>	44.4 $\pm$ 0.4 **	44.6 $\pm$ 0.6	46.0 $\pm$ 0.5	50.0 $\pm$ 0.8 **
			Litter Mean of Adjusted $\pm$ SE <sup>e</sup>	45.8 $\pm$ 0.4 *	45.0 $\pm$ 0.5	45.7 $\pm$ 0.5	47.7 $\pm$ 0.6
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p=0.021	p=0.539	p=0.798	p=0.404
			BW at Attainment (g) <sup>h</sup>	198.8 $\pm$ 2.8 **	194.6 $\pm$ 2.7	194.7 $\pm$ 2.9	175.7 $\pm$ 4.0 **
			BW at Weaning (g) <sup>h</sup>	51.2 $\pm$ 1.3 **	46.7 $\pm$ 1.3	44.0 $\pm$ 1.4 **	36.0 $\pm$ 1.5 **

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Generation	Litter	Cohort	Male				
			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	F1c NonParent Males	No. Examined (litters)	43 (16)	34 (12)	46 (18)	6 (5)
			No. Removed (litters) <sup>a</sup>	1 (1)	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			Day of BPS				
			Mean Analysis <sup>c</sup>				
			Litter Mean ± SE <sup>d</sup>	44.9 ± 0.6 **	45.5 ± 0.6	46.7 ± 0.6	51.8 ± 1.7 **
			Litter Mean of Adjusted ± SE <sup>e</sup>	45.9 ± 0.5	45.6 ± 0.4	46.2 ± 0.6	48.4 ± 1.1
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p=0.052	p=0.695	p=0.695	p=0.630
			BW at Attainment (g) <sup>h</sup>	195.9 ± 3.6 *	196.5 ± 2.7	198.0 ± 3.8	174.7 ± 5.3 *
			BW at Weaning (g) <sup>h</sup>	49.4 ± 1.9 **	45.7 ± 1.6	43.5 ± 1.4 *	32.2 ± 3.3 **

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Generation	Litter	Cohort	Male				
			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	F1c Parental Males	No. Examined (litters)	40 (19)	40 (14)	40 (18)	26 (7)
			No. Removed (litters) <sup>a</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			Day of BPS				
			Mean Analysis <sup>c</sup>				
			Litter Mean $\pm$ SE <sup>d</sup>	44.2 $\pm$ 0.5 **	44.3 $\pm$ 0.6	45.4 $\pm$ 0.5	49.7 $\pm$ 0.7 **
			Litter Mean of Adjusted $\pm$ SE <sup>e</sup>	45.4 $\pm$ 0.5 **	44.7 $\pm$ 0.5	45.1 $\pm$ 0.4	47.9 $\pm$ 0.5 *
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p=0.002	p=0.775	p=0.936	p=0.065
			BW at Attainment (g) <sup>h</sup>	200.0 $\pm$ 3.4 **	195.0 $\pm$ 3.2	191.6 $\pm$ 2.4	175.7 $\pm$ 4.3 **
			BW at Weaning (g) <sup>h</sup>	51.7 $\pm$ 1.1 **	47.5 $\pm$ 1.2	44.4 $\pm$ 1.6 **	36.5 $\pm$ 1.5 **

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Generation	Litter	Cohort	Female				
			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	All Females	No. Examined (litters)	89 (20)	78 (14)	87 (19)	26 (8)
			No. Removed (litters) <sup>a</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			No. Not Attaining VO (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			Day of VO				
			Mean Analysis <sup>c</sup>				
			Litter Mean $\pm$ SE <sup>d</sup>	34.5 $\pm$ 0.4 **	35.0 $\pm$ 0.4	37.7 $\pm$ 0.4 **	43.3 $\pm$ 1.1 **
			Litter Mean of Adjusted $\pm$ SE <sup>e</sup>	35.4 $\pm$ 0.4 **	35.0 $\pm$ 0.4	37.4 $\pm$ 0.4 **	42.1 $\pm$ 0.9 **
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p<0.001	p=0.508	p<0.001	p<0.001
			BW at Attainment (g) <sup>h</sup>	114.3 $\pm$ 1.7	111.1 $\pm$ 2.7	116.8 $\pm$ 2.9	117.8 $\pm$ 3.4
			BW at Weaning (g) <sup>h</sup>	50.1 $\pm$ 1.1 **	43.7 $\pm$ 1.4 **	41.3 $\pm$ 1.6 **	35.5 $\pm$ 2.0 **

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Generation	Litter	Cohort	Female				
			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	F1c NonParent Female	No. Examined (litters)	40 (17)	35 (14)	41 (15)	NR
			No. Removed (litters) <sup>a</sup>	0 (0)	0 (0)	0 (0)	NR
			No. Not Attaining VO (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	NR
			Day of VO				
			Mean Analysis <sup>c</sup>				
			Litter Mean $\pm$ SE <sup>d</sup>	34.3 $\pm$ 0.4 **	35.0 $\pm$ 0.4	37.4 $\pm$ 0.4 **	NR
			Litter Mean of Adjusted $\pm$ SE <sup>e</sup>	35.1 $\pm$ 0.4 **	34.9 $\pm$ 0.4	36.8 $\pm$ 0.4 *	NR
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p=0.002	p=0.275	p=0.049	NR
BW at Attainment (g) <sup>h</sup>	113.5 $\pm$ 2.1	110.2 $\pm$ 3.2	113.6 $\pm$ 2.8	NR			
BW at Weaning (g) <sup>h</sup>	50.1 $\pm$ 1.3 **	43.6 $\pm$ 1.7 **	39.9 $\pm$ 1.4 **	NR			

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			Treatment Groups (ppm)				
			0	5000	15000	40000	
F1	C	F1c Parental Females	No. Examined (litters)	40 (20)	40 (14)	40 (18)	26 (8)
			No. Removed (litters) <sup>a</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			No. Not Attaining VO (litters) <sup>b</sup>	0 (0)	0 (0)	0 (0)	0 (0)
			Day of VO				
			Mean Analysis <sup>c</sup>				
			Litter Mean $\pm$ SE <sup>d</sup>	34.3 $\pm$ 0.4 **	34.9 $\pm$ 0.4	37.8 $\pm$ 0.6 **	43.3 $\pm$ 1.1 **
			Litter Mean of Adjusted $\pm$ SE <sup>e</sup>	35.1 $\pm$ 0.4 **	35.0 $\pm$ 0.4	37.6 $\pm$ 0.6 **	42.4 $\pm$ 0.9 **
			Proportional Hazards Analysis <sup>f</sup>				
			Litter-based Model <sup>g</sup>	p<0.001	p=0.819	p<0.001	p<0.001
BW at Attainment (g) <sup>h</sup>	113.7 $\pm$ 1.8	112.1 $\pm$ 2.6	117.4 $\pm$ 3.0	117.8 $\pm$ 3.4			
BW at Weaning (g) <sup>h</sup>	50.1 $\pm$ 1.2 **	44.1 $\pm$ 1.2 **	41.2 $\pm$ 1.6 **	35.5 $\pm$ 2.0 **			

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## LEGEND

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

In multiple breeding/littering studies Litter A is the default designation for the first litter; subsequent litters would be B, C etc.

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

<sup>a</sup>Animals that died or were removed prior to the end of the observation period and did not attain. These animals were excluded from all analyses.

<sup>b</sup>Animals that survived to the end of the observation period without attaining.

<sup>c</sup>Summary statistics and mixed model results are presented for animals that attained during the observation period.

<sup>d</sup>Means of litter means presented. 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.

<sup>e</sup>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.

<sup>f</sup>Animals that did not attain by the end of the observation period were included in the proportional hazards analysis.

<sup>g</sup>P-values for trend and pairwise comparisons 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.

<sup>h</sup>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.

Endpoints including weaning weight use PND 21 body weight. The 40000 ppm group pups were weaned on PND 28.

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 Non-Parental Females in the 40000 ppm group were removed by postnatal day 26 due to toxicity.

NR not recorded

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