C Number:	R92025B
Study Gender:	Both
PWG Approval Date	See web page for date of PWG Approval

Study Number: R92025B
Test Type: RACB
Route: Dosing in Feed
Species/Strain: Rat/Sprague-Dawley

R16: Pubertal Markers Summary
Test Compound: 4-Methylimidazole
CAS Number: 822-36-6

Date Report Requested: 02/12/2019
Time Report Requested: 10:05:36
Lab: RTI

Male

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	All Males	No. Examined (litters)	89 (18)	100 (22)	60 (15)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of BPS			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	43.5 ± 0.4 **	46.2 ± 0.4 **	47.2 ± 0.6 **
			Litter Mean of Adjusted ± SE[e]	44.3 ± 0.3 *	46.4 ± 0.4 **	46.4 ± 0.5 *
			Proportional Hazards Analysis[f]			
			Individual Median Day of BPS[g]	44 **	46 **	46 **
			Litter-based Model[h]	p=0.002	p<0.001	p<0.001
			BW at Attainment (g)[i]	195.8 ± 2.7	205.6 ± 2.9 *	190.4 ± 3.4
			BW at Weaning (g)[i]	85.4 ± 1.6 **	80.4 ± 1.7	73.1 ± 2.9 **

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Male

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	F1 NonParental Male	No. Examined (litters)	49 (18)	56 (22)	20 (8)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of BPS			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	43.4 ± 0.4 **	46.3 ± 0.5 **	47.3 ± 0.7 **
			Litter Mean of Adjusted ± SE[e]	44.3 ± 0.3	46.4 ± 0.5 **	45.4 ± 0.6
			Proportional Hazards Analysis[f]			
			Individual Median Day of BPS[g]	44	47 **	45
			Litter-based Model[h]	p=0.067	p<0.001	p=0.138
			BW at Attainment (g)[i]	197.8 ± 2.4 **	206.1 ± 4.4	180.8 ± 3.7 **
			BW at Weaning (g)[i]	86.0 ± 1.8 **	80.6 ± 2.0 *	66.3 ± 2.3 **

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Male

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	F1 Parental Males	No. Examined (litters)	40 (18)	44 (22)	40 (15)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining BPS (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of BPS			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	43.4 ± 0.5 **	46.1 ± 0.4 **	47.5 ± 0.7 **
			Litter Mean of Adjusted ± SE[e]	44.4 ± 0.5 *	46.4 ± 0.4 **	46.6 ± 0.6 *
			Proportional Hazards Analysis[f]			
			Individual Median Day of BPS[g]	45 *	46 **	46 **
			Litter-based Model[h]	p=0.004	p=0.001	p=0.002
			BW at Attainment (g)[i]	193.3 ± 4.1	206.1 ± 3.0 *	193.1 ± 4.0
			BW at Weaning (g)[i]	84.7 ± 1.8 **	80.6 ± 1.8	73.6 ± 2.9 **

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Female

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	All Females	No. Examined (litters)	96 (19)	111 (22)	67 (15)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining VO (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of VO			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	33.8 ± 0.2 **	37.2 ± 0.3 **	39.4 ± 0.3 **
			Litter Mean of Adjusted ± SE[e]	34.1 ± 0.2 **	37.2 ± 0.3 **	39.0 ± 0.3 **
			Proportional Hazards Analysis[f]			
			Individual Median Day of VO[g]	34 **	38 **	40 **
			Litter-based Model[h]	p<0.001	p<0.001	p<0.001
			BW at Attainment (g)[i]	106.2 ± 2.0 **	117.2 ± 2.0 **	117.1 ± 1.8 **
			BW at Weaning (g)[i]	76.7 ± 1.6 **	71.3 ± 1.5 *	64.3 ± 2.3 **

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Female

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	F1 NonParent Female	No. Examined (litters)	47 (17)	58 (22)	27 (12)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining VO (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of VO			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	33.8 ± 0.2 **	37.0 ± 0.3 **	39.0 ± 0.4 **
			Litter Mean of Adjusted ± SE[e]	34.2 ± 0.2 **	37.0 ± 0.3 **	38.6 ± 0.4 **
			Proportional Hazards Analysis[f]			
			Individual Median Day of VO[g]	34 **	38 **	39 **
			Litter-based Model[h]	p<0.001	p<0.001	p<0.001
			BW at Attainment (g)[i]	107.2 ± 1.9 *	116.9 ± 2.0 **	116.2 ± 2.3 *
			BW at Weaning (g)[i]	77.3 ± 1.8 **	71.3 ± 1.3 *	65.2 ± 2.2 **

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Female

Generation	Litter	Cohort		Treatment Groups (ppm)		
				0	750	2500
F1	C	F1 Parental Females	No. Examined (litters)	40 (19)	44 (22)	40 (15)
			No. Removed (litters)[a]	0 (0)	0 (0)	0 (0)
			No. Not Attaining VO (litters)[b]	0 (0)	0 (0)	0 (0)
			Day of VO			
			Mean Analysis[c]			
			Litter Mean ± SE[d]	33.8 ± 0.2 **	37.5 ± 0.4 **	39.8 ± 0.4 **
			Litter Mean of Adjusted ± SE[e]	34.1 ± 0.2 **	37.5 ± 0.4 **	39.5 ± 0.3 **
			Proportional Hazards Analysis[f]			
			Individual Median Day of VO[g]	34 **	38 **	40 **
			Litter-based Model[h]	p<0.001	p<0.001	p<0.001
			BW at Attainment (g)[i]	106.9 ± 1.9 *	118.3 ± 2.9 **	118.4 ± 2.7 **
			BW at Weaning (g)[i]	77.1 ± 1.6 **	70.9 ± 2.0	63.9 ± 2.8 **

LEGEND

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

Animals in the F1 Extra Males, F1 Extra Females and F1 Male MGWM PND 28 seclections were not included in the analysis.

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

[a]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.

[b]Animals that survived to the end of the observation period without attaining.

[c]Summary statistics and mixed model results are presented for animals that attained during the observation period.

[d]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.

[e]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.

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

[g]Individual medians and trend and pairwise tests were calculated from a Cox proportional hazards model with dose and weaning weight as covariates, using the Hommel adjustment for multiple comparisons. Litter structure was not included in these results.

[h]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.

[i]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.

* Statistically significant at P <= 0.05

** Statistically significant at P <= 0.01

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

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

** END OF REPORT **