

**Study Number:** R10997  
**Test Type:** RACB  
**Route:** Dosing in Feed  
**Species/Strain:** Rat/Sprague-Dawley

**R04: Anogenital Distance Summary**  
**Test Compound:** Diisobutyl Phthalate  
**CAS Number:** 84-69-5

**Date Report Requested:** 01/09/2020  
**Time Report Requested:** 09:08:45  
**Lab:** RTI

**C Number:** R10997  
**Study Gender:** Both  
**PWG Approval Date** See web page for date of PWG Approval

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**Male Pups**

Generation	Litter ID	Cohort	PND1	Treatment Groups (ppm)			
				0	1000	5000	10000
F1	A		No. Examined (litters)	130 (20)	136 (23)	128 (21)	110 (22)
			Anogenital Distance (mm)	2.08 ± 0.06 **	2.01 ± 0.06	1.96 ± 0.04	1.87 ± 0.04 *
			Bodyweight (g)	7.18 ± 0.15	6.73 ± 0.13	6.95 ± 0.13	6.86 ± 0.15
			Adjusted AGD (mm)	2.06 ± 0.06 **	2.02 ± 0.06	1.95 ± 0.04	1.88 ± 0.04 *
F1	B		No. Examined (litters)	124 (21)	132 (23)	126 (20)	149 (22)
			Anogenital Distance (mm)	2.06 ± 0.10 *	1.96 ± 0.07	1.99 ± 0.06	1.78 ± 0.05 *
			Bodyweight (g)	7.23 ± 0.16 *	6.70 ± 0.12 *	6.95 ± 0.14	6.58 ± 0.15 **
			Adjusted AGD (mm)	2.05 ± 0.10 *	1.96 ± 0.07	1.98 ± 0.06	1.79 ± 0.05 *
F1	C		No. Examined (litters)	109 (21)	91 (21)	100 (19)	112 (20)
			Anogenital Distance (mm)	1.89 ± 0.06 **	2.01 ± 0.07	1.79 ± 0.06	1.64 ± 0.04 **
			Bodyweight (g)	7.16 ± 0.12 **	6.91 ± 0.11	7.26 ± 0.17	6.56 ± 0.11 **
			Adjusted AGD (mm)	1.87 ± 0.06 **	2.01 ± 0.07	1.77 ± 0.05	1.66 ± 0.04 *
F2	A		No. Examined (litters)	200 (32)	168 (29)	168 (32)	137 (27)
			Anogenital Distance (mm)	2.04 ± 0.04 **	2.04 ± 0.04	1.96 ± 0.03	1.88 ± 0.04 **
			Bodyweight (g)	7.06 ± 0.13	7.11 ± 0.14	7.34 ± 0.15	7.04 ± 0.10
			Adjusted AGD (mm)	2.04 ± 0.04 **	2.03 ± 0.04	1.95 ± 0.03	1.89 ± 0.03 **
F2	B		No. Examined (litters)	224 (34)	178 (30)	177 (31)	174 (33)
			Anogenital Distance (mm)	1.95 ± 0.04 **	1.89 ± 0.03	1.87 ± 0.03	1.79 ± 0.04 **
			Bodyweight (g)	7.00 ± 0.13	7.10 ± 0.12	7.20 ± 0.10	7.08 ± 0.13
			Adjusted AGD (mm)	1.95 ± 0.04 **	1.87 ± 0.03	1.84 ± 0.03	1.77 ± 0.03 **
F2	C		No. Examined (litters)	212 (31)	193 (35)	178 (28)	147 (33)
			Anogenital Distance (mm)	2.04 ± 0.06	2.34 ± 0.07 **	2.07 ± 0.08	2.27 ± 0.08

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**Male Pups**

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Generation	Litter ID	Cohort	PND1	Treatment Groups (ppm)			
				0	1000	5000	10000
			Bodyweight (g)	7.02 ± 0.11	7.13 ± 0.11	7.08 ± 0.13	7.18 ± 0.14
			Adjusted AGD (mm)	2.04 ± 0.06	2.34 ± 0.07 *	2.06 ± 0.08	2.26 ± 0.08

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**Female Pups**

Generation	Litter ID	Cohort	PND1	Treatment Groups (ppm)			
				0	1000	5000	10000
F1	A		No. Examined (litters)	127 (20)	137 (23)	130 (21)	128 (22)
			Anogenital Distance (mm)	1.03 ± 0.03	0.97 ± 0.03	1.03 ± 0.03	1.02 ± 0.02
			Bodyweight (g)	6.93 ± 0.11	6.52 ± 0.10 *	6.61 ± 0.13	6.62 ± 0.14
			Adjusted AGD (mm)	1.01 ± 0.03	0.98 ± 0.03	1.03 ± 0.03	1.02 ± 0.02
F1	B		No. Examined (litters)	124 (21)	155 (23)	138 (21)	122 (22)
			Anogenital Distance (mm)	0.98 ± 0.06	0.93 ± 0.03	0.99 ± 0.03	0.89 ± 0.04
			Bodyweight (g)	6.91 ± 0.15 **	6.51 ± 0.12	6.55 ± 0.14	6.26 ± 0.17 **
			Adjusted AGD (mm)	0.97 ± 0.06	0.93 ± 0.04	0.99 ± 0.04	0.89 ± 0.04
F1	C		No. Examined (litters)	81 (21)	107 (21)	97 (20)	96 (19)
			Anogenital Distance (mm)	0.83 ± 0.03	0.94 ± 0.04 *	0.87 ± 0.03	0.79 ± 0.03
			Bodyweight (g)	6.65 ± 0.15	6.63 ± 0.18	6.95 ± 0.15	6.29 ± 0.10
			Adjusted AGD (mm)	0.83 ± 0.02	0.94 ± 0.04 *	0.85 ± 0.03	0.81 ± 0.03
F2	A		No. Examined (litters)	200 (32)	178 (30)	211 (31)	145 (29)
			Anogenital Distance (mm)	0.99 ± 0.02	0.98 ± 0.02	1.02 ± 0.02	1.02 ± 0.03
			Bodyweight (g)	6.74 ± 0.12	6.86 ± 0.14	6.94 ± 0.14	6.71 ± 0.09
			Adjusted AGD (mm)	0.99 ± 0.02	0.98 ± 0.02	1.01 ± 0.02	1.02 ± 0.03
F2	B		No. Examined (litters)	218 (34)	205 (30)	196 (31)	175 (33)
			Anogenital Distance (mm)	0.96 ± 0.02	0.94 ± 0.02	0.93 ± 0.01	0.95 ± 0.02
			Bodyweight (g)	6.73 ± 0.12	6.79 ± 0.14	6.83 ± 0.10	6.79 ± 0.13
			Adjusted AGD (mm)	0.96 ± 0.02	0.94 ± 0.02	0.93 ± 0.02	0.95 ± 0.02
F2	C		No. Examined (litters)	192 (32)	215 (36)	177 (28)	178 (33)
			Anogenital Distance (mm)	0.96 ± 0.02	1.10 ± 0.03 **	1.00 ± 0.03	1.11 ± 0.03 **

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**Female Pups**

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Generation	Litter ID	Cohort	PND1	Treatment Groups (ppm)			
				0	1000	5000	10000
			Bodyweight (g)	6.67 ± 0.12	6.86 ± 0.10	6.82 ± 0.13	6.83 ± 0.12
			Adjusted AGD (mm)	0.96 ± 0.03	1.09 ± 0.03 **	0.99 ± 0.03	1.10 ± 0.03 **

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## LEGEND

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Data are displayed as mean of litter means  $\pm$  SEM of litter means.

Adjusted AGD is anogenital distance adjusted for body weight on PND 1.

Statistical analysis was performed using mixed models, with dam ID as random effect for trend and pairwise tests, with the Dunnett-Hsu adjustment for multiple comparisons.

Animals dead, cannibalized or missing presumed dead were excluded from 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  $P \leq 0.01$

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

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