

Statistical Analysis of Anogenital Distance on PND 90

Table 1. Summary Statistics for Females and Males PND 90 Absolute Anogenital Distance (mm) and AGD Index										
Group	AGD			AGD Index						
	Females		Males		Females		Males			
Group	N	Mean	S.E.	N	Mean	S.E.	Mean	S.E.	Mean	S.E.
Vehicle	20	10.9	0.2	20	27.6	0.5	1.65	0.03	3.53	0.06
BPA 2.5 ($\mu\text{g/kg}$)	23	11.2	0.3	23	29.3	0.4	1.71	0.03	3.70	0.05
BPA 8 ($\mu\text{g/kg}$)	18	10.8	0.2	18	28.6	0.6	1.66	0.03	3.62	0.07
BPA 25 ($\mu\text{g/kg}$)	21	10.8	0.1	21	28.6	0.6	1.66	0.03	3.64	0.07
BPA 80 ($\mu\text{g/kg}$)	20	11.1	0.2	20	28.5	0.4	1.70	0.03	3.63	0.05
BPA 260 ($\mu\text{g/kg}$)	20	11.0	0.2	20	28.5	0.5	1.66	0.03	3.62	0.06
BPA 840 ($\mu\text{g/kg}$)	20	11.0	0.2	20	28.3	0.4	1.67	0.03	3.60	0.05
BPA 2700 ($\mu\text{g/kg}$)	20	11.1	0.2	20	29.1	0.5	1.70	0.03	3.71	0.07
BPA 100,000 ($\mu\text{g/kg}$)	20	10.9	0.2	20	28.5	0.3	1.71	0.03	3.68	0.05
BPA 300,000 ($\mu\text{g/kg}$)	19	10.8	0.2	18	28.4	0.4	1.71	0.03	3.76	0.05
EE ₂ 0.5 ($\mu\text{g/kg}$)	20	11.0	0.4	20	28.9	0.6	1.66	0.06	3.70	0.07
EE ₂ 5.0 ($\mu\text{g/kg}$)	20	11.5	0.2	20	27.7	0.4	1.69	0.03	3.53	0.04
Naive Control	20	11.1	0.2	20	29.5	0.4	1.71	0.03	3.76	0.04

1 For analysis of anogenital index, the average of three measurements of anogenital distance was divided by the cube root of body weight.

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Table 2. ANOVA ¹ Results for Group Effect on Anogenital Distance on PND 90					
Sex	Effect	NumDF	DenDF	FValue	ProbF
F	Group	12	248	0.446	0.943
M	Group	12	247	1.629	0.084

1 ANOVA was performed separately for each sex using anogenital index.

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Table 3. Females and Males ANOVA Comparison¹ of Least Square Mean Anogenital Distance³ Across Dose Groups on PND 90

Vehicle			BPA 2.5 ($\mu\text{g/kg}$)			BPA 8 ($\mu\text{g/kg}$)			BPA 25 ($\mu\text{g/kg}$)			BPA 80 ($\mu\text{g/kg}$)			BPA 260 ($\mu\text{g/kg}$)			BPA 840 ($\mu\text{g/kg}$)			BPA 2700 ($\mu\text{g/kg}$)			
Sex	Mean	S.E.	P-val ²	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val
F	1.654	0.033	0.462	1.705	0.031	0.770	1.660	0.035	1.000	1.661	0.032	1.000	1.700	0.033	0.863	1.664	0.033	1.000	1.667	0.033	1.000	1.703	0.033	0.831
M	3.534	0.057	0.211	3.702	0.053	0.160	3.624	0.060	0.807	3.636	0.056	0.677	3.630	0.057	0.744	3.617	0.057	0.844	3.604	0.057	0.924	3.708	0.057	0.160

Table 3. Females and Males ANOVA Comparison¹ of Least Square Mean Anogenital Distance³ Across Dose Groups on PND 90

BPA 100,000 ($\mu\text{g/kg}$)			BPA 300,000 ($\mu\text{g/kg}$)			EE ₂ 0.5 ($\mu\text{g/kg}$)			EE ₂ 5.0 ($\mu\text{g/kg}$)			Naive Control			
Sex	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val
F	1.705	0.033	0.433	1.707	0.034	0.427	1.665	0.033	0.963	1.691	0.033	0.642	1.708	0.033	0.244
M	3.680	0.057	0.132	3.758	0.060	0.015	3.703	0.057	0.070	3.534	0.057	1.000	3.761	0.057	0.006

¹ All p-values and % are relative to the control group, except p-values for the linear trend are presented under the control group² Correlation between litter-mates is accounted for in the analysis with litter as the unit of analysis.³ Measurements of anogenital distance were divided by the cube root of body weight.

Table 4. Female and Male ANOVA Anogenital Distance Least Square Mean Treatment Percent of Vehicle with Anogenital Index

Sex	Treatments												Naive Control
	BPA 2.5 ($\mu\text{g/kg}$)	BPA 8 ($\mu\text{g/kg}$)	BPA 25 ($\mu\text{g/kg}$)	BPA 80 ($\mu\text{g/kg}$)	BPA 260 ($\mu\text{g/kg}$)	BPA 840 ($\mu\text{g/kg}$)	BPA 2700 ($\mu\text{g/kg}$)	BPA 100,000 ($\mu\text{g/kg}$)	BPA 300,000 ($\mu\text{g/kg}$)	EE ₂ 0.5 ($\mu\text{g/kg}$)	EE ₂ 5.0 ($\mu\text{g/kg}$)	Naive Control	
F	103.1	100.4	100.4	102.8	100.6	100.8	102.9	103.1	103.2	100.6	102.2	103.3	
M	104.8	102.6	102.9	102.7	102.3	102.0	104.9	104.1	106.3	104.8	100.0	106.4	

Table 5. Female and Male ANOVA Anogenital Distance Unadjusted P-values for Females and Males with Anogenital Index

Sex	Treatments												Naive Control
	BPA 2.5 ($\mu\text{g/kg}$)	BPA 8 ($\mu\text{g/kg}$)	BPA 25 ($\mu\text{g/kg}$)	BPA 80 ($\mu\text{g/kg}$)	BPA 260 ($\mu\text{g/kg}$)	BPA 840 ($\mu\text{g/kg}$)	BPA 2700 ($\mu\text{g/kg}$)	BPA 100,000 ($\mu\text{g/kg}$)	BPA 300,000 ($\mu\text{g/kg}$)	EE ₂ 0.5 ($\mu\text{g/kg}$)	EE ₂ 5.0 ($\mu\text{g/kg}$)	Naive Control	
F	0.255	0.898	0.886	0.323	0.833	0.782	0.297	0.268	0.264	0.820	0.428	0.244	
M	0.033	0.280	0.206	0.241	0.307	0.389	0.033	0.074	0.008	0.038	0.993	0.006	

Table 6. ANOCOVA ¹ Results for Group Effect on Anogenital Distance on PND 90					
Sex	Effect	NumDF	DenDF	FValue	ProbF
F	Group	12	247	0.385	0.968
	Body Weight	1	247	24.105	0.000
M	Group	12	246	1.456	0.142
	Body Weight	1	246	20.675	0.000

1 ANOCOVA was performed separately for each sex using absolute anogenital distance with covariate body weight.

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Table 7. Females and Males ANOCOVA Comparison¹ of Least Square Mean Anogenital Distance³ Across Dose Groups on PND 90

	Vehicle			BPA 2.5 ($\mu\text{g/kg}$)			BPA 8 ($\mu\text{g/kg}$)			BPA 25 ($\mu\text{g/kg}$)			BPA 80 ($\mu\text{g/kg}$)			BPA 260 ($\mu\text{g/kg}$)			BPA 840 ($\mu\text{g/kg}$)			BPA 2700 ($\mu\text{g/kg}$)		
Sex	Mean	S.E.	P-val ²	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val
F	10.86	0.21	0.485	11.20	0.20	0.765	10.86	0.23	1.000	10.86	0.21	1.000	11.12	0.21	0.929	10.93	0.22	1.000	10.95	0.22	1.000	11.14	0.21	0.894
M	27.63	0.45	0.245	29.06	0.42	0.106	28.42	0.47	0.724	28.50	0.44	0.589	28.42	0.45	0.688	28.35	0.45	0.773	28.24	0.45	0.876	29.01	0.45	0.150

Table 7. Females and Males ANOCOVA Comparison¹ of Least Square Mean Anogenital Distance³ Across Dose Groups on PND 90

	BPA 100,000 ($\mu\text{g/kg}$)			BPA 300,000 ($\mu\text{g/kg}$)			EE ₂ 0.5 ($\mu\text{g/kg}$)			EE ₂ 5.0 ($\mu\text{g/kg}$)			Naïve Control		
Sex	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val	Mean	S.E.	P-val
F	11.10	0.22	0.649	11.05	0.23	0.764	10.94	0.22	0.952	11.19	0.23	0.457	11.16	0.22	0.319
M	28.70	0.45	0.161	29.04	0.49	0.062	28.96	0.45	0.068	27.68	0.45	0.997	29.45	0.45	0.004

¹ All p-values and % are relative to the control group, except p-values for the linear trend presented under the control group.² Correlation between litter-mates is accounted for in the analysis.

Table 8. Female and Male ANOCOVA Anogenital Distance Least Square Mean Treatment Percent of Vehicle with Body Weight Covariate

Sex	Treatments											
	BPA 2.5 ($\mu\text{g/kg}$)	BPA 8 ($\mu\text{g/kg}$)	BPA 25 ($\mu\text{g/kg}$)	BPA 80 ($\mu\text{g/kg}$)	BPA 260 ($\mu\text{g/kg}$)	BPA 840 ($\mu\text{g/kg}$)	BPA 2700 ($\mu\text{g/kg}$)	BPA 100,000 ($\mu\text{g/kg}$)	BPA 300,000 ($\mu\text{g/kg}$)	EE ₂ 0.5 ($\mu\text{g/kg}$)	EE ₂ 5.0 ($\mu\text{g/kg}$)	Naïve Control
F	103.1	100.0	100.0	102.4	100.6	100.8	102.6	102.2	101.8	100.7	103.1	102.8
M	105.2	102.8	103.1	102.9	102.6	102.2	105.0	103.9	105.1	104.8	100.2	106.6

Table 9. Female and Male ANOCOVA Anogenital Distance Unadjusted P-values with Body Weight Covariate

Sex	Treatments											
	BPA 2.5 ($\mu\text{g/kg}$)	BPA 8 ($\mu\text{g/kg}$)	BPA 25 ($\mu\text{g/kg}$)	BPA 80 ($\mu\text{g/kg}$)	BPA 260 ($\mu\text{g/kg}$)	BPA 840 ($\mu\text{g/kg}$)	BPA 2700 ($\mu\text{g/kg}$)	BPA 100,000 ($\mu\text{g/kg}$)	BPA 300,000 ($\mu\text{g/kg}$)	EE ₂ 0.5 ($\mu\text{g/kg}$)	EE ₂ 5.0 ($\mu\text{g/kg}$)	Naïve Control
F	0.253	0.997	0.993	0.395	0.821	0.766	0.353	0.433	0.540	0.795	0.285	0.319
M	0.021	0.229	0.167	0.211	0.257	0.335	0.031	0.092	0.034	0.037	0.947	0.004