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Supplemental Material

Evaluation of Prenatal Exposure to Bisphenol Analogues on Development and Long-Term Health of the Mammary Gland in Female Mice

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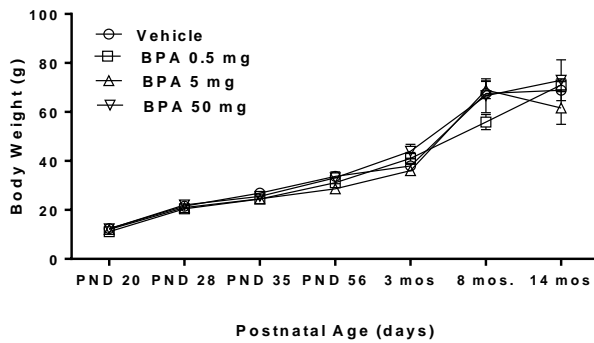
Figure S1. Pubertal and adult body weight gain in female offspring of CD-1 dams treated from gestation day (GD) 10 - GD 17 with A) bisphenol A (BPA), B) bisphenol AF (BPAF), or C) bisphenol S (BPS). * $p < 0.05$ is statistically significant on PND 35 in BPAF 0.5 mg/kg/bw and BPS 5 mg/kg/bw groups compared to vehicle control using ANOVA and a Dunnett's t-test. Litters: Vehicle (n = 9), BPA 0.5 (n = 12), BPA 5 (n = 12), BPA 50 (n = 9), BPAF 0.05 (n = 9), BPAF 0.5 (n = 11), BPAF 5 (n = 11), BPS 0.05 (n = 12), BPS 0.5 (n = 11) and BPS (n = 10).

Figure S2. Effects of prenatal bisphenol A (BPA), bisphenol AF (BPAF), or bisphenol S (BPS) exposure on female serum hormone levels at 3, 8, and 14 months. A, D, G, and J = 3 months; B, E, H, and K = 8 months; and C, F, I, and L = 14 months. Mean \pm SEM. Litter n=2-6 per treatment group. * $p < 0.05$ and ** $p < 0.01$ is statistically significant compared to vehicle control by ANOVA and Dunnett's test.

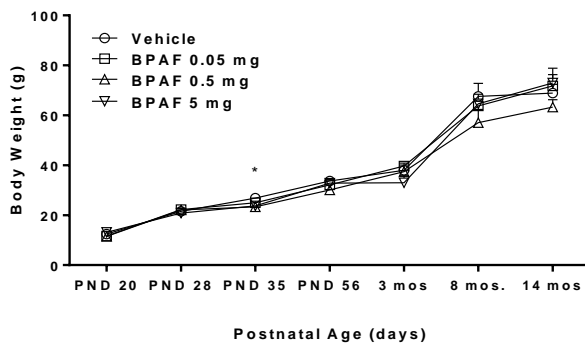
Table S1. Record of vehicle and treated animals \geq 3 months of age with mammary gland diagnoses by age.

Table S2. Number of days (d) spent in estrus, diestrus and proestrus following in utero exposure to bisphenol A (BPA), bisphenol AF (BPAF), or bisphenol S (BPS) during postnatal days (PND) 63-83.

A)



B)



C)

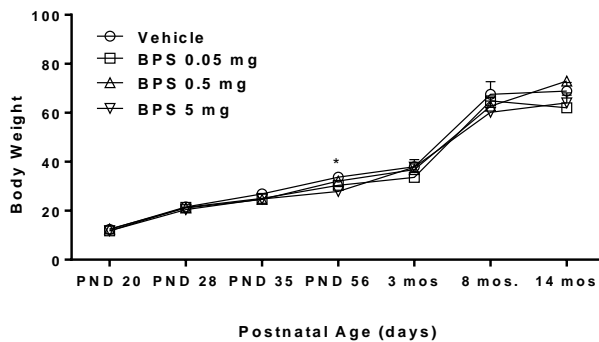


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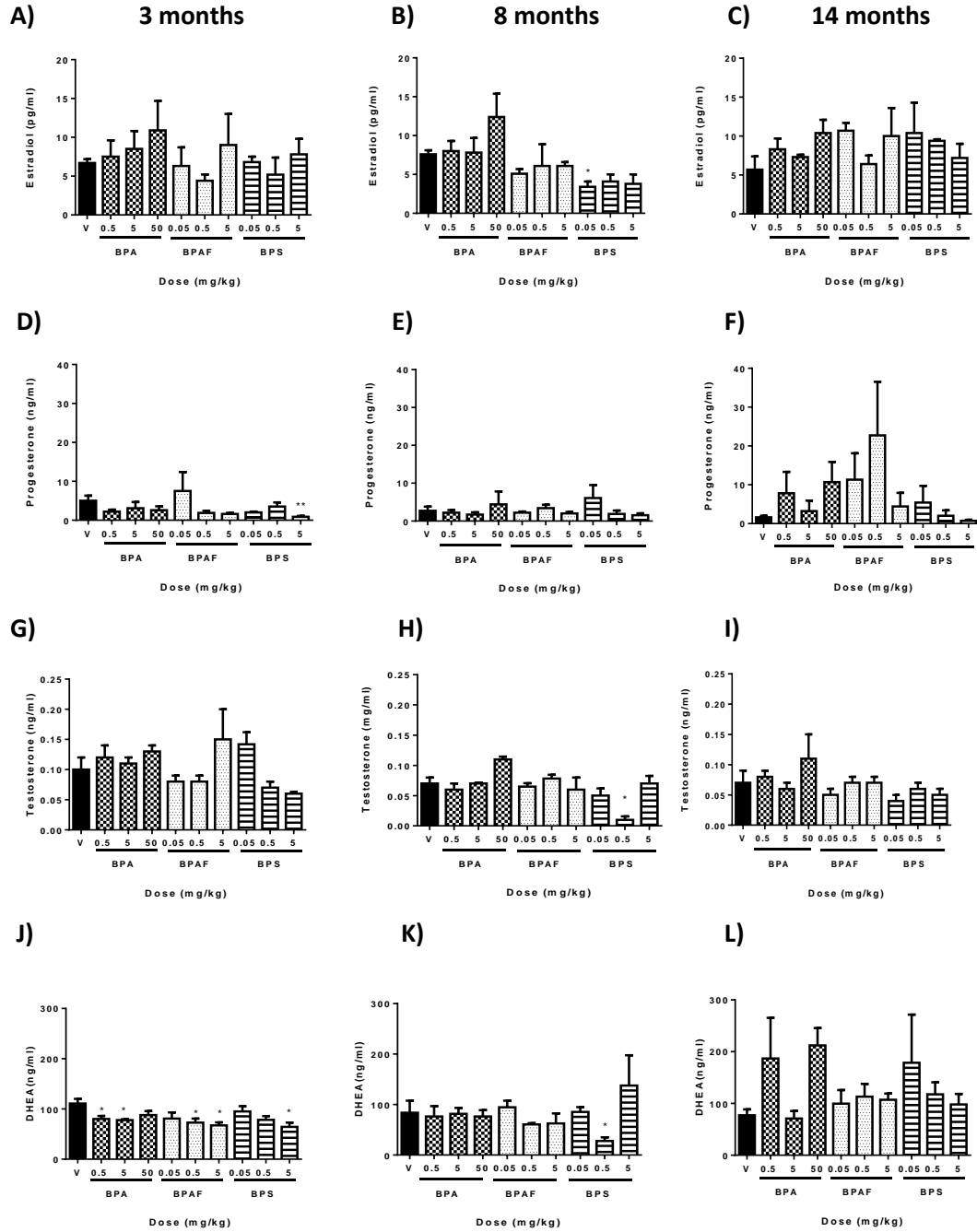


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Table S1. Record of vehicle and treated animals \geq 3 months of age with mammary gland diagnoses by age

Age at Necropsy (Weeks)	11-20*	21-30	31-40*	41-52	53 - 68*†	Total between weeks 1-68
Total number of animals	90 [2]	[4]	54 [1]	[27]	140 [10]	284 [44]
Inflammation	12		1	6	74	93
Ductal Squamous metaplasia			1		15	16
Lobuloalveolar hyperplasia			1	4	19	24
Miscellaneous (cyst, keratin, hemorrhage)					9	9
Histiocytic Sarcoma					1	1
Fibroadenoma					1	1
Papillary Carcinoma					1	1
Squamous Cell Carcinoma					2	2
Adenocarcinoma/Carcinoma				2	1	3
Total Neoplasia				2	6	8

[n] Animals with dermatitis, moribund, or found dead. Reason for death was determined or pathology was conducted, when possible.

*Includes animals from scheduled necropsies at 3 mo (12 wk), 8 mo (32 wk) or 14 mo (56 wk)

†Some animals may have had multiple mammary diagnoses

Table S2: Number of days (d) spent in estrus, diestrus and proestrus following in utero exposure to bisphenol A (BPA), bisphenol AF (BPAF), or bisphenol S (BPS) during postnatal days (PND) 63-83

	Estrus (d)	Diestrus (d)	Proestrus (d)
Vehicle	7.2 ± 4.0	11.7 ± 3.3	2.0 ± 1.0
BPA 0.5 mg/kg	7.6 ± 2.9	11.4 ± 2.7	1.2 ± 1.3
BPA 5 mg/kg	7.7 ± 2.7	11.0 ± 2.4	1.0 ± 0.6*
BPA 50 mg/kg	6.1 ± 2.4	12.1 ± 3.1	1.4 ± 1.2
BPAF 0.05 mg/kg	7.3 ± 2.2	11.2 ± 2.9	1.5 ± 1.2
BPAF 0.5 mg/kg	7.0 ± 2.3	11.7 ± 2.6	1.6 ± 1.0
BPAF 5 mg/kg	5.6 ± 2.4	12.8 ± 1.4	1.4 ± 0.7
BPS 0.05 mg/kg	7.5 ± 3.3	11.2 ± 3.8	1.7 ± 1.0
BPS 0.5 mg/kg	6.0 ± 3.7	12.4 ± 4.0	1.3 ± 1.3
BPS 5 mg/kg	6.7 ± 2.0	11.4 ± 2.2	1.7 ± 1.3

Data are presented as the mean number of days in that stage ± SEM.

Litter numbers: Vehicle (n = 9), BPA 0.5 (n = 12), BPA 5 (n = 12), BPA 50 (n = 9), BPAF 0.05 (n = 9), BPAF 0.5 (n = 11), BPAF 5 (n = 11), BPS 0.05 (n = 12), BPS 0.5 (n = 11) and BPS (n = 10).

Statistically significantly different from the control group by Dunnett's test at *p < 0.05.