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## **Supplemental Material**

### **Prostate Cancer Risk and DNA Methylation Signatures in Aging Rats following Developmental BPA Exposure: A Dose–Response Analysis**

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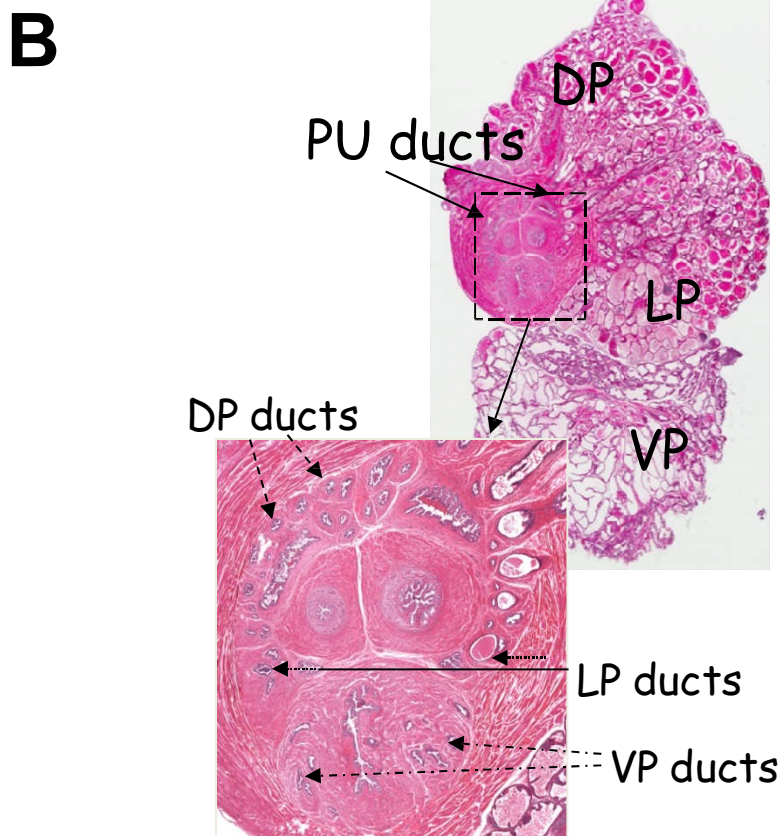
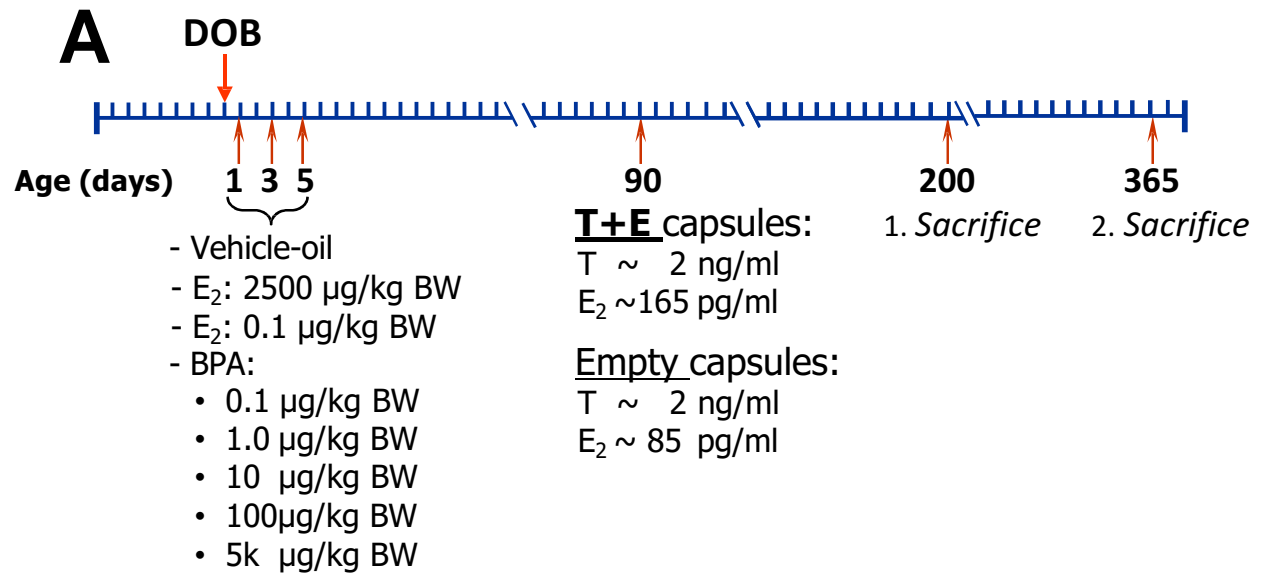
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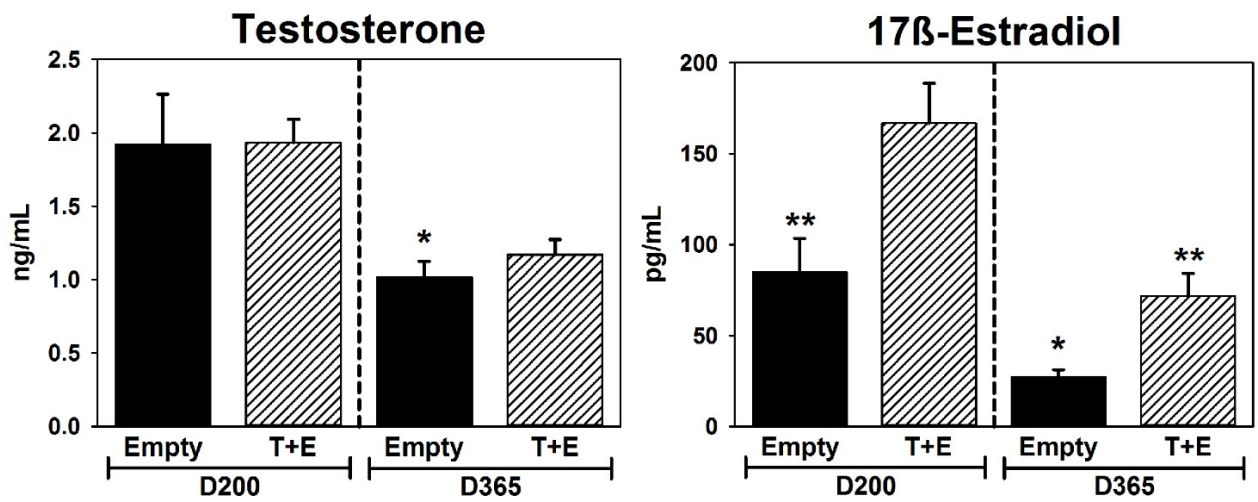
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**Figure S1:** **A:** Schematic representation of the experimental design for the animal studies. **B:** Transverse section through the Day 200 rat prostate-urogenital complex with the prostate lobes labeled as DP=dorsal, LP=lateral, VP=ventral and PU ducts = periurethral prostatic ducts. Magnified inset demarcates the regional periurethral prostate ducts that drain into the urethra. The section includes half of the bilateral prostatic lobes since the remaining VP, LP and DP was removed at autopsy and snap-frozen for molecular analysis.



**Figure S2:** Serum testosterone and 17 $\beta$ -estradiol levels in Day 200 and Day 365 rats given empty or T+E capsules on Day 90 of life. N=8 for each group. For testosterone data, \* denotes P<0.05 versus empty capsule rats at Day 200. For estradiol data, \* denotes P<0.01 versus T+E Day 365 rats; \*\* denotes P<0.001 vs T+E Day 200 rats.

**Table S1: Prostate (T&E) Pathology in day 200 Rats**

µg/kg BW	PIN			Hyperplasia		Inflammation	
	Score	Incidence		Score	Incidence	Score	Incidence
		All	HGPIN				
<b>Dorsal Lobes</b>							
<b>Vehicle + empty</b>	0.3	10%	0%	0.10	10%	0	0%
<b>Vehicle + T&amp;E</b>	0.56	33%	0%	0.89	56%	0.10	9%
<b>Low E2</b>	0.12	12%	0%	0.50	12%	0	0%
<b>High E2</b>	1.43	50%	25%	0.45	60%	0	0%
<b>0.1 BPA</b>	0.86	40%	20%	0.20	10%	0.10	20%
<b>1.0 BPA</b>	1.00	78%	11%	0.90	67%	0.30	33%
<b>10 BPA</b>	1.00	50%	11%	0.55	56%	0.10	11%
<b>100 BPA</b>	2.00 <sup>a</sup>	75%	50% <sup>a</sup>	0.75	38%	0.50	38%
<b>5K BPA</b>	1.20	60%	20%	1.10	90% <sup>b</sup>	0.10	10%
<b>(a) vs Veh + T&amp;E</b>	P<0.05	n.s.	P<0.05	n.s.		n.s.	n.s.
<b>(b) vs Low E2</b>					P<0.02		
<b>(b) vs 0.1 BPA</b>					P<0.001		
<b>Ventral Lobes</b>							
<b>Vehicle + empty</b>	0.2	10%	0%	0.20	20%	0	0%
<b>Vehicle + T&amp;E</b>	0.11	10%	0%	0.67	44%	0	0%
<b>Low E2</b>	0.25	25%	0%	0.50	25%	0.25	25%
<b>High E2</b>	1.00 <sup>a</sup>	45%	9%	2.00 <sup>a</sup>	91% <sup>a,b</sup>	0.18	9%
<b>0.1 BPA</b>	0.20	20%	0%	1.40	90% <sup>a,b</sup>	0.30	30%
<b>1.0 BPA</b>	0.67	44%	11%	1.22	78% <sup>a,b</sup>	0.11	22%
<b>10 BPA</b>	0.67	44%	0%	1.56	100% <sup>a,b</sup>	0.44	22%
<b>100 BPA</b>	1.00 <sup>a</sup>	50%	12%	1.88	75% <sup>a,b</sup>	0.12	12%
<b>5K BPA</b>	0.50	40%	0%	1.70 <sup>a</sup>	100% <sup>a,b</sup>	0.10	10%
<b>(a) vs Veh+T&amp;E</b>	P<0.05	n.s.	n.s.	P<0.05	P<0.02	n.s.	n.s.
<b>(a) vs Low E2</b>				P<0.05			
<b>(b) vs Low E2</b>					P<0.005		
<b>Periurethral Prostatic Ducts</b>							
<b>Vehicle + empty</b>	0.2	20%	0%	0.44	32%	0	0%
<b>Vehicle + T&amp;E</b>	0.11	20%	0%	0.33	29%	0	0%
<b>Low E2</b>	0.00	0%	0%	0.43	50%	0	0%
<b>High E2</b>	1.33	67% <sup>a,c</sup>	33% <sup>a</sup>	1.20	78%	0	0%
<b>0.1 BPA</b>	1.43 <sup>c</sup>	70% <sup>a,c</sup>	0%	1.90 <sup>b,c</sup>	100% <sup>b</sup>	0	0%
<b>1.0 BPA</b>	1.55 <sup>a</sup>	78% <sup>a,c</sup>	33% <sup>b</sup>	1.88 <sup>a,c</sup>	67% <sup>a</sup>	0.25	25%
<b>10 BPA</b>	2.00 <sup>b,d</sup>	100% <sup>b,d</sup>	33% <sup>b</sup>	1.89 <sup>a,c</sup>	89% <sup>b</sup>	0.11	11%
<b>100 BPA</b>	2.00 <sup>a,c</sup>	80% <sup>a,c</sup>	40% <sup>a</sup>	2.40 <sup>b,c</sup>	100% <sup>b</sup>	0	0%
<b>5K BPA</b>	1.67 <sup>a,c</sup>	71% <sup>a,c</sup>	29%	1.29	86% <sup>a</sup>	0.11	11%
<b>(a) vs Veh+T&amp;E</b>	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	n.s.	n.s.
<b>(b) vs Veh+T&amp;E</b>	P<0.01	P<0.001	P<0.01	P<0.01	P<0.01		
<b>(c) vs Low E2</b>	P<0.05	P<0.05		P<0.05			
<b>(d) vs Low E2</b>	P<0.01	P<0.001					

**Table S1:** Day 200 lesion incidence and scores (0-3) for PIN, hyperplasia and inflammation in the dorsal lobes, ventral lobes and periurethral prostatic ducts.

**Table S2: Prostate (T&E) Pathology in Aged (day 365) Rats**

µg/kg BW	PIN			Carcinoma-Microinvasion		Carcinoma-Glandular		Hyperplasia		Inflammation	
	Score	Incidence		Incidence		Incidence		Score	Incidence	Score	Incidence
<b>Dorsal Lobes</b>											
<b>Vehicle + Empty</b>	0.06	1/17	6%	0/17	0%	0/17	0%	0.41	35%	0.00	0%
<b>Vehicle + T&amp;E</b>	0.44	4/18	22%	1/18	6%	0/18	0%	0.56	39%	0.14	11%
<b>Low E2</b>	0.21	3/14	21%	1/14	7%	0/14	0%	1.07	71% <sup>a</sup>	0.14	7%
<b>High E2</b>	1.23 <sup>a,b</sup>	14/26	54% <sup>a,b</sup>	5/26	19%	2/26	8%	1.36	54%	0.62	31% <sup>a</sup>
<b>10 BPA</b>	0.38	5/21	24%	3/21	14%	1/21	5%	0.86	52%	0.00	0%
<b>(a) vs Veh+empty</b>	P<0.01	P<0.01		n.s.		n.s.		n.s.	P<0.05	n.s.	P<0.01
<b>(b) vs Veh+T&amp;E</b>	P<0.05	P<0.05		n.s.		n.s.		n.s.	n.s.	n.s.	n.s.
<b>Ventral Lobes</b>											
<b>Vehicle + Empty</b>	0.18	1/17	6%	0/17	0%	0/17	0%	0.24	12%	0.00	0%
<b>Vehicle + T&amp;E</b>	0.17	3/18	17%	0/18	0%	0/18	0%	0.67 <sup>a</sup>	50% <sup>a</sup>	0.11	6%
<b>Low E2</b>	0.36	3/14	21%	1/14	7%	0/14	0%	0.86 <sup>a</sup>	57% <sup>a</sup>	0.14	7%
<b>High E2</b>	1.88 <sup>a,b</sup>	22/26	85% <sup>a,b</sup>	5/26	19% <sup>a</sup>	2/26	8%	1.73 <sup>a,b</sup>	69% <sup>a</sup>	1.12	38% <sup>a,b</sup>
<b>10 BPA</b>	0.67	9/21	43% <sup>c</sup>	1/21	5%	0/21	0%	1.05 <sup>a</sup>	71% <sup>a</sup>	0.19	10%
<b>(a) vs Veh+empty</b>	P<0.001	P<0.001		P=0.06		n.s.		P<0.01	P<0.01	n.s.	P<0.01
<b>(b) vs Veh+T&amp;E</b>	P<0.001	P<0.001		n.s.		n.s.		n.s.	n.s.	n.s.	P<0.01
<b>(c) vs Veh+T&amp;E</b>		P<0.01									
<b>Periurethral Prostatic Ducts</b>											
<b>Vehicle + Empty</b>	0.24	2/17	12%	1/17	6%	1/17	6%	0.71	35%	0.00	0%
<b>Vehicle + T&amp;E</b>	0.44	5/18	28%	1/18	6%	0/18	0%	0.67	56%	0.00	0%
<b>Low E2</b>	0.79	6/14	43% <sup>a</sup>	1/14	7%	0/14	0%	1.07	64%	0.00	0%
<b>High E2</b>	1.54 <sup>a,b</sup>	17/26	65% <sup>a,b</sup>	7/26	27%	8/26	31% <sup>a,b</sup>	1.35 <sup>b</sup>	62% <sup>b</sup>	0.65 <sup>a,b</sup>	23% <sup>a,b</sup>
<b>10 BPA</b>	0.71	9/21	43% <sup>a</sup>	2/21	10%	3/21	14%	0.86	57%	0.05	5%
<b>(a) vs Veh+empty</b>	P<0.05	P<0.05		n.s.		P<0.05		n.s.	n.s.	P<0.05	P<0.05
<b>(b) vs Veh+T&amp;E</b>	P<0.05	P<0.05		n.s.		P<0.05		P<0.05	P<0.05	P<0.05	P<0.05

**Table S2:** Day 365 lesion incidence and scores (0-3) -for HGPIN, microinvasion, carcinoma-glandular, hyperplasia and inflammation in the dorsal lobes, ventral lobes and periurethral prostatic ducts.