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RESEARCH ARTICLE

Bisphenol S modulates concentrations of bisphenol A and oestradiol in female and male mice

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ABSTRACT

1. Concern over endocrine-disrupting actions of bisphenol A (BPA) has prompted some manufacturers to remove it from consumer products. Among the chemical replacements in "BPA-free" products are other bisphenol analogues, such as bisphenol S (BPS). Given evidence that BPA and BPS possess similar oestrogenic activity, their capacity to interact and disrupt oestrogen homeostasis should be examined.
2. We investigated whether BPS can modulate concentrations of ^{14}C-BPA, exogenous ^{3}H-oestradiol (E2), or natural E2. CF-1 mice were each given a single subcutaneous injection of oil containing 0 (vehicle), 1, 3, or 9 mg BPS, then given a dietary supplement containing either 50 μg/kg ^{14}C-BPA or 5 μCi (14.5 ng) ^{3}H-E2. BPS treatment elevated ^{14}C-BPA concentrations in blood serum and certain reproductive organs of both sexes, but reduced ^{3}H-E2 concentrations in blood serum of females. In another experiment, natural E2 was measured in urine 2–12 h after injection of 0 (vehicle), 1, or 3 mg BPS. BPS reduced E2 concentrations at 10 h after injection in both sexes.
3. These results are consistent with evidence that BPS and BPA compete for access to metabolic enzymes, and that BPS can disrupt oestrogen homeostasis. These findings demonstrate the importance of considering multiple toxicants when determining regulatory exposure limits.

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Bisphenol analogues; endocrine disruptors; oestradiol; oestrogen homeostasis; xeno-oestrogens

Introduction

Bisphenols are used in the production of polycarbonate plastics that are found in many household, commercial, and medical products, including food packaging, thermal receipt paper, dental sealants, and electronics (Vandenberg et al., 2007). The ubiquitous presence of bisphenol A (BPA) (Vandenberg et al., 2010) and its actions as an endocrine-disrupting chemical (EDC) (Rochester, 2013; Seachrist et al., 2016; Ziv-Gal & Flaws, 2016) have led to increasing concerns by researchers, regulators, and the public. This has prompted manufacturers to remove BPA from products and, in some cases, to use other bisphenol analogues. One such replacement is bisphenol S (BPS), which is found in "BPA-free" thermal paper (Liao et al., 2012c), personal care products (Liao & Kannan, 2014), and food (Liao & Kannan, 2013). BPS has been detected in surface water (Yamazaki et al., 2015), sediment (Yang et al., 2014), and indoor house dust (Liao et al., 2012b). BPS has also been measured in human urine (Liao et al., 2012a; Thayer et al., 2016) and blood serum (Thayer et al., 2016) at concentrations and detection frequencies that are comparable to those of BPA.

Recent evidence from our laboratory has shown that exposure to common EDCs can elevate concentrations of BPA in tissues and blood serum (Borman et al., 2017; Pollock, 2017; Pollock et al., 2014, 2017a,b, 2018). In these studies, mice were administered a single dose of one EDC then given 50 μg/kg ^{14}C-BPA, which is the oral reference dose set by the EPA (U.S. EPA, 1988). Administration of 0.6–18 mg triclosan (Pollock et al., 2014), 1–27 mg tetrabromobisphenol A (TBBPA) (Pollock et al., 2017a), 1–9 mg butyl paraben (BP) (Pollock et al., 2017b), 9 mg propyl paraben (PP) (Pollock et al., 2017b), or 3–18 mg diethylhexyl phthalate (DEHP) (Borman et al., 2017) significantly elevated BPA concentrations in tissues and blood serum of female and/or male mice. Concurrent administration can have effects at doses below those required for the individual EDCs to do so (Pollock et al., 2017a); a mixture of 0.1 mg each of triclosan, TBBPA, BP, PP, and DEHP significantly elevated BPA concentrations in tissues and blood serum of female mice (Pollock et al., 2018). Some of these EDCs also modulate concentrations of oestradiol (E2), the most potent natural oestrogen. Elevated urinary E2 concentrations were observed in mice

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administered a single dose of 1–2 mg triclosan (Pollock et al., 2016), 1 mg TBBPA (Pollock et al., 2017a), or 3 mg BP (Pollock et al., 2017b). Such actions could be pertinent to human health given that elevated oestrogen levels are implicated in hormone-dependent cancers (Million Women Study Collaborators, 2003, 2005, 2007) and can be damaging to female fertility (deCatanzaro, 2015; Gidley-Baird et al., 1986; Ma et al., 2003; Thorpe et al., 2013).

These findings are consistent with evidence that EDCs compete with each other and natural oestrogens for access to metabolic enzymes. Various enzymes are involved in EDC and natural oestrogen metabolism, including cytochrome p450 (CYP), hydroxysteroid dehydrogenase (HSD), sulphotransferase (SULT), and UDP-glucuronosyltransferase (UGT) (Dumas & Diorio, 2011). BPA is an established EDC with oestrogenic activity (Rochester, 2013; Seachrist et al., 2016; Ziv-Gal & Flaws, 2016), and several studies have shown that BPS exhibits similar oestrogenic activity (Catanese & Vandenberg, 2017; Chen et al., 2016; Eladak et al., 2015; Rochester & Bolden, 2015; Žalmanová et al., 2016). Given the co-occurrence of BPA and BPS in certain products and metabolically within individuals (Russo et al., 2017; Thayer et al., 2016), it is critical to assess their capacity to interact with each other and disrupt natural oestrogen homeostasis.

The present study was designed to assess *in vivo* interaction between BPS and BPA, and to assess the impacts of BPS on endogenous and exogenous E2 concentrations. We employed the same paradigm as used to study the EDCs mentioned above to allow direct comparison of the potency of BPS and these other EDCs. Consistent with evidence of shared detoxification pathways for BPS and BPA (Kurebayashi et al., 2010; Skledar et al., 2016), we hypothesised that BPS would elevate concentrations of BPA by competing for access to metabolic enzymes. We tested this hypothesis by measuring the impact of BPS injection on concentrations of dietary ^{14}C-BPA in female and male mice. We also hypothesised that BPS would modulate concentrations of endogenous and exogenous E2 by competing for access to metabolic enzymes and/or receptors. We tested this hypothesis by measuring the impact of BPS injection on concentrations of either dietary ^{3}H-E2 or natural urinary E2.

Materials and methods

Animals and housing

Female and male CF-1 mice aged 3–4 months were obtained from Charles River (St. Constant, QC). We used females in dioestrus, as this is an easily detected point in the cycle where oestrogen levels are relatively stable (Miller & Takahashi, 2014). Females were identified from a colony of mice by vaginal cytology using published procedures (Byers et al., 2012). The colony was maintained at 21 °C with a reversed 14 h light:10 h darkness cycle. Animals were housed in polypropylene cages measuring 28 × 16 × 11 (l × w × h) cm with *ad libitum* access to food (8640 Teklad Certified Rodent Chow; Harlan Teklad, Madison, WI) and water, except where otherwise stated. All procedures adhered to the standards of the Canadian Council on Animal Care and were approved by the Animal Research Ethics Board of McMaster University.

Chemicals and materials

BPS (CAS 80-09-1, ≥98% purity), E2 (CAS 50-28-2, ≥98% purity), and creatinine standards were obtained from Sigma-Aldrich, St. Louis, MO. ^{14}C-BPA ([ring-[^{14}C](U)]-BPA, in ethanol, 0.1 mCi/ml, 50 mCi/mmol) was obtained from Moravek Biochemicals, Brea, CA. ^{3}H-E2 ([2,4,6,7-[^{3}H](N)]-E2, in ethanol, 1.0 mCi/ml, 94 Ci/mmol), SOLVABLE solubilisation cocktail, Ultima Gold scintillation cocktail, and 8 ml midi-vial scintillation vials were obtained from PerkinElmer, Waltham, MA. E2 antibodies and HRP conjugates were obtained from the Department of Population Health and Reproduction at the University of California, Davis, CA.

Experimental design and dosing

This research followed previously published procedures (Borman et al., 2017; Pollock et al., 2014, 2016, 2017a,b, 2018). Mice were weighed, individually housed, and each given a dietary supplement of 1 g peanut butter. About 14–16 h later, at the onset of darkness on the following day (8:00 AM), animals were randomly assigned to treatment conditions involving a single subcutaneous (sc) injection. Dioestrous females and males received 0 (vehicle), 1, 3, or 9 mg BPS in 0.1 ml peanut oil ($n = 7$ per dose). Table 1 provides animal weights in g and BPS doses in mg/kg for each treatment condition. At 30 min after injection, each animal was given a 0.2 g peanut butter dietary supplement containing 50 μg/kg ^{14}C-BPA in Experiment 1 or 5 μCi ^{3}H-E2 (equivalent to 14.5 ng E2) in Experiment 2. Food, water, and bedding were removed to prevent contamination of the

Table 1. Mean (± SD) animal weights in g and BPS doses in mg/kg for each treatment condition.

	n	Animal weights (g)	BPS dose (mg)	Dose (mg/kg)
Experiment 1				
Females	7	29.2 ± 2.3	Vehicle	0.0 ± 0.0
	7	27.4 ± 2.3	1	36.7 ± 3.0
	7	26.3 ± 1.9	3	114.6 ± 8.1
	7	27.6 ± 3.0	9	330.0 ± 36.6
Males	7	36.5 ± 1.7	Vehicle	0.0 ± 0.0
	7	36.6 ± 2.6	1	27.4 ± 2.0
	7	37.0 ± 2.8	3	81.4 ± 5.9
	7	36.6 ± 3.2	9	247.3 ± 21.9
Experiment 2				
Females	7	34.4 ± 2.2	Vehicle	0.0 ± 0.0
	7	36.3 ± 3.0	1	27.7 ± 2.3
	7	38.4 ± 3.1	3	78.5 ± 6.0
	7	35.8 ± 2.6	9	252.7 ± 18.3
Males	7	43.9 ± 5.7	Vehicle	0.0 ± 0.0
	7	40.7 ± 8.0	1	25.4 ± 4.6
	7	40.1 ± 6.9	3	76.8 ± 13.5
	7	41.5 ± 4.0	9	218.9 ± 23.4
Experiment 3				
Females	10	38.3 ± 3.5	Vehicle	0.0 ± 0.0
	10	39.8 ± 2.9	1	25.2 ± 1.8
	10	37.9 ± 3.7	3	79.8 ± 7.6
Males	10	43.5 ± 4.4	Vehicle	0.0 ± 0.0
	10	44.9 ± 4.2	1	22.5 ± 2.1
	10	46.5 ± 3.7	3	65.0 ± 5.2

n = number of animals.

^{14}C-BPA or ^{3}H-E2 treatment. At 1 h after ^{14}C-BPA or ^{3}H-E2 administration, each animal was anaesthetised with isoflurane and blood was collected via cardiac puncture. Each animal was perfused with 20 ml phosphate-buffered saline (PBS) and tissues were collected in pre-weighed scintillation vials. Tissue samples taken included the heart, lung, superficial adductor muscle from the hind leg, abdominal adipose, liver, and a cross-section of the kidney encompassing both the medulla and cortex. Female reproductive tissues taken included the whole uterus and both ovaries. Male reproductive tissues were one testis, one epididymis, one vesicular-coagulating (VC) gland, and one preputial gland. Vials were re-weighed following tissue collection to determine the sample wet mass.

In Experiment 3, mice were weighed and individually placed in a Plexiglas apparatus measuring 21 × 15 × 13 (l × w × h) cm with a wire-mesh grid floor raised approximately 1 cm above a Teflon-coated stainless-steel surface covered with wax paper. Animals acclimated to the novel cages for 3 days prior to the start of the experiment. At the onset of darkness on the fourth day, dioestrous females and males were injected sc with 0 (vehicle), 1, or 3 mg BPS in 0.1 ml peanut oil ($n = 10$ per dose). Urine was collected non-invasively at 2, 4, 6, 8, 10, and 12 h post-injection. All urine samples were placed into labelled vials and frozen at −20 °C at the time of collection.

We administered ^{14}C-BPA in a dietary supplement to mimic ingestion of BPA from food, beverages, and indoor dust, which accounts for approximately 85–95% of total exposure in adults (EFSA, 2015). We also administered ^{3}H-E2 in a dietary supplement to compare findings with those of ^{14}C-BPA, and to resemble human oestrogen supplementation (van den Heuvel et al., 2005). We followed an effective paradigm used in previous studies (Borman et al., 2017; Pollock et al., 2014, 2016, 2017a,b, 2018) for other parameters, including BPS administration via sc injection, the 30-min latency between BPS and ^{14}C-BPA or ^{3}H-E2 administration, and the 1-h latency between ^{14}C-BPA or ^{3}H-E2 administration and tissue collection. Maintaining identical parameters across studies permits comparison of the impacts of BPS on concentrations of BPA with those previously shown for triclosan (Pollock et al., 2014), TBBPA (Pollock et al., 2017a), parabens (Pollock et al., 2017b), and DEHP (Borman et al., 2017).

Blood and tissue processing for liquid scintillation counting

Blood and tissue samples were processed for liquid scintillation counting following previously published procedures (deCatanzaro et al., 2014; deCatanzaro & Pollock, 2016; Greville et al., 2017). Blood samples were centrifuged at 1500 *g* for 10 min and 10 µl serum was added to a scintillation vial containing 5 ml Ultima Gold. Tissue samples were solubilised by adding 1 ml SOLVABLE to each vial and placing vials in a 50 °C water bath for 18 h until completely dissolved. Following the addition of 5 ml Ultima Gold, vials were agitated to promote mixing of the sample and scintillation cocktail. Each vial was stored in the darkness chamber of a TriCarb 2910 TR Liquid Scintillation Analyzer (PerkinElmer, Waltham, MA) for 5 min to eliminate noise in the form of heat and luminescence. Radioactivity was then measured for 5 min per vial. The amount of radioactivity per sample, in disintegrations per minute (dpm), was automatically calculated via Quanta-Smart software (PerkinElmer, Waltham, MA) by subtracting background radiation, which is continually monitored by the scintillation counter. Frequent cleaning and monitoring of all work surfaces and equipment ensured that contamination of samples did not occur. The final dpm measures were normalised to the weight of the sample wet mass and reported as equivalent ng BPA/g tissue or ng BPA/ml serum.

Measurement of urinary E2

Full procedures and validations for enzyme immunoassays for mouse urine were previously reported (Muir et al., 2001). Cross-reactivities for anti-E2 are E2 100%, oestrone 3.3%, progesterone 0.8%, testosterone 1.0%, androstenedione 1.0%, and all other measured steroids <0.1%. Assay precision was determined by interplate and intraplate coefficients of variation (CV) using pooled urine samples calibrated to span the range of physiological E2 concentrations. Interplate CV was 6.1% and 9.0% for urine samples calibrated to bind at 30% and 70%, respectively. Intraplate CV was 8.7% for 40 aliquots of a urine sample calibrated to bind at 50%. Urinary E2 levels were considered with and without adjustment for urinary creatinine, which corrects for differential hydration and urinary concentration among animals, and reported as ng E2/mg creatinine and ng E2/ml urine respectively.

Statistical analyses

All analyses were performed using the R software environment (R Core Team, 2017), with a comparison-wise error rate of $\alpha < 0.05$ for all tests. Differences among treatments in Experiments 1 and 2 were analysed by univariate analysis of variance (ANOVA) for each tissue, using false discovery rate adjustments to correct for the number of tissues (Benjamini & Hochberg, 1995). Significant effects in ANOVA were followed by pair-wise Newman–Keuls multiple comparisons. Differences between urinary E2 concentrations of animals in Experiment 3 were analysed by factorial ANOVA comparing the effects of treatment and collection time-point (repeated measures), followed by Newman–Keuls multiple comparisons at each time-point.

Results

Experiment 1: measurement of ^{14}C-BPA in mice given BPS

Pre-treatment with BPS elevated concentrations of ^{14}C-BPA in blood serum and certain tissues of females (Figure 1) and males (Figure 2). Concentrations of ^{14}C-BPA in the liver and kidney are reported in Table 2 and tissue/serum concentration ratios are reported in Supplemental Table S1. Comparisons were made among the four treatments for each of nine tissues in females. ANOVA using false discovery rate adjustment produced significant effects of treatment for the

uterus, $F(3,24) = 9.25$, $p = .003$, and serum, $F(3,24) = 6.24$, $p = .012$. Multiple comparisons revealed that the vehicle treatment differed from the 3 and 9 mg treatments for the uterus, as well as the 9 mg treatment for serum. Comparisons were made among the four treatments for each of eleven tissues in males. ANOVA using false discovery rate adjustment produced significant effects of treatment for the heart, $F(3, 24) = 5.83$, $p = .014$; VC glands, $F(3,24) = 6.34$, $p = .014$; and serum, $F(3,24) = 6.24$, $p = .014$. Multiple comparisons revealed that the vehicle treatment differed from the 9 mg treatment for the heart, VC glands, and serum.

Experiment 2: measurement of ^{3}H-E2 in mice given BPS

Pre-treatment with BPS reduced concentrations of ^{3}H-E2 in females (Figure 3). Concentrations of ^{3}H-E2 in the liver and kidney are reported in Table 3 and tissue/serum concentration ratios are reported in Supplemental Table S2. Comparisons among the four treatments for each of nine

Table 2. Mean (± SE) concentration of ^{14}C-BPA in the liver and kidney of dioestrous females and males following sc injection of 0 (vehicle), 1, 3, or 9 mg BPS and subsequent dietary administration of 50 µg/kg ^{14}C-BPA in Experiment 1.

	BPS dose (mg)	Liver (ng BPA/g)	Kidney (ng BPA/g)
Experiment 1			
Females	Vehicle	37.3 ± 7.2	19.8 ± 3.1
	1	40.7 ± 7.8	21.5 ± 2.9
	3	48.3 ± 5.6	35.8 ± 5.8
	9	53.9 ± 10.3	33.3 ± 4.5
Males	Vehicle	47.7 ± 6.2	97.1 ± 10.7
	1	57.0 ± 10.7	98.0 ± 22.9
	3	50.3 ± 8.5	93.5 ± 10.3
	9	56.9 ± 13.4	114.3 ± 29.6

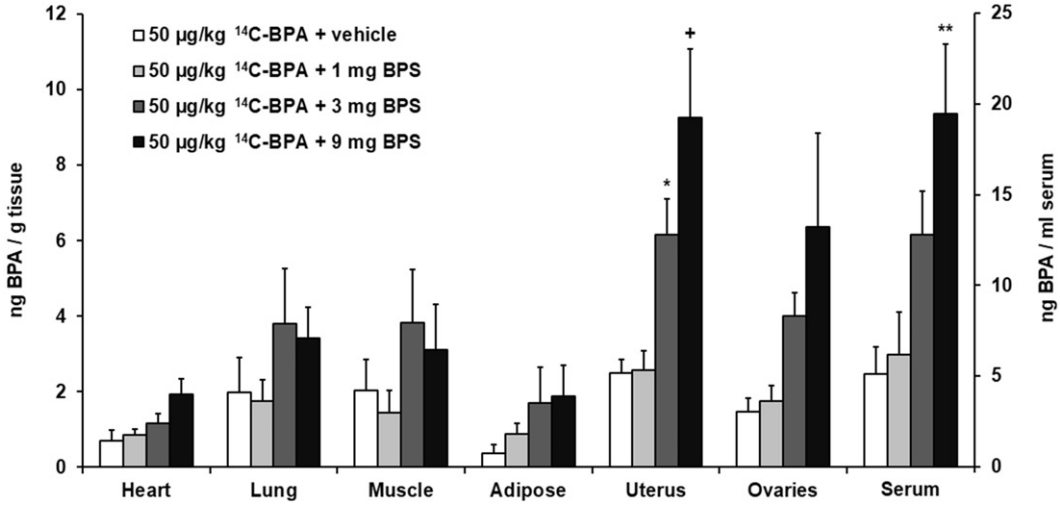

Figure 1. Mean (+SE) concentration of ^{14}C-BPA in the heart, lung, muscle, adipose, uterus, ovaries, and serum of dioestrous females in Experiment 1. Animals received sc injection of 0 (vehicle), 1, 3, or 9 mg BPS followed by dietary administration of 50 µg/kg ^{14}C-BPA ($n = 7$ per dose). Significant difference from vehicle treatment in the same tissue: $^{*}p < .05$; $^{**}p < .01$; $+p < .001$

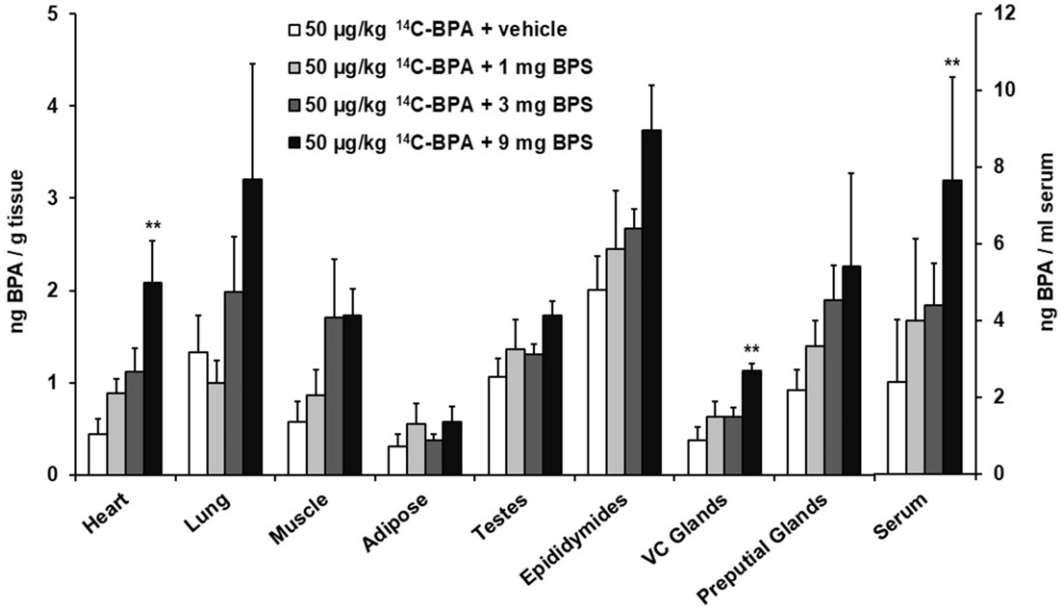

Figure 2. Mean (+SE) concentration of ^{14}C-BPA in the heart, lung, muscle, adipose, testes, epididymides, VC glands, preputial glands, and serum of males in Experiment 1. Animals received sc injection of 0 (vehicle), 1, 3, or 9 mg BPS followed by dietary administration of 50 µg/kg ^{14}C-BPA ($n = 7$ per dose). Significant difference from vehicle treatment in the same tissue: $^{**}p < .01$.

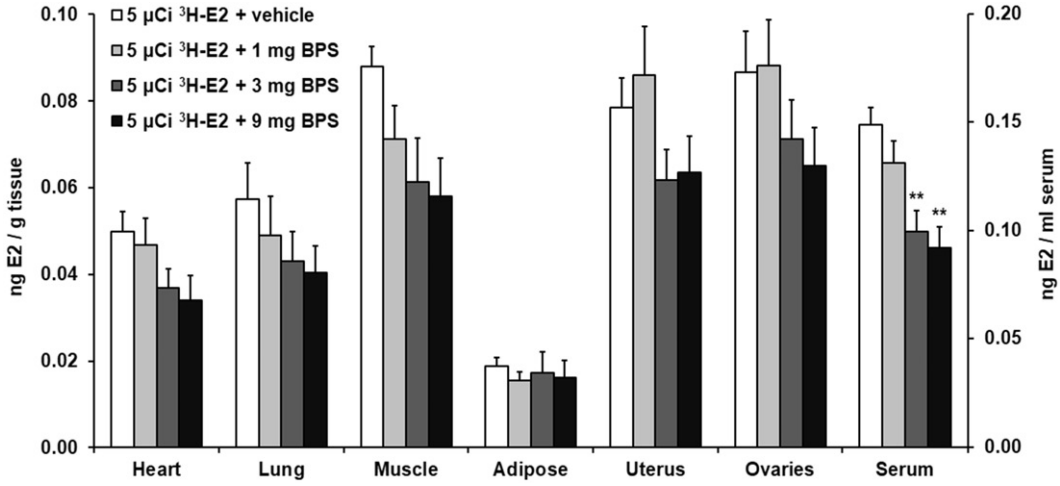

Figure 3. Mean (+SE) concentration of ^{3}H-E2 in the heart, lung, muscle, adipose, uterus, ovaries, and serum of dioestrous females in Experiment 2. Animals received sc injection of 0 (vehicle), 1, 3, or 9 mg BPS followed by dietary administration of 5 µCi ^{3}H-E2 ($n = 7$ per dose). Significant difference from vehicle treatment in the same tissue: $^{**}p < .01$.

Table 3. Mean (± SE) concentration of ^{3}H-E2 in the liver and kidney of dioestrous females and males following sc injection of 0 (vehicle), 1, 3, or 9 mg BPS and subsequent dietary administration of 5 µCi ^{3}H-E2 in Experiment 2.

	BPS dose (mg)	Liver (ng E2/g)	Kidney (ng E2/g)
Experiment 2			
Females	Vehicle	0.49 ± 0.04	0.15 ± 0.01
	1	0.44 ± 0.08	0.18 ± 0.05
	3	0.31 ± 0.03	0.11 ± 0.02
	9	0.27 ± 0.04	0.09 ± 0.02
Males	Vehicle	0.53 ± 0.03	0.14 ± 0.01
	1	0.48 ± 0.07	0.17 ± 0.02
	3	0.62 ± 0.12	0.15 ± 0.03
	9	0.50 ± 0.06	0.15 ± 0.02

tissues in females showed a significant effect of treatment for serum, $F(3,24) = 7.86$, $p = .007$. Multiple comparisons revealed that the vehicle treatment differed from the 3 and 9 mg treatments for serum. Reduced ^{3}H-E2 concentrations were noted in most peripheral tissues of females given 3 or 9 mg BPS, although these measures did not reach statistical significance. Comparable trends were also observed in males (Figure 4); however, comparisons among the four treatments for each of 11 tissues in males showed no statistically significant differences.

Experiment 3: measurement of urinary E2 in mice given BPS

Concentrations of E2 are reported for creatinine-adjusted (ng E2/mg creatinine) and unadjusted (ng E2/ml urine) measures. In females (Figure 5), ANOVA showed a significant main effect of collection time-point on adjusted measures, $F(5,65) = 3.54$, $p = .007$, and on unadjusted measures, $F(5,65) = 8.89$, $p < .001$. Multiple comparisons revealed that females receiving vehicle differed from those receiving 1 or 3 mg BPS at 10 h after injection for adjusted and unadjusted measures. In males (Figure 6), ANOVA showed a significant main effect of collection time-point on adjusted measures, $F(5,20) = 5.08$, $p = .004$, and on unadjusted measures, $F(5,20) = 5.62$, $p = .002$. For unadjusted measures, there was also a significant main effect of treatment, $F(2,4) = 9.96$, $p = .028$, and a significant interaction, $F(10,20) = 2.94$, $p = .019$. Multiple comparisons revealed that males receiving vehicle differed from those receiving 1 or 3 mg BPS at 10 h after injection for adjusted and unadjusted measures. Multiple comparisons also showed that males receiving 3 mg BPS differed from those receiving vehicle or 1 mg BPS at 4 h after injection for adjusted and unadjusted measures.

Discussion

To the best of our knowledge, these data are the first to demonstrate *in vivo* interaction between BPS and BPA. When animals were given a dietary supplement containing ^{14}C-BPA, pre-treatment with 9 mg BPS increased radioactivity in the blood serum of female and male mice. Pre-treatment with 9 mg BPS also elevated radioactivity in the heart and VC glands of males, while 3 mg BPS was sufficient to increase radioactivity in the uterus of females. Such tissues contain moderate-to-high oestrogen receptor (ER) expression (Couse et al., 1997; Kuiper et al., 1997). Consistent with previous studies measuring the distribution of BPA (Kim et al., 2004; Kurebayashi et al., 2005; Pollock & deCatanzaro, 2014), concentrations of ^{14}C-BPA were highest in the liver and kidney; these organs are involved in metabolism and excretion and may not necessarily reflect tissue deposition or receptor interaction. Tissue/serum concentration ratios for ^{14}C-BPA were similar to those previously reported in female rats administered 100 µg/kg BPA via oral gavage (Doerge et al., 2011).

The findings of Experiment 1 are consistent with previous studies of the effects of triclosan, TBBPA, BP, PP, or DEHP on distribution of ^{14}C-BPA (Borman et al., 2017; Pollock et al., 2014, 2017a,b). Elevation of ^{14}C-BPA concentrations by BPS is most likely due to competition among these chemicals for access to metabolic enzymes. Bisphenols are largely detoxified by phase II conjugative enzymes, including UGT and SULT (Hanioka et al., 2008; Pritchett et al., 2002;

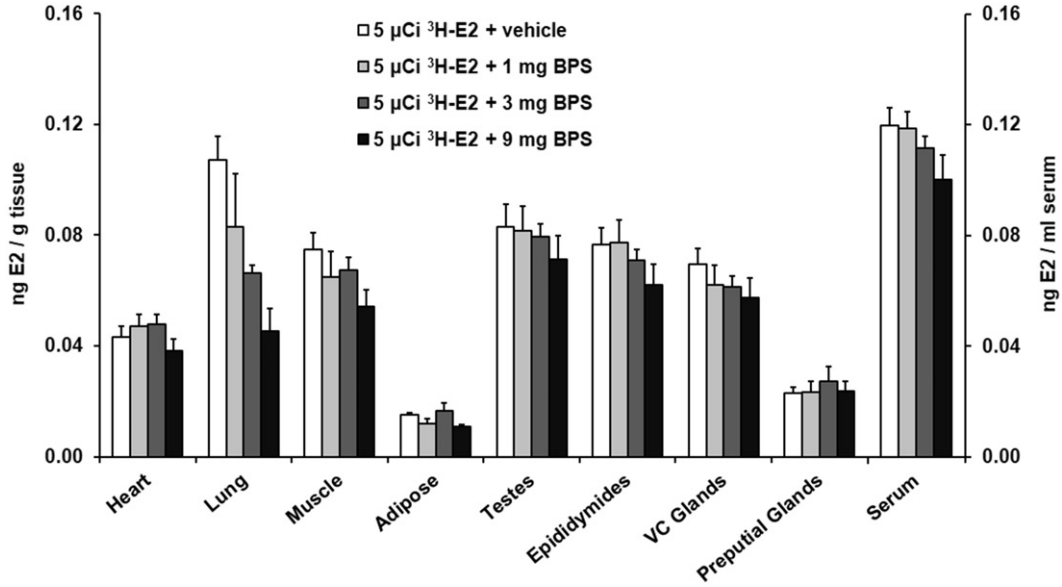

Figure 4. Mean (+SE) concentration of ^{3}H-E2 in the heart, lung, muscle, adipose, testes, epididymides, VC glands, preputial glands, and serum of males in Experiment 2. Animals received sc injection of 0 (vehicle), 1, 3, or 9 mg BPS followed by dietary administration of 5 µCi ^{3}H-E2 ($n = 7$ per dose).

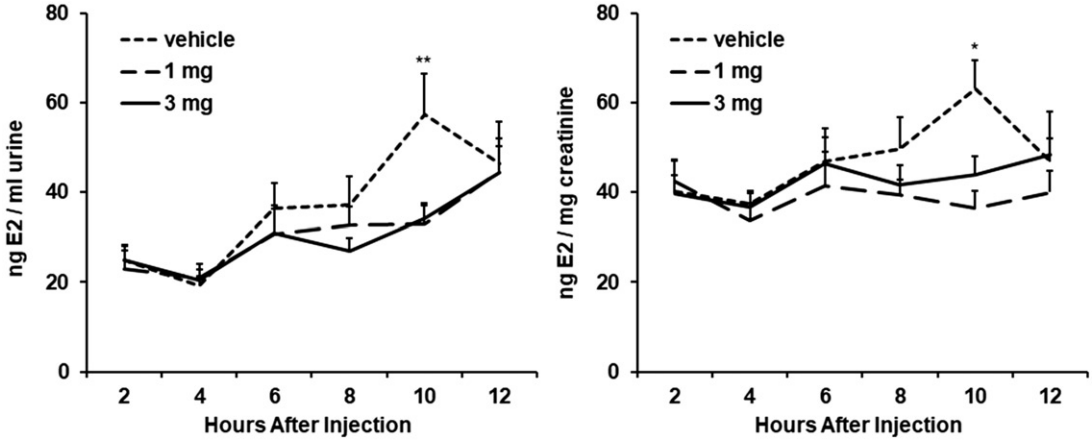

Figure 5. Mean (+SE) concentration of urinary E2, expressed as ng E2/ml urine and ng E2/mg creatinine, of dioestrous females in Experiment 3. Animals received sc injection of 0 (vehicle), 1, or 3 mg BPS ($n = 10$ per dose). Significant difference from all other treatments at the same time-point: $^{*}p < .05$; $^{**}p < .01$.

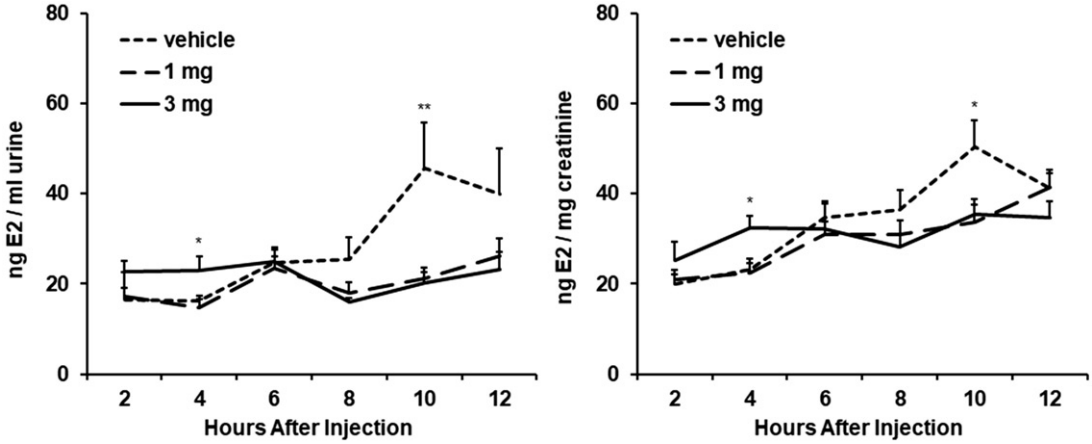

Figure 6. Mean (+SE) concentration of urinary E2, expressed as ng E2/ml urine and ng E2/mg creatinine, of males in Experiment 3. Animals received sc injection of 0 (vehicle), 1, or 3 mg BPS ($n = 10$ per dose). Significant difference from all other treatments at the same time-point: $^{*}p < .05$; $^{**}p < .01$.

Skledar et al., 2016; Zalko et al., 2002). Resulting from interaction with UGT, the monoglucuronide conjugate is a major metabolite of BPS (Skledar et al., 2016) and BPA (Hanioka et al., 2008; Kurebayashi et al., 2010). Other BPA metabolites, and presumably also BPS metabolites given structural similarities between the two substances, include the monosulphate conjugate produced by SULT (Yalcin et al., 2016), and various sulphate/glucuronide diconjugates (Inoue et al., 2016; Yalcin et al., 2016).

Concentrations of exogenous ^{3}H-E2 were reduced in blood serum, and to a lesser degree in tissues, by pre-treatment with BPS. These findings contrast with those previously observed for triclosan, which elevated exogenous ^{3}H-E2 concentrations in tissues and blood serum of female mice (Pollock et al., 2016). Whereas triclosan is a highly potent inhibitor of oestrogen sulphonation by SULT (James et al., 2010, 2015; Wang et al., 2004), bisphenols are weaker substrates of SULT (Yalcin et al., 2016). Thus, the findings of Experiment 2 are not well explained by competition between BPS and E2 for access to metabolic enzymes. Rather, reduction of ^{3}H-E2 in serum could be due to competition with BPS for access to carrier proteins. Given that endogenous E2 is lipophilic, a large fraction exists as a complex with sex hormone-binding proteins, such as globulin and albumin (Hammond 1990, 1995). Certain xeno-oestrogens, including BPA and BPS, can similarly interact with sex hormone-binding proteins (Déchaud et al., 1999; Wang et al., 2014; Xie et al., 2010) and displace E2 *in vitro* (Déchaud et al., 1999). Impacts of BPS on exogenous ^{3}H-E2 uptake into circulation may be relevant to human oestrogen supplementation, albeit with certain caveats. Whereas we gave E2 in a dietary supplement, human oestrogen supplementation usually involves E2 derivatives such as ethinyl oestradiol administered via an oral pill, vaginal ring, or transdermal patch (van den Heuvel et al., 2005). Nevertheless, modulation of exogenous oestrogen concentrations and actions by BPS could disrupt human oestrogen supplementation and warrants further study.

Concentrations of natural E2 in urine were also modulated by exposure to BPS. Urinary E2 generally reflects systemic oestrogen trends, and it has advantages over blood measures because urine can be collected non-invasively at multiple time-points (deCatanzaro et al., 2003, 2004; Muir et al., 2001; Thorpe et al., 2014). Urinary E2 was elevated by BPS at 4 h after injection in males, but reduced at 10 h after injection in females and males. The discrepancy in E2 modulation at 4 h and 10 h could be explained, at least in part, by differential effects on oestrogen production via aromatase (CYP19A). BPA can stimulate aromatase activity in JEG-3 cells following short-term exposure (between 10 min and 2 h), while longer exposures (up to 18 h) inhibit aromatase activity (Nativelle-Serpentini et al., 2003). BPS has also been previously shown to increase aromatase expression in zebrafish (Ji et al., 2013), thereby reducing testosterone concentrations and elevating E2 concentrations (Ji et al., 2013; Naderi et al., 2014). Another potential mechanism of urinary E2 modulation by BPS involves hormonal regulation of renal function. Oestrogens regulate expression of various transporters involved in renal tubular uptake of substrates from circulation, including organic anion transporters (OAT), organic cation transporters (OCT), and organic anion-transporting polypeptide (OATP) (Cheng & Klaassen, 2009; Ljubojević et al., 2004). Substrates of these transporters include endogenous steroid hormones, as well as exogenous pharmaceuticals and xenobiotics (Roth et al., 2012). In the US population, reduced glomerular filtration rate through the kidney was associated with lower concentrations of urinary xeno-oestrogens, such as triclosan and BPA (You et al., 2010). In the context of our findings, oestrogenic actions of BPS in the kidney could modulate excretion of E2 in urine.

Concentrations of biologically active oestrogens in circulation and tissues are tightly regulated by actions of several enzymes (Dumas & Diorio, 2011). Generally, E2 plays critical roles in reproduction, including sexual differentiation, reproductive organ development, sexual behaviour and receptivity, and oestrous/menstrual cycling (Alonso & Rosenfield, 2002; deCatanzaro, 2015). Increased E2 can lead to pregnancy failure by disrupting intrauterine blastocyst implantation in mice and other mammals (deCatanzaro, 2015; Gidley-Baird et al., 1986; Ma et al., 2003; Thorpe et al., 2013). Heightened oestrogen actions from hormone-replacement therapy are associated with increased risk of hormone-dependent cancers (Million Women Study Collaborators, 2003, 2005, 2007). Elevated maternal serum E2 in the first trimester is also associated with low birth weight in humans (Hu et al., 2014).

The doses of BPS administered in the present studies are much greater than typical exposures in the general public. Whereas we administered a single dose of 1–9 mg BPS (corresponding to around 22–330 mg/kg), human exposures are likely several orders of magnitude lower (Chen et al., 2016; Liao et al., 2012a). Based on BPS output in urine, one study (Chen et al., 2016) estimates that mean exposure levels range from 0.001 to 0.115 μg/kg/d across several countries. We have previously shown that BPA concentrations are elevated by a single administration of triclosan, TBBPA, BP, PP, or DEHP (Borman et al., 2017; Pollock et al., 2014, 2017a, 2017b). Concurrent exposure to a mixture of all five of these chemicals at doses as low as 0.1 mg can significantly elevate concentrations of BPA in tissues and E2 in urine (Pollock et al., 2018). Thus, BPS could have similar effects at much lower doses when studied in combination with other EDCs. Furthermore, we only investigated the capacity of BPS to modulate concentrations of BPA and E2 acutely via one exposure route. Future studies should explore impacts of repeated administrations over several days and via other routes of exposure.

BPA is an EDC with known oestrogenic properties (Rochester, 2013; Seachrist et al., 2016; Ziv-Gal & Flaws, 2016), and BPS shows similar mechanisms of action and potencies (Rochester & Bolden, 2015). BPS can bind to ER (Mansouri et al., 2016; Rochester & Bolden, 2015; Rosenmai et al., 2014; Yamasaki et al., 2004), and can also produce an oestrogenic response in a rat uterotrophic assay (Yamasaki et al., 2004). Concurrent exposure to BPA and BPS could become even more harmful to human health as manufacturers continue to substitute BPA with bisphenol analogues in consumer products. Further assessment of interaction between BPA and BPS is necessary to ensure that replacement of BPA with BPS is not a case of regrettable

substitution, whereby the new substance is similar or even worse than the substance(s) that it was designed to replace. Our findings of the interaction between BPA and BPS highlight the importance of studies of multiple toxicants, which should be emphasised when conducting human health risk assessments and determining regulatory exposure limits.

Disclosure statement

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