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## Effects of *In Utero* Exposure to Bisphenol A or Diethylstilbestrol on the Adult Male Reproductive System

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### Abstract

The objective of this study was to determine if *in utero* exposure to Bisphenol A (BPA) induced reproductive tract abnormalities in the adult male testis. Using the C57/B16 mouse, we examined sex-organ weights, anogenital distance (AGD), and testis histopathology in adult males exposed *in utero* via oral gavage to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg diethylstilbestrol (DES) as a positive control from gestational days 10–16. No changes in sperm production or germ cell apoptosis were observed in adult testes following exposure to either chemical. Adult mRNA levels of genes associated with sexual maturation and differentiation, *GATA4* and *ID2*, were significantly lower only in DES-exposed testes. In summary, the data indicate no gross alterations in spermatogenesis following *in utero* exposure to BPA or DES. At the molecular level, *in utero* exposure to DES, but not BPA, leads to decreased mRNA expression of genes associated with Sertoli cell differentiation.

### Keywords

Endocrine disruptors; gene expression; hormones/cyclicality; molecular biology; postnatal evaluation; spermatogenesis

## 1. INTRODUCTION

Within the environment there are numerous chemicals that have hormone mimicking effects which interfere with the normal endocrine functions of humans and wildlife (Hotchkiss et al., 2008). These chemical compounds have been given the name endocrine disruptors. A growing number of these compounds have been reported to have estrogenic activity acting as estrogen agonists or antagonists within the human population. Animal studies have demonstrated that exposure of mice and rats to relatively high concentrations of certain chemicals with estrogenic activity during fetal or postnatal development lead to developmental abnormalities in the male reproductive system including cancer (Sharpe and Skakkebaek, 2008; Sonne et al., 2008). Therefore, one aim of the present study was to determine the effects on adult male reproductive parameters induced in the testis by *in utero* exposure to two estrogen disruptors of different potency, bisphenol A (BPA), a weak estrogenic compound, and diethylstilbestrol (DES), a potent estrogenic compound, during organogenesis of the male reproductive system. The second aim of the study was to determine if changes in gene expression associated with apoptosis, steroidogenesis, or testis maturation occurred in the adult male testis following *in utero* exposure to either of these estrogenic compounds.

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BPA, a weak estrogenic phenolic compound, is a building block of polycarbonate plastic and epoxy resins that grosses six billion dollars a year for the companies that produce it in the United States (Borrell, 2010). BPA has generated increasing concern in the scientific and public health arenas over its potential to induce reproductive abnormalities similar to DES due to its similar structure and high production volume. BPA is found in plastic food containers, baby bottles, and the lining of canned goods to prevent corrosion. Unfortunately, one of the deleterious characteristics of BPA is its ability to leach from plastic, thus presenting a route for human exposure by ingestion. This suggests that humans are ubiquitously exposed to BPA, and measurable levels have been found in human breast milk, urine, serum, fetal and maternal plasma (Willhite et al., 2008). BPA at high doses has been shown to result in gene expression changes associated with apoptosis (Li et al., 2009) in the adult male, and with changes in ICaBP, IL4R, and progesterone receptor in the fetal testis (Naciff et al., 2005). Alterations in Sertoli cell number have also been found following intrauterine exposure to BPA (Wistuba et al., 2003).

In the 1940's, DES was developed as a chemotherapeutic agent for the treatment of metastatic prostate cancer (Huggins and Hodges, 1972) and was used as an off-label drug to prevent adverse pregnancy outcomes in women with a history of miscarriage (Dieckmann et al., 1953; Dutton, 1988). Since then, DES effects on the reproductive tract have been widely documented in both humans and experimental animals (Brouwers et al., 2006; Herbst et al., 1971; Klip et al., 2002; Newbold et al., 2009). Exposure to potent estrogenic agents like DES, *in utero* or postnatally, have resulted in induced male reproductive abnormalities including reduction in sperm count, cryptorchidism, and other reproductive tract abnormalities (Fielden et al., 2002; Rivas et al., 2002). Importantly, *in utero* exposure to DES can alter sex-specific genetic pathways governing early differentiation and cell proliferation of both the male and female gonads (Ikeda et al., 2008). Moreover, functional alterations in the Sertoli cell have been found following exposure to DES (Sharpe et al., 1998); and changes in gene expression in steroidogenesis and the INSL3 have also been observed (Zhang et al., 2009).

Understanding that the developing organism is uniquely sensitive to perturbations by chemical compounds with estrogenic activity, we designed the current study utilizing C57/BL6 mice to determine (1.) if *in utero* exposure during the major period of organogenesis (gestational days 10 through 16) to either BPA or DES resulted in male reproductive abnormalities in adulthood and (2.) if changes in gene expression were associated with defects in testis growth and/or maturation occurred in the adult mouse testis following *in utero* exposure to these estrogenic chemicals. We utilized oral gavage as our route of exposure because it is the main route through which humans are exposed to BPA, and also allows for first pass metabolism. Mice were exposed to BPA at either a moderate dose of 50 µg/kg, which corresponds to the Environmental Protection Agency's (EPA) acceptable daily intake, or a high dose of 1,000 µg/kg, or sesame oil control. We chose to expose an additional group of dams to 2 µg/kg DES as a positive control. We chose this dose because the estrogenic potency of DES has typically been 100 to 1,000-fold times higher than that of BPA, and this dose of 2 µg/kg is 500 times lower than our high dose of BPA (vom Saal and Welshons, 2006). This dose of DES is also below the 5 µg/kg cutoff for a low dose of DES, as higher doses can result in opposite effects of lower doses (Richter et al., 2007). Our model utilized C57/BL6 mice, an appropriate model system to study effects of xenoestrogen exposure in mammary glands (Wadia et al., 2007). We found that BPA failed at either dose to induce changes in gene expression in the adult testes of mice. Although no overt histopathological changes were observed following *in utero* exposure to either BPA or DES at the doses employed, at the molecular level *in utero* exposure to DES resulted in gene expression changes associated with the functional maturation of Sertoli cells. Taken

together, while BPA displayed little effect on male reproductive development, DES disrupted the normal pattern of Sertoli cell differentiation.

## 2. MATERIAL AND METHODS

### 2.1 Exposure paradigm and animal treatment

BPA (>99% purity) and DES (97.5% purity) were purchased from Sigma and dissolved in sesame seed oil (Sigma Aldrich, St. Louis, MO). Timed pregnant females purchased from Charles River (Wilmington, MA) were treated via oral gavage with either sesame oil as a vehicle control (N=12), 50µg BPA/kg (N=11), or 1,000µg BPA/kg (N=14) from gestational days 10 through 16. As a positive control, DES was administered at 2µg/kg (N=14). Doses were administered on a milligram per kilogram body weight basis and adjusted daily for weight changes. The weight of the dams during dosing and the length of gestation were recorded. Offspring were weaned at PND 21. Animals were housed in polycarbonate cages and given access to chow (Purina 5010) and water in a central pipe system *ad libitum*. The Brown University Institutional Animal Care and Use Committee approved all experimental animal protocols in compliance with National Institute of Health guidelines.

### 2.2 Body weights, testis weights, spermatid head counts, anogenital distance

The body and testis weights of F1 male mice were weighed and recorded at PND 56. Anogenital distance was also measured at PND 56 utilizing digital calipers (Control Company, Friendswood, Texas). Testes were detunicated, homogenized separately for 30 seconds in 1.5 ml saline merthiolate triton, and sperm heads were counted on TC-10 automated cell counter (Bio-Rad, Hercules, CA). This method resulted in similar sperm counts using a hemacytometer method as previously described (Rasoulpour et al., 2006).

### 2.3 Testis histopathology

The testes from adult male C57/BL6 mice exposed *in utero* with sesame oil vehicle, BPA, or DES were fixed in 10% neutral buffered formalin and paraffin embedded. Testis sections (7µm) were stained with hematoxylin and eosin. Images were captured using an Aperio Scan Scope (Aperio Technologies, Vista, CA) and images were analyzed using Aperio ImageScope v10.2 (Aperio Technologies). Testicular histopathological examination was conducted at the gross anatomy level to identify exposure-related effects such as retained spermatids, missing germ cell layers, multinucleated giant cells, or sloughing of spermatogenic cells into the lumen. Seminiferous tubule diameters were measured for each treatment group, and the average of at least 100 seminiferous tubule cross sections per animal and of at least five animals per treatment group were used for statistical analysis.

### 2.4 TUNEL staining and quantification

Germ cell apoptosis was detected in sections of paraffin-embedded tissue by the terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) labeling method using the ApopTag kit (Chemicon, Temecula, CA). Tissue was counterstained with methyl green. Testis sections were viewed using a Nikon E800 microscope (Nikon, Melville, NY) using differential interference contrast microscopy for TUNEL quantification. The images were captured with Aperio Imagescope. TUNEL-positive germ cells were quantified in each tissue section by counting the number of TUNEL-positive cells in each essentially round seminiferous tubule. For each testis section, approximately 100–200 tubules were counted from at least six different mice. The incidence of apoptosis was then categorized into either of three groups, defined as none, one to three, or more than three TUNEL-positive germ cells per seminiferous tubule cross-section as previously described (Rasoulpour et al., 2006). In the control mouse testis, the percentage of seminiferous tubules with more than three

TUNEL-positive cells is less than 10%, so that an increase in apoptosis is easily determined using this data presentation.

## 2.5 RNA isolation

The testes from C57/BL6 mice exposed *in utero* were detunicated, weighed, and homogenized in TriReagent (Sigma Aldrich) and further RNA isolation was performed according to the TriReagent manufacturer's protocols.

## 2.6 Quantitative RT-PCR

Total RNA (1 µg) was DNase (Promega, Madison, WI) treated and reverse-transcribed using the iScript cDNA Synthesis Kit (Bio-Rad) according to the manufacturer's protocols. cDNA templates were amplified with either self-designed primers from Invitrogen (Invitrogen, Madison, WI) or pre-optimized mouse-specific QuantiTect® primer assays (Qiagen, Valencia, CA) using iQ SYBR Green Supermix (Bio-Rad) on an iCycler iQ Multicolor Real-time PCR Detection System (Bio-Rad). Each sample was run in triplicate, and mRNA levels were analyzed relative to hypoxanthine phosphoribosyltransferase (HPRT). Log<sub>2</sub>-transformed relative expression ratios were calculated as described using the equation set forth by Pfaffl (Pfaffl, 2001). Primer pairs and their respective sequences where available are listed in Table 1.

## 2.7 Serum isolation and hormone measurement

To measure serum testosterone, blood samples were collected by cardiac puncture. Serum was separated via centrifugation of clotted samples and stored at -80 °C for later analyses. Serum concentrations of testosterone were analyzed by the ELISA method. All samples were run in duplicate, and each n represented a different litter.

## 2.8 Statistical analysis

A one-way ANOVA followed by a Tukey HSD test was used to determine statistical significance for BPA treated groups using the sesame oil group as a reference. Given that DES is a different toxicant, a two-tailed student t-test was performed to determine statistical significance between sesame oil and DES treated groups. Litter averages were used for body weights, anogenital distance, and organ weights. Males from separate litters were used for other experiments. A  $p < 0.05$  was considered statistically significant. Statistics were calculated using R ([www.r-project.org](http://www.r-project.org)).

# 3. RESULTS

## 3.1 Assessment of litter outcomes and adult testicular parameters following *in utero* exposure to BPA or DES

To determine if maternal exposure to either BPA or DES resulted in differences in litter outcomes, we examined litter sizes and viability at birth and weaning. Litter size at birth, litter size at weaning, and percent viability of offspring remained consistent across the BPA treatment groups compared to sesame oil control (Table 2). In contrast, *in utero* exposure to DES resulted in a significant decrease in litter size at birth, and a strong trend for decreased litter size at weaning. These data indicate that BPA exposure had little effect on litter outcomes, while DES had negative effects. To characterize adult reproductive parameters of adult males previously exposed to BPA or DES *in utero*, we examined the body weight, testis weight, seminal vesicle weight, and AGD from BPA and DES exposed F1 males and compared them with adult F1 sesame oil control males. As table 3 illustrates, we found no significant differences in male body weight in any of the F1 exposed adult males relative to F1 control male body weights. No significant differences in the adult testis weights at both

the gross and body-weight adjusted levels of F1 male mice exposed to either BPA or DES were observed when compared with that of sesame oil F1 control animals. Seminal vesical weights at both the gross and bodyweight adjusted levels were also not affected by either BPA or DES *in utero* exposure. However, *in utero* exposure to DES, but not BPA, resulted in a significant increase in anogenital distance at both the gross measurement and after adjustment for body weight.

### **3.2 No gross alteration in the seminiferous tubules or sperm production of adult F1 males following in utero exposure to BPA or DES**

To determine if spermatogenesis was altered in the adult F1 males, we examined cross sections of testis seminiferous tubules. Gross testis histopathology was not altered in BPA (Figure 1b and 1c) or DES (Figure 1d) exposure groups, and no differences were detected in seminiferous tubule diameters (Table 4). In addition to testis weights and histopathology, the measurement of spermatid heads at PND 56 (Figure 2) did not reveal significant differences between any of the F1 exposure groups or sesame oil control animals, suggesting a full complement of germ cells in the testis.

### **3.3 No increase in germ cell apoptosis or Fas and FasL mRNA levels in the adult testis following in utero exposure to BPA or DES**

Exposure to high doses of BPA in adult male mice ranging from 160 mg/kg to 960 mg/kg body weight has been shown to lead to increased germ cell apoptosis in the testis with concomitant increases in Fas and FasL gene expression (Li et al., 2009). Therefore, to determine the potential consequences of *in utero* exposure to BPA and DES in the adult testis, we examined the level of germ cell apoptosis in F1 exposed and F1 control seminiferous tubules at PND 56. Apoptosis was assessed by counting seminiferous tubules with more than three apoptotic germ cells per cross section. At the doses examined, we found no significant differences in germ cell apoptosis in the F1 *in utero* exposed groups relative to control animals (Figure 3). Fas and FasL mRNA expression also remained similar in the F1 sesame oil control group compared to either BPA or DES at all doses examined (Figure 4).

### **3.4 In utero exposure to DES, but not BPA, alters the mRNA levels of GATA4 and ID2 in the adult testis**

In the adult testis, mRNA levels of genes associated with sexual differentiation and maturation of the testis, *GATA4* and *ID2*, were significantly lower in the F1 adult male DES exposure group; and there was a trend for decreased expression of *inhibin B* ( $p=0.07$ ) a marker of Sertoli cell function suggesting that *in utero* exposure to DES alters gene expression in the testis (Figure 4). In contrast to the gene changes observed following *in utero* exposure to DES, no changes in gene expression in the panel of genes examined (Table 1) were observed in the adult testes of F1 male mice exposed *in utero* to BPA.

### **3.5 In utero exposure to BPA or DES does not affect testosterone levels in adult male serum**

Given that DES altered a number of genes related to sexual maturation in the testis, we decided to investigate the levels of testosterone in serum from BPA and DES exposed F1 males. We found no significant changes in testosterone from either exposure group (Table 5).

## 4. DISCUSSION

Although BPA has been extensively studied, questions remain about whether BPA causes male reproductive abnormalities in rodents and/or humans (Goodman et al., 2009; Howdeshell et al., 2008; Maffini et al., 2006; Ryan et al., 2010; Vandenberg et al., 2009; Willhite et al., 2008). Differences in experimental outcomes which have been attributed to differences in species and/or strains of animals used, routes of administration of chemical, and critical window(s) of exposure (Myers et al., 2009; Vandenberg et al., 2009). The developing fetus and children are of particular concern for toxicant exposure due to their small size and vulnerability. Human exposure to BPA is primarily through the oral route, and based on this, we chose to study the major period of organogenesis in the C57/Bl6 mouse (gestation days 10–16) with the dam receiving the dosage via oral gavage. The oral gavage route allows for the BPA to enter first pass metabolism in the dam, thereby resulting in less bioavailability to the fetus rather than through intraperitoneal or subcutaneous injections of the same dosage. Mice were housed in polycarbonate cages, which previously work has indicated could potentially expose animals to additional BPA, with the highest exposure in very old cages (Howdeshell et al., 2003). Our animals were housed in new polycarbonate cages with metal wire food trays and a centralized metal pipe system for water, and we believe that exposure to BPA through leaching was kept at a minimum.

The C57/Bl6 mouse is a more aggressive mouse, and as a result we observed high levels of infanticide following birth in all exposure groups. This behavior is normally observed in this strain, and resulted in relatively low rates of viability in all the exposure groups, but no observable difference in litter viability was found between BPA, DES, or sesame oil exposed litters (Table 2) We did however observe differences in litter size at birth and litter size at weaning for the DES-treatment group, indicating potential maternal toxicity or higher levels of infanticide following stress from DES exposure. It should be noted that very occasionally entire litters were eaten, which is why the F1 litter numbers are less than the original number of dams as described in the materials and methods (section 2.1). While litter culling was not performed, we instead chose to use litter averages for male reproductive outcomes, and randomly selected 1 male offspring per litter for molecular experiments so each n would represent a different litter.

Similar to previously published results (Howdeshell et al., 2008; Ryan et al., 2010), we observed few significant differences in body weight, testis weight, or seminal vesicle weight following *in utero* exposure to BPA. Histological analyses revealed little apparent difference between either BPA or DES-exposed F1 seminiferous tubules when compared to sesame oil controls. Moreover, we found no significant increases in germ cell apoptosis or in the genes, Fas and FasL, which have previously been reported to be up-regulated in the rodent testis following exposure to extremely high doses of BPA (Li et al., 2009; Ryan et al., 2010). However, DES, but not BPA, exposure resulted in a significant increase in anogenital distance. As DES is a potent estrogen, high doses will result in anti-androgenic effects in males. For example, it has been reported that prenatal exposure to high levels of DES (200 µg/kg/day) results in a significant decrease in anogenital distance (Palanza et al., 2001). However, we did not find any differences in serum testosterone levels in any of the exposure groups compared to sesame oil controls, indicating normal testosterone synthesis. While there is a trend for decreased levels of serum testosterone in the DES treated males compared to sesame oil controls, this did not reach statistical significance ( $p=0.14$ ). This is similar to a prior study which indicated that BPA and the positive control ethinyl estradiol did not alter serum hormone levels in rats exposed during gestation and lactation (Howdeshell et al., 2008). Based on these results we propose that at lower doses of DES, there is a potentially agonistic effect rather than an antagonist effect on androgen receptor signaling.

At the molecular level, high levels of estrogens have been shown to affect gene expression in the testis early in life (Saunders et al., 1997). Therefore, we examined a panel of genes associated with steroidogenesis, germ cell apoptosis, and Sertoli cell maturation which have been shown by other laboratories to be altered by endocrine disrupting chemicals including BPA (Li et al., 2009; Naciff et al., 2005) and DES (Li et al., 2009; Naciff et al., 2005; Saunders et al., 1997). We found that DES reduced the expression of two transcription factors, *GATA4* and *ID2*, in the testes of adult F1 males. The transcription factor *GATA4* is expressed in Sertoli cells, steroidogenic Leydig cells, and other testicular somatic cells, and *GATA4*, along with its cofactor *FOG2*, is necessary for proper Sry expression and all subsequent steps in testicular organogenesis, including testis cord formation and differentiation of both Sertoli and fetal Leydig cells (Bielinska et al., 2007). Interestingly a recent study in which *GATA4* was conditionally deleted in Sertoli cells in the testis of mice resulted in Sertoli cell vacuolization, impaired spermatogenesis, increased permeability of the blood-testis barrier, and infertility (Kyronlahti et al., 2011). *ID2*, similar to *GATA4*, is a transcription factor and is associated with the inhibition of differentiation of various cell types (Chaudhary et al., 2005). *ID2* is most abundant in Sertoli cell nuclei, but also detectable in pachytene and diplotene spermatocytes (Sablitzky et al., 1998). *Inhibin B* is associated with disorders of sexual and gonadal differentiation (Barrionuevo et al., 2009; Toulis et al., 2010). Collectively, the gene expression changes in our DES exposed mice may indicate future problems with fertility in the DES-exposed males, and future studies are warranted. The data indicate no gross spermatogenic changes following *in utero* exposure to DES or BPA at the doses employed by adulthood. However, at the molecular level, *in utero* exposure to DES results in gene expression changes in the adult testis associated with alterations in Sertoli cell function.

In summary, we found that moderate (50 µg/kg) to high exposure (1,000 µg/kg) to BPA *in utero* does not lead to gross changes in adult body weight, testis weight, AGD, spermatogenesis, sperm production, or specific changes in gene expression associated with steroidogenesis, apoptosis, or Sertoli cell maturation in the F1 generation of adult C57/BL6 mice. However, *in utero* exposure to DES resulted in increased AGD in F1 males, and gene expression changes associated with an altered Sertoli cell phenotype. Overall these results indicate that at the doses employed via oral gavage, BPA does not affect young adult male reproductive health in regards to the testis. The use of the C57/Bl6 mouse in this study may be useful for future studies using transgenic mouse models to study gene-environment interactions with BPA. Future studies in our laboratory will focus on other target organs as well as additional lower doses as BPA may elicit its effects differently in other organ systems.

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## Abbreviations

<b>BPA</b>	Bisphenol A
<b>DES</b>	Diethylstilbestrol
<b>AGD</b>	Anogenital Distance

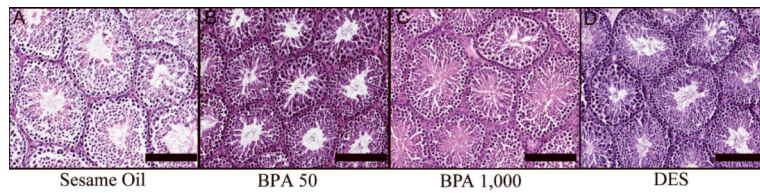
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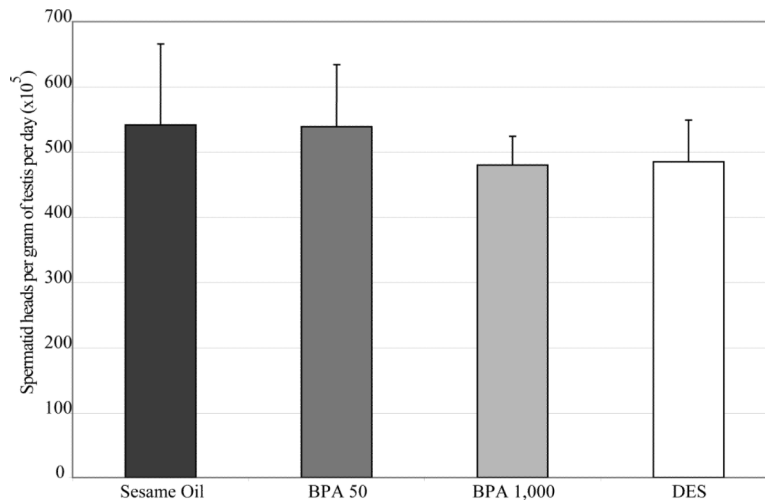
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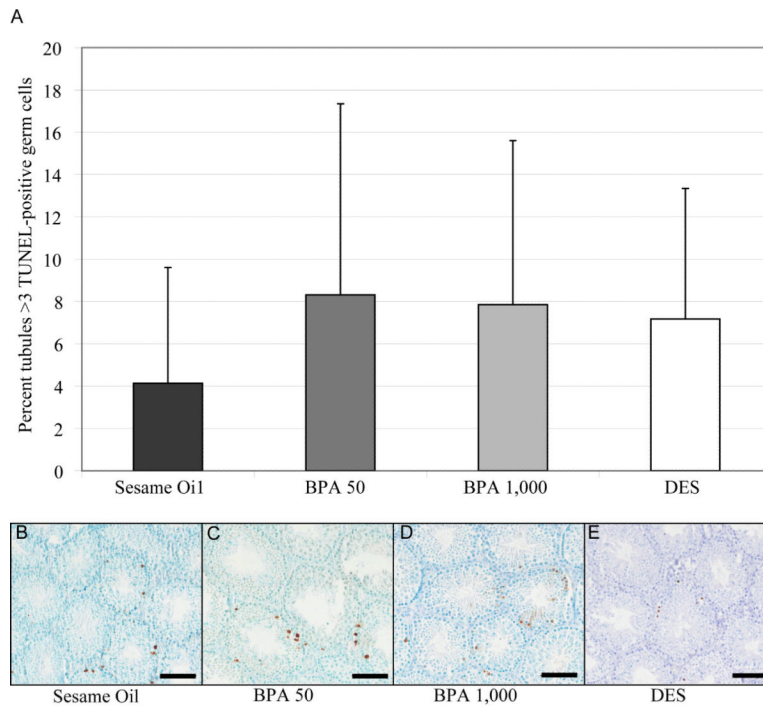
**Figure 1. Testis histopathology**

H&E sections of sesame oil (a), BPA 50  $\mu\text{g}/\text{kg}$  (b), BPA 1,000  $\mu\text{g}/\text{kg}$  (c), and DES 2  $\mu\text{g}/\text{kg}$  (d) treated testis. No observable histopathological changes were detected in any treatment group. Scale bars indicate 1,000  $\mu\text{m}$ .



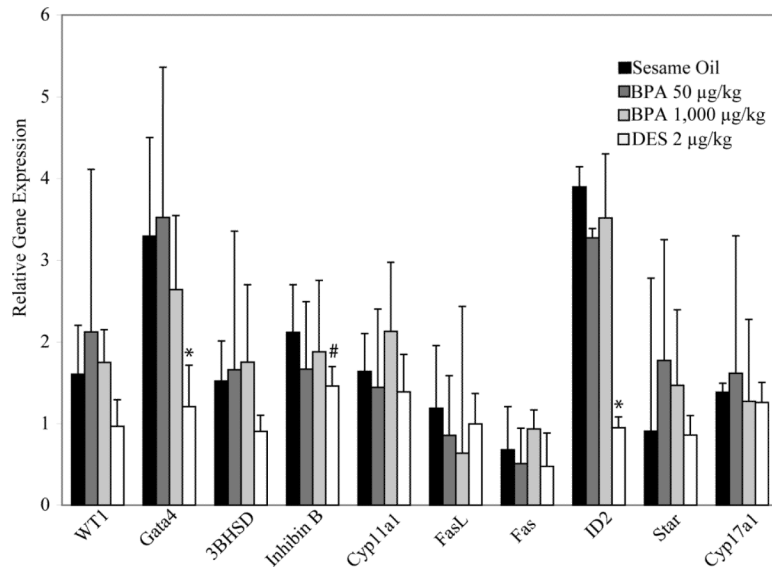
**Figure 2. Spermatid head counts of adult C57/Bl6 male mice following *in utero* exposure to sesame oil, 50  $\mu\text{g}/\text{kg}$  BPA, 1,000  $\mu\text{g}/\text{kg}$  BPA, or 2  $\mu\text{g}/\text{kg}$  DES**

Spermatid heads were counted using a TC-10 automated cell counter (Bio Rad). No statistical significance was found amongst any treatment groups compared to sesame oil control. Data represented as mean + standard deviation. Statistical analyses were conducted using one-way ANOVA followed by a Tukey HSD test for BPA and a two-tailed t-test for DES.  $n=6-7$  samples per treatment group, with each  $n$  representing a different litter.



**Figure 3. Germ cell apoptosis in adult C57/B16 male mice following *in utero* exposure to sesame oil, 50  $\mu\text{g}/\text{kg}$  BPA, 1,000  $\mu\text{g}/\text{kg}$  BPA, or 2  $\mu\text{g}/\text{kg}$  DES**

The apoptotic incidence in seminiferous tubules was quantified by using TUNEL staining (a). The percent of TUNEL-positive tubules with greater than 3 apoptotic cells is represented as mean + standard deviation. No significant differences in apoptotic incidence were found for BPA or DES treated testes. Statistical analyses were conducted using one-way ANOVA followed by a Tukey HSD test for BPA and a two-tailed t-test for DES.  $n=6$  per treatment group, with each  $n$  representing a different litter. Representative images of TUNEL staining in the testis for sesame oil (b), BPA 50  $\mu\text{g}/\text{kg}$  (c), BPA 1,000  $\mu\text{g}/\text{kg}$  (d), and DES 2  $\mu\text{g}/\text{kg}$  (e). Scale bars indicate 1,000  $\mu\text{m}$ .



**Figure 4. Gene expression of adult C57/Bl6 male mice following *in utero* exposure to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg DES**

Quantitative RT-PCR was conducted on a panel of genes associated with steroidogenesis, apoptosis, or differentiation of the testis. Statistical analyses were conducted using one-way ANOVA followed by a Tukey HSD test for BPA and a two-tailed t-test for DES. Data represented as mean + standard deviation. n=3–4 per treatment group, with each n representing a different litter. Asterisk (\*) indicates significance compared with sesame oil control (p<0.05). # indicates p<0.1.

**Table 1**

Primer sequences for real-time RT-PCR analysis

Gene	Primer Pair
WT1	F: CCCATTTACTAACTGCCCTAGAAAG R: TCTGCCTGGTGCCTTGC
GATA4	F: GTGAAAGGACAAAGGTGATAGAC R: GTGGGTGATGAGGACAAGG
3 $\beta$ HSD	QuantiTect® primer assay
Inhibin B	F:GGCTGAGTGTGGTTGTTC R:TGCGTGAGTCTGGCTATG
Cyp11a1	QuantiTect® primer assay
FasL	F: GCAAATAGCCAACCCCAGTACAC R: GCCACCTTCTTATACTTCACTCCAG
Fas	F: TGCACCCTGACCCAGAATAC R: GCCAGGAGAATCGCAGTAGAA
ID2	F:ACTGTGATACCGTTATTTATGAGAGAC R: TCCAAGTGTAGAAAGGGCACTG
STAR	QuantiTect® primer assay
HPRT	F:TTGCGCTCATCTTAGGCTTT R:CAGGCCAGACTTTGTGGAT Or QuantiTect® primer assay
Cyp17a1	QuantiTect® primer assay

**Table 2**

Litter outcomes following exposure to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg DES.

Mouse Exposure Group	Number of Initial Litters	Litter Size at Birth (n)	Litter Size at Weaning (n)	Viability (%)
Sesame oil	12	7.1 +/- 1.0	5.5 +/- 2.8	73.9 +/- 35.7
BPA 50 µg/kg	11	8.2 +/- 1.0	5.5 +/- 3.5	66.8 +/- 42.0
BPA 1,000 µg/kg	14	6.2 +/- 2.5	4.0 +/- 3.2	63.5 +/- 47.8
DES 2 µg/kg	14	5.8 +/- 1.8*	3.1 +/- 3.2 <sup>#</sup>	51.3 +/- 44.7

<sup>#</sup>  
p=0.05

\*  
p<0.05



**Table 3**

Male reproductive parameters in adult C57/Bl6 male mice following *in utero* exposure to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg DES

Mouse Exposure Group (N=litters examined)	Age (days)	Body Weight (g)	Combined Testis Weight (mg)	Adjusted Combined Testis Weight (mg/g)	Seminal Vesicle Weight (mg/g)	Adjusted Seminal Vesicle Weight (mg/g)	AGD (mm)	Adjusted AGD (mm/g)
Sesame oil N=8	56	23.5 +/- 1.2	154.6 +/- 23.5	6.8 +/- 0.9	72.5 +/- 13.5	3.2 +/- 0.5	10.9 +/- 1.3	0.5 +/- 0.1
BPA 50 µg/kg N=8	56	23.0 +/- 1.4	164.5 +/- 19.6	7.2 +/- 0.9	73.1 +/- 13.3	3.2 +/- 0.5	11.1 +/- 0.5	0.5 +/- 0.1
BPA 1,000 µg/kg N=10	56	23.2 +/- 1.0	167.8 +/- 8.3	7.2 +/- 0.5	76.6 +/- 9.6	3.3 +/- 0.3	10.8 +/- 0.4	0.5 +/- 0.1
DES 2 µg/kg N=6	56	22.5 +/- 1.1	143.8 +/- 41.1	6.6 +/- 2.0	76.9 +/- 13.3	3.5 +/- 0.6	12.6 +/- 0.6 <sup>***</sup>	0.6 +/- 0.0 <sup>***</sup>

\*\* p<0.01

**Table 4**

Seminiferous tubule diameters in adult C57/Bl6 male mice following *in utero* exposure to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg DES

Mouse Exposure Group	Tubule Diameter (µm)
Sesame oil N=5	144.82 +/- 9.76
BPA 50 µg/kg N=6	155.53 +/- 19.35
BPA 1,000 µg/kg N=6	148.54 +/- 20.88
DES 2 µg/kg N=6	137.87 +/- 3.59

**Table 5**

Serum testosterone levels in adult C57/Bl6 male mice following *in utero* exposure to sesame oil, 50 µg/kg BPA, 1,000 µg/kg BPA, or 2 µg/kg DES

Mouse Exposure Group	Serum Testosterone (ng/ml)
Sesame oil N=5	0.16 +/- 0.09
BPA 50 µg/kg N=5	0.18 +/- 0.15
BPA 1,000 µg/kg N=4	0.12 +/- 0.08
DES 2 µg/kg N=4	0.08 +/- 0.05