OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Evidence on the Male Reproductive Toxicity of

Bisphenol S

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Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment
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PREFACE

This document presents evidence relevant to the evaluation of the male reproductive toxicity of bisphenol S. On December 12, 2024, the Developmental and Reproductive Toxicant Identification Committee (DARTIC) is scheduled to deliberate on whether bisphenol S has been clearly shown to cause male reproductive toxicity.

Proposition 65[1] requires the publication of a list of chemicals known to the State to cause cancer or reproductive toxicity within the meaning of the Act (Health and Safety Code section 25249.8). The Office of Environmental Health Hazard Assessment (OEHHA) of the California Environmental Protection Agency maintains this list in its role as lead agency for implementing Proposition 65. The DARTIC advises and assists OEHHA in adding chemicals to the Proposition 65 list of chemicals that cause reproductive toxicity, as required by Health and Safety Code section 25249.8. The DARTIC serves as the state's qualified experts for determining whether a chemical has been clearly shown to cause reproductive toxicity.

The Committee also provides advice and consultation regarding which chemicals should receive their review. At their meeting in December 2020, the DARTIC recommended that bisphenol S be placed in a 'high' priority group for future listing consideration. OEHHA selected bisphenol S for consideration for listing by the DARTIC, and in March 2022, OEHHA solicited from the public information relevant to the assessment of the evidence on the reproductive toxicity of this chemical. In response to this solicitation, several studies were submitted by the BASF Corporation. The submitted information was considered in the development of this document. On December 12, 2023, the DARTIC considered the female reproductive toxicity of this chemical and determined that bisphenol S has been clearly shown through scientifically valid testing according to generally accepted principles to cause female reproductive toxicity. Consequently, on December 29, 2023, bisphenol S was added to the Proposition 65 list based on female reproductive toxicity.

OEHHA is providing this document to the DARTIC to assist the Committee in its deliberations on whether or not bisphenol S should be listed under Proposition 65 for the male reproductive toxicity endpoint. The original papers and reports discussed in this document are provided to the DARTIC.

[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code section 25249.5 *et seq*.).

OEHHA is holding a public comment period on this hazard identification document. For information on how to comment go to https://oehha.ca.gov/comments. Comments on this document will be included in the hazard identification materials that are provided to the DARTIC members prior to the meeting.

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LIST OF ABBREVIATIONS

Abbreviation	Full name
8-OHdG	8-hydroxy-2-deoxyguanosine
µg	microgram
ADME	absorption, distribution, metabolism, excretion
AGD	anogenital distance
BMI	body mass index
BPA	bisphenol A
BPF	bisphenol F
BPS	bisphenol S
BTB	blood-testis barrier
CAT	catalase
CI	confidence interval
C_{max}	maximum plasma concentration
DART	developmental and reproductive toxicity
DARTIC	Developmental and Reproductive Toxicant Identification Committee
dL	deciliter
DNA	deoxyribonucleic acid
dpf	days post fertilization
DSB	double-stranded break
DSP	daily sperm production
E2	estradiol
ECHA	European Chemicals Agency
EDC	endocrine-disrupting chemical
EPA	Environmental Protection Agency
EOGRT	extended-one-generation reproductive toxicity
F0–3	filial generation 0, 1, 2, 3
FAI	free androgen index
FDA	Food and Drug Administration
FT	free testosterone
g	gram
GD	gestational day
GSH	glutathione
GSI	gonadosomatic index
HAWC	Health Assessment Workspace Collaborative
hpf	hours post fertilization
HPG	hypothalamic-pituitary-gonadal
IQR	interquartile range
KC	key characteristic
kg	kilogram
L	liter
LH	luteinizing hormone
LOD	limit of detection
LOQ	limit of quantification
LPO	lipid peroxidation
m	meter
MDA	malondialdehyde
mg	milligram
mil	million
mL	milliliter
mRNA	messenger ribonucleic acid
N	population sample size
n	sample size
ng	nanogram

Abbreviation	Full name
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
OR	odds ratio
p	p-value
PCNA	proliferating cell nuclear antigen
pg	picogram
PND	postnatal day
Q1–4	quartile 1, 2, 3, 4
r	Pearson correlation coefficient
RNA	ribonucleic acid
ROS	reactive oxygen species
RR	relative risk
S	supplemental
SD	standard deviation

Abbreviation	Full name
SHBG	sex hormone-binding globulin
SOD	superoxide dismutase
T	testosterone
T_{max}	time to maximum plasma concentration
TBARS	thiobarbituric acid reactive substances
TSH	thyroid-stimulating hormone
TT	total testosterone
T3	triiodothyronine
T4	thyroxine
TUNEL	terminal deoxynucleotidyl transferase dUTP nick-end labeling
US	United States
WHO	World Health Organization
β	beta coefficient
µg	microgram

LIST OF GENES AND PROTEINS

Gene	Description
5αred1, Srd5a1	5-alpha reductase 1
Akap4	A kinase anchor protein 4
Akt	thymoma viral proto-oncogene 1
Apaf1	apoptotic peptidase activating factor 1
Ape1, Apex1	apurinic/apyrimidinic endonuclease 1
Ar	androgen receptor
Atf4	activating transcription factor 4
Atg	autophagy related
Atg16l1	autophagy related 16 like 1
Bak1	BCL2-antagonist/killer 1
Bad	BCL2-associated agonist of cell death
Bax	BCL2-associated X protein
Bcl2l1	BCL2-like 1
Cat	catalase
Catsper	cation channel, sperm associated
Ckit	KIT proto-oncogene receptor tyrosine kinase
Cycs	cytochrome c, somatic
Cyp	cytochrome p450
Ddit3	DNA-damage inducible transcript 3
Ddx4	DEAD box helicase 4
Dnmt	DNA methyltransferase
Dnmt3l	DNA methyltransferase 3-like
Dot1	disruptor of telomeric silencing 1
Dot1l	DOT1-like histone lysine methyltransferase
Eed	embryonic ectoderm development
Eif2ak3	eukaryotic translation initiation factor 2 alpha kinase 3
Er, Esr	estrogen receptor
Ern1	endoplasmic reticulum to nucleus signaling 1
Err, Esrr	estrogen-related receptor
Ezh2	enhancer of zeste 2 polycomb repressive complex 2 subunit
Fsh	follicle-stimulating hormone
Fshr	follicle-stimulating hormone receptor
Gnrh	gonadotropin-releasing hormone
Gnrhr	gonadotropin-releasing hormone receptor
Gpr30, Gper1	G protein-coupled estrogen receptor 1
Gpr56, Adgrg1	adhesion G protein-coupled receptor G1
Gpx	glutathione peroxidase
Gsr	glutathione reductase
Hmgcr, Hmgr	3-hydroxy-3-methylglutaryl-coenzyme A reductase
Ho1, Hmox1	heme oxygenase 1
Hsd	hydroxysteroid dehydrogenase

Gene	Description
Hspa5	heat shock protein 5
Insl3	insulin-like factor 3
Keap1	kelch-like ECH associated protein 1
Kmt2	histone-lysine N-methyltransferase 2
Lh	luteinizing hormone
Lhcgr	luteinizing hormone/choriogonadotropin receptor
Lhr	luteinizing hormone receptor
Mt1, Mtnr1	melatonin receptor 1
Mmp9	matrix metalloproteinase 9
Myh, Mutyh	mutY DNA glycosylase
Nrf, Nfe	nuclear factor, erythroid derived
Ogg	8-oxoguanine DNA-glycosylase
Pgr	progesterone receptor
Polb	DNA polymerase beta
Por	cytochrome p450 oxidoreductase
Ppargc 1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha
Prlr	prolactin receptor
Rhox	X-linked reproductive homeobox
Rictor	RPTOR independent companion of MTOR, complex 2
Rps6	ribosomal protein S6
Setd	SET domain containing
Sirt1	sirtuin 1
Sod	superoxide dismutase
Spata	spermatogenesis-associated
Spo11	SPO11 initiator of meiotic double-stranded breaks
Star	steroidogenic acute regulatory protein
Suz12	suppressor of zest 12
Tet1	ten-eleven translocation
Tex	testis expressed gene
Thr	thyroid hormone receptor
Tp53, Trp53	transformation related protein 53
Tssk1	testis-specific serine kinase 1
Vtg1	vitellogenin 1

Protein	Description
AKT	thymoma viral proto-oncogene 1
AMH	anti-Mullerian hormone
ARP3	actin-related protein 3
BAX	BCL2-associated X-protein
BCL2	B-cell leukemia/lymphoma 2
BECN1	beclin 1; autophagy related 6
CASP	caspase; cysteine-aspartic protease
CAT	catalase
CDK2	cyclin-dependent kinase 2
CHOP, DDIT3	DNA damage-inducible transcript 3
CLDN11	claudin 11
COX	cyclooxygenase
CX43	connexin-43; gap junction protein, alpha 1
CYP	cytochrome p450
DDX4	DEAD box helicase 4
DHH	desert hedgehog
DIO2	iodothyronine deiodinase type II
DNMT	DNA methyltransferase
EGFR	epidermal growth factor receptor
EPS8	epidermal growth factor receptor pathway substrate 8
ER, ESR	estrogen receptor
ERK	extracellular signal-regulated kinases
ERR, ESRR	estrogen-related receptor
FAS	Fas cell surface death receptor
FASL	Fas ligand
FOXO	forkhead box O
FTO	fat mass and obesity-associated
GATA4	GATA binding protein 4
GLUT1	solute carrier family 2, facilitated glucose transporter member 1
GPX	glutathione peroxidase
H3K4me2	histone 3 lysine 4 dimethylation
H3K4me3	histone 3 lysine 4 trimethylation
H3K9me2	histone 3 lysine 9 dimethylation
H3K9me3	histone 3 lysine 9 trimethylation
H3K27ac	histone 3 lysine 27 acetylation
H3K27me3	histone 3 lysine 27 trimethylation
HO1, HMOX1	heme oxygenase
HSD	hydroxysteroid dehydrogenase
HSP90	heat shock protein 90
IL6	interleukin 6
IR/INSR	insulin receptor
JAK2	janus kinase 2
KEAP1	kelch-like ECH associated protein 1
LC3B	microtube-associated protein 1 light chain 3, beta isoform
METTL3	methyltransferase-like 3
MLH1	MutL protein homolog 1

Protein	Description
MMP	matrix metalloproteinase
MT1, MTNR1A	melatonin receptor 1A
NFKB	nuclear factor kappa B
NRF, NEF	nuclear factor, erythroid derived
OCL, OCLN	occludin
PRDX6	peroxiredoxin 6
RICTOR	RPTOR independent companion of MTOR, complex 2
RPS6	small ribosomal subunit protein eS6
SIRT1	silent information regulator sirtuin 1
SOD	superoxide dismutase
STAR	steroidogenic acute regulatory protein
STAT3	signal transducer and activator of transcription 3
TEX	testis-expressed protein
TH	tyrosine 3-hydroxylase
THR	thyroid hormone receptor
TSHR	thyroid-stimulating hormone receptor
TIMP	tissue inhibitor of metalloproteinases
VCAM	vascular cell adhesion molecule
YTHDF1	YTH N6-methyladenosine RNA binding protein F1
ZO	zona occludens

SUMMARY

Introduction

This document presents evidence relevant to the evaluation of the male reproductive toxicity of bisphenol S (BPS), also identified as 4,4'-sulfonyldiphenol. BPS consists of two hydroxyphenyl groups connected by a sulfonyl group and is an analogue of bisphenol A (BPA).

Uses, Occurrence, and Exposure

BPS is part of polyethersulfone plastic, which is used to make hard plastic items and synthetic fibers for clothing and other textiles, and as a color developer in thermal paper. It has been detected in cash register receipts, personal care products, foods, baby bottles, and other products. Recent data from Biomonitoring California (https://biomonitoring.ca.gov) found BPS was detected in approximately two-thirds of Californians tested. Data from other United States (US)-based human biomonitoring studies indicate that as BPA has been removed from many products over the last decade, the levels of BPS detected in human samples has increased.

Systematic Literature Review Approach

Using a systematic approach, the Office of Environmental Health Hazard Assessment (OEHHA) conducted literature searches on the developmental and reproductive toxicity of BPS, including primary searches in major biomedical databases, searches in other data sources such as reports by other health agencies, and additional focused searches. In addition, OEHHA conducted a data call-in from March 4 to April 18, 2022 to solicit relevant information. This document focuses on literature relevant to male reproductive toxicity (last comprehensive search, January 2024).

Pharmacokinetics of BPS

BPS is rapidly absorbed in humans by the oral and dermal routes and is distributed throughout the body. BPS has been detected in human blood, umbilical cord blood, amniotic fluid, breast milk, semen, skin, and urine. BPS does not accumulate in tissues or blood over time. Once absorbed, BPS undergoes metabolism primarily in the liver, where it can be conjugated, and then excreted in urine and feces. Estimates of the half-life of BPS in humans exposed by the oral route range between 7 to 9 hours.

Male Reproductive Toxicity

Studies in Humans

OEHHA identified eleven epidemiologic studies of possible effects of BPS on the male reproductive system. The limits of detection were varied, with some being relatively high, and the percentage of the participants with BPS exposure above the limits of detection was low in some of these studies.

Sperm quality was the focus of five studies. The epidemiologic evidence for an effect of BPS exposure on sperm quality was strongest for sperm total motility with two studies reporting significantly lower total motility. One of these studies also observed an inverted U-shaped curve for both lower total motility and progressive motility. The second study reported a borderline ($p = 0.06$) lower association between BPS exposure and total motility in men with detectable urinary BPS concentrations, and significantly lower total motility in men with a body mass index (BMI) ≥ 25 kilograms per square meter (kg/m^2). Three other studies reported no significant decreases in sperm motility.

Sperm concentration and sperm count were examined in five studies. Higher BPS exposure was associated with lower sperm concentration and count in two studies, with stronger associations observed in men with a BMI ≥ 25 kg/m^2 in one of the studies. Three other studies did not find such effects. While these three studies did include BMI as a covariate, they did not report stratifying by BMI or testing for an interaction.

Ejaculate volume was examined in four studies and three reported significant associations with BPS. One study reported significantly lower volume in men with detectable BPS concentrations compared to those with non-detectable concentrations. The second study reported a significant decrease in ejaculate volume in the third quartile versus the first quartile of urine BPS. The third study reported a decrease in ejaculate volume per ten-fold increase in seminal plasma BPS concentrations. The fourth study reported a non-significant association in the same direction.

Reproductive hormone levels were examined in four studies. Three studies reported that higher BPS exposure was associated with significantly lower serum estradiol (E2) in males. Two of these studies used National Health and Nutrition Examination Survey (NHANES) data from the same survey cycles and observed lower E2 levels in male children. The third study of adult men attending an infertility clinic, observed lower E2, lower estradiol / testosterone (E2/T) ratios, and lower sex hormone-binding globulin (SHBG). No significant association was reported for BPS and E2 in the fourth study using NHANES data of adult men; however, there was a significant interaction with BMI, in which the association was negative in normal and overweight men and positive in

obese men. Also reported in this study was a significant increase in SHBG associated with higher BPS exposure.

Additionally, higher BPS exposure was significantly associated with lower total testosterone (TT) levels, free androgen index, and TT/E2 ratios in male children. In adult males, exposure to BPS was associated with significantly lower free testosterone levels in one study, but with no effect on TT levels. Other findings were less consistent across studies.

Two other outcomes, *time to pregnancy* and *male offspring reproductive development* were examined in one paper each. Neither study reported significant associations with BPS.

Studies in Animals

Overview

BPS-mediated effects on the male reproductive system were evaluated in 41 *in vivo* studies including 14 studies in mice, 20 studies in rats, one study in hamsters, one study in gerbils, four studies in zebrafish, and one study in guppies. BPS-related outcomes reported in the reviewed studies included effects on organ weight or histology (of testis, epididymis, seminal vesicle, and the prostate, adrenal, pituitary, and thyroid glands), effects on sperm, the endocrine system, mammary gland development, and reproductive performance. For most rodent studies, BPS was administered orally via gavage, diet, or drinking water, while zebrafish and guppies were exposed to BPS in water. Exposures varied by life stage (e.g., gestational, pubertal, and adult) and duration (ranging from a single exposure via subcutaneous injection to continuous exposure via drinking water for 48 weeks).

Testis weight and histology

The effects of BPS on testicular weight were mixed. Five mouse studies, 12 rat studies, and one study each in gerbils and hamsters assessed testicular weight, and of these, two mouse studies, four rat studies, and the hamster study reported weight changes. Increased testicular weight was observed across all life stages, while decreased testicular weight was observed only with postnatal exposure.

Histopathological effects were assessed and reported in four of the mouse studies, 11 rat studies, and one hamster study. The most frequently reported findings included alterations in the height, diameter, and area of the seminiferous tubule epithelium, changes to the interstitial space, vacuolization and cellular damage, and aberrant absence or presence of sperm cells across the epithelium.

Epididymis weight and histology

Nine rat studies assessed the effects of BPS on epididymal weight, and four reported decreases in epididymis weight. Six rat studies examined epididymal histology of which four reported no histopathological findings.

Seminal vesicle weight and histology

Eleven studies in rats assessed the effects of BPS on seminal vesicle weight, and six reported decreases in seminal vesicle weight. There were no histopathological findings in the two rat studies that assessed seminal vesicle histology.

Prostate weight and histology

The effects of BPS on prostate weight were assessed in one mouse study, ten rat studies, and one gerbil study, with mixed results. Prostate weight was increased in the mouse study and decreased in four of the rat studies, with no discernible exposure-associated patterns. While no effects on prostate weight were reported in gerbils, this study reported histopathological findings in the ventral prostate including alterations to the lumen, epithelium, and muscular and non-muscular stroma, and hyperplasia.

Adrenal weight and histology

Five rat studies assessed the effects of BPS on adrenal gland weight, and four reported increases. Adrenal gland histology was also examined in four studies, and adrenal hypertrophy and/or hyperplasia were observed in three of the studies.

Pituitary weight and histology

Two rat studies assessed the effects of BPS on pituitary weight, with one study reporting increased weight and the other study reporting no effects. Histology of the pituitary gland was assessed in two rat studies, the study reporting no effects on weight and another rat study that did not examine weight. Neither study reported any histopathological changes.

Thyroid weight and histology

Three rat studies assessed the effects of BPS on thyroid weight and histopathology. One study reported increased thyroid weight and the other two reported no effects. All three rat studies reported no histopathological findings. Two studies in mice assessed the effects of BPS on thyroid gland histoarchitecture and both reported histopathological findings including alterations to the height of the thyroid follicular epithelium and/or vacuolation.

Gonadosomatic index

Four rat studies and four zebrafish studies assessed the effects of BPS on gonadosomatic index, with decreased gonadosomatic index reported in two of the rat studies and three of the zebrafish studies.

Effects on sperm

Several studies assessed the effects of BPS on spermatogenesis, sperm count, daily sperm production, and sperm morphology and motility. Consistently, altered spermatogenesis and morphology, and decreased sperm count, daily sperm production, and sperm motility were observed across exposure paradigms, species, and life stages.

Spermatogenesis

Six mouse studies, five rat studies, one hamster study, and one zebrafish study assessed the effects of BPS on germ cell development. All but one mouse study reported effects, including altered progression through the spermatogenic cycle, decreases in germ cell and spermatozoa numbers, and effects on germ cell division.

Sperm count

Seven mouse studies, six rat studies, and one study each in hamsters and zebrafish assessed the effects of BPS on sperm count, and all but two mouse studies and one rat study reported decreases. In mice, decreased sperm count was observed at doses ranging from 0.5 µg/kg-day to 200 mg/kg-day, and included gestational, juvenile, and adult exposure windows. In the two mouse studies reporting no effects on sperm count, one was a lactational exposure study (0.02 mg/kg-day administered to the dam from PND 0 to 15) and the other study exposed adults (0.1 mg/kg-day) for 8 weeks.

Daily sperm production

Five rat studies assessed the effects of BPS exposure on daily sperm production and all five reported decreases.

Sperm morphology

Two studies in mice and two studies in rats reported morphological abnormalities in sperm including double-headed and banana-shaped sperm while one study in rat reported no effects on sperm morphology in F0 and F1 males.

Sperm motility

Seven studies each in mice and rats assessed the effects of BPS exposure on sperm motility, and all but one mouse study and two rat studies reported decreases in sperm motility.

Endocrine effects

Several endocrine effects, including altered hormone levels, steroidogenic enzymes, and endocrine-related gene expression, were reported from studies in animals and *in vitro* systems and are summarized below under Mechanistic Considerations.

In addition, two mouse studies and five rat studies assessed the effects of BPS exposure on anogenital distance (AGD) with mixed results. In one mouse study, decreased and increased AGD were observed with lactational exposure to 0.2 and 20 µg/kg-day, respectively, while the other study did not observe effects on anogenital index (AGD divided by body weight) with gestational and lactational exposure to up to 2,000 µg/kg-day. Decreased AGD was observed in the two rat studies that reported effects.

Mammary gland development

The effects of BPS exposure on male mammary gland histology were assessed in one mouse study and four rat studies, and all studies reported histopathological findings. In mice, observations included altered mammary epithelial tree sizes with lateral effects in pre-pubertal mice. All four rat studies reported diffuse or multifocal atrophy of the mammary glands.

Reproductive performance

Two mouse studies assessed the effects of BPS on male reproductive performance. One study in male mice exposed to BPS during lactation and mated to untreated female mice reported decreases in zygote cleavage rate and number of blastomeres per blastocyst. The second study reported a non-significant increase in the number of days to successful mating and no effects on pregnancy rate or litter size.

Mechanistic Considerations

There is mechanistic evidence relevant to male reproductive toxicity from *in vivo* and *in vitro* studies that suggests that BPS induced oxidative stress, genetic damage, apoptosis, epigenetic alterations, and disruptions to the endocrine system and other cellular functions. Across studies, these effects were observed in tissues and cells relevant for male reproduction, including testicular tissue, sperm cells, Sertoli cells,

Leydig cells, and prostate cells, as well as altered serum or plasma hormone levels. One or more of these effects were observed in animal models that additionally showed gross and histopathologic findings in reproductive organs, altered spermatogenesis and sperm parameters, diminished reproductive performance, AGD effects, and/or altered mammary gland development.

BPS increased reactive oxygen species (ROS) production and lipid peroxidation in rat testicular tissue, rat sperm cells, and a mouse Leydig cell line, while one study in bovine spermatozoa reported decreased ROS levels. Studies reported evidence of an overwhelmed antioxidant system in mouse, rat, and hamster testicular tissues, rat sperm cells, a mouse Leydig cell line, and a human prostate cell line exposed to BPS, including changes in glutathione (GSH) levels and the activities of GSH reductase, superoxide dismutase (SOD), GSH peroxidase (GPX), and catalase (CAT). GSH levels and SOD, GPX, and CAT activities demonstrated directionality consistent with oxidative stress (increased with short-term exposure and decreased with longer exposures). Numerous oxidative stress-related genes and proteins were altered in animal testes. There is evidence that BPS can cause genetic damage, specifically, data showed that BPS increased 8-hydroxy-2′-deoxyguanosine (a marker of oxidative damage to DNA) in testicular tissue, increased DNA fragmentation index in sperm, increased DNA damage as indicated by positive comet assays in rat sperm and a human prostate cell line, and increased phosphorylated γH2AX (a marker of double-strand breaks) in mouse testes, a spermatogonial cell line, and zygotes. Also, the expression of the DNA damage repair gene, *MUTYH*, was decreased in a human prostate cell line.

DNA fragmentation was observed in the germ cells of male mice exposed to BPS during gestation suggesting that BPS induced apoptosis. There was also evidence that BPS exposure increased spermatogenic apoptotic index in mouse sperm and increased the apoptosis rate in a mouse Leydig cell line. Numerous apoptosis-related genes and proteins were altered in mouse, rat, and hamster testes, a human prostate cell line, and/or a mouse Leydig cell line.

BPS induced epigenetic alterations in zebrafish testicular tissue and a mouse spermatocyte cell line, affecting global DNA methylation and several genes involved in DNA and histone methylation and demethylation and other epigenetic-regulated processes in mouse, rat, and zebrafish testicular tissues. BPS also affected epigenetic-related protein expression and modification in rat sperm cells, mouse germ and Sertoli cells, and a mouse Leydig cell line.

There is abundant evidence that BPS affects various aspects of the endocrine system. BPS altered the blood, testicular, whole body, urine, and/or Leydig cell levels of

gonadotropins, testosterone, estradiol, and the testosterone/estradiol ratio. BPS increased aromatase, an enzyme responsible for converting androgens into estrogen, in zebrafish brain, mouse testicular tissue, and a mouse spermatocyte cell line, and decreased aromatase in zebrafish testicular tissue. BPS increased pregnenolone and progesterone in a mouse Leydig cell line, and decreased 11-ketotestosterone, a testosterone derivative, in zebrafish testicular tissue. BPS affected spermatocyte viability via estrogen-sensitive signaling cascades in a mouse cell line. BPS also altered thyroid hormones in mice and zebrafish and thyroid hormone receptors in mice. In hamsters, BPS decreased serum melatonin, a hormone that influences gonadotropin-releasing hormone and luteinizing hormone release, gonadal testosterone synthesis, testicular function, and antioxidant status. Further, BPS altered the expression of several endocrine-related genes and proteins in mice, rats, hamsters, gerbils, guppies, zebrafish, and mouse cell lines.

Additional reports of mechanistic effects of BPS included: impaired mitochondrial function in rat testicular tissue, a mouse Leydig cell line, and a mouse spermatocyte cell line; endoplasmic reticulum stress in a human prostate cell line; decreased lysosome activity with decreased cell membrane integrity and inhibition of gap junctions in a mouse Leydig cell line; and altered spermatogonial and testicular cell cytoskeletal structure and/or nuclear morphology. BPS also interrupted the integrity of the blood-testes barrier, altered the cell cycle and/or cell proliferation in testicular and prostate cells, and altered gene and protein expression and post-translational modifications in sperm development and maturation.

Key characteristics

Exogenous agents that cause male reproductive toxicity frequently exhibit one or more of the key characteristics (KCs) of male reproductive toxicants (see Table 4.3.1 in Section 4). The available mechanistic evidence suggests that BPS exhibits several of these KCs, including KC 1: alters development, function, or death in germ cells; KC 2: alters development, functions, or death in somatic cells; KC 3: alters production and levels of reproductive hormones; KC 4: alters hormone receptor levels/function; KC 5: induces genotoxicity; KC 6: induces epigenetic alterations; and KC 7: induces oxidative stress. In addition, at least two of the KCs of male reproductive toxicants (KC 3 and KC 4) overlap with a related set of key characteristics of endocrine-disrupting chemicals (see Table 4.3.7). Overall, the findings from mechanistic and toxicity studies of BPS reflect many of the KCs of male reproductive toxicants and of endocrine-disrupting chemicals.

1. INTRODUCTION

1.1 Identity of Bisphenol S (BPS)

BPS consists of two hydroxyphenyl groups connected by a sulfonyl group and has the molecular formula $C_{12}H_{10}O_4S$. It is an analog of bisphenol A (BPA), and its IUPAC name is 4-(4-hydroxyphenyl) sulfonylphenol. Other names for BPS include 4,4'-sulfonyldiphenol, 4-hydroxyphenyl sulfone, and 4,4′-sulfonylbisphenol. The chemical structure of BPS is shown in Figure 1. The Chemical Abstracts Service (CAS) Registry Number for BPS is 80-09-1.

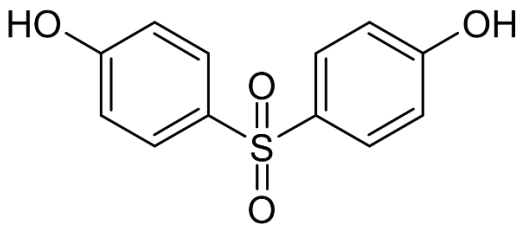

Figure 1 Structure of BPS

1.2 Uses, Occurrence, and Exposure

Use and Production

BPS, a BPA analog, is used to make plastics and is part of polyethersulfone plastic used to make hard plastic items and synthetic fibers for clothing and textiles. BPS can be used to make colors last longer in some fabrics. It is a common replacement for BPA in some types of paper receipts. It can be used in coatings of nonstick pans, baby bottles, and parts of electronics (e.g. screens for mobile phones) (Biomonitoring California 2020).

According to the US Environmental Protection Agency (US EPA), the 2019 national aggregated production volume of BPS was 1,000,000 to 10,000,000 pounds. Almost all of the BPS reported to the US EPA was imported (US EPA 2020). The European Chemicals Agency (ECHA) reported that at least 10,000 million metric tons of BPS are manufactured or imported annually into the European Economic Area per year (ECHA 2023).

Some increases in BPS use are associated with actions taken to reduce the use of BPA. In 2011, Governor Brown signed into law a ban on the use of BPA in baby bottles

and spill-proof cups; the US Food and Drug Administration (FDA) similarly banned this use in 2012, and restricted use of BPA-based epoxy resins in packaging materials for infant formula in 2013 (US FDA 2014). The European Union (EU) instituted greater restrictions on BPA that became effective in 2020, including an EU-wide ban on its use in thermal paper. Since the restrictions on BPA use and public concerns regarding its safety, manufacturers have been gradually replacing BPA with BPS and other analogs such as bisphenol F (BPF). ECHA projected the use of BPS in thermal paper in the EU to be 61% in 2022, and for BPA to be mainly replaced by BPS for this use (ECHA 2022). Between 2020 and 2030 the online market analysis platform ChemAnalyst (2021) projects a possible 3.6-fold increase in market global demand for BPS.

ChemAnalyst (2021) notes that:

> "More than 42% of the demand is generated by Heat Sensitive Developers that are used for the manufacture of Thermal Papers. … Polyurethane systems have been associated with offering strength, durability, and resilience while dealing with challenging conditions such as heavy footfall, physical impacts, extreme temperatures, and corrosive chemicals. These offerings by [polyurethane] resins are driving the BPS market growth" (ChemAnalyst 2021).

Occurrence

The European Chemicals Agency (ECHA 2023) notes that BPS:

> "can be found in products with material based on: "leather (e.g. gloves, shoes, purses, furniture), paper (e.g. tissues, feminine hygiene products, diapers, books, magazines, wallpaper), paper used for articles with intense direct dermal (skin) contact during normal use such as printed articles (e.g. newspapers, books, magazines, printed photographs) and paper used for articles with intense direct dermal (skin) contact during normal use such as personal hygiene articles (e.g. diapers, feminine hygiene products, adult incontinence products, tissues, towels, and toilet paper)" (ECHA 2023).

BPS has also been detected in personal care products (e.g., hair care products, makeup, lotions, toothpaste), paper products (e.g., currency, tickets, mailing envelopes, airplane boarding passes), and food (dairy products, meat, vegetables, canned foods). BPS has been detected in environmental samples such as dust and sediment and human biological samples which is expanded on in Section 3. Pharmacokinetics (Fan et al. 2021; Liao et al. 2012; Liao and Kannan 2013, 2014; Pelch et al. 2017).

Some examples of BPS detections in environmental samples and consumer products are provided below.

- House dust. BPS was detected in all house dust samples from 38 families in Northern California, collected between May 2015 to August 2016 (Shin et al. 2020). Similarly, BPS was detected in over 75% of house dust samples collected from 2007 to 2010 in 13 cities across Canada under the Canadian House Dust Study. The median concentration was 0.242 micrograms per gram (µg/g), and the range was < 0.017 to 35.1 (Fan et al. 2021).
- Paper receipts. BPS was detected in 75% of receipts submitted by volunteers from several US locations, with the majority from southeast Michigan (Miller and Olson 2018).
- Food. Fifty adults in North Carolina collected 776 duplicate-diet solid food samples over a six-week monitoring period in 2009–2011. A 32% detection frequency was reported for BPS. The researchers calculated a daily dietary exposure of 13,640 nanograms per day (ng/day) and a dietary intake dose of 238 nanograms per kilogram per day (ng/kg/day) (Morgan and Clifton 2021).
- Fish. Fish packaged with thermal labels on plastic film were found with BPS with a mean of 147 ng/g, and a maximum level of 1,140 ng/g, exceeding the European Union specific migration limit (50 ng/g wet weight) (Xu et al. 2023).
- Meat. In a study in Albany, NY, BPS was detected in 43% of fresh meat and meat products (n = 51) with a mean of 0.609 ng/g, and 95th percentile of 0.780 (Liao and Kannan 2013).

Cao et al. (2022a, b) reported concentrations of BPS in composite samples of meat and meat products in the Canadian Total Diet Study from 2008 to 2020. In 2008, BPS was detected in meat samples with measured concentrations ranging from 0.070 ng/g in canned luncheon meats to 105 ng/g in fresh pork and 140 ng/g in veal cutlets. In 2020, measured concentrations of BPS in meat samples ranged from 0.14 ng/g in cured pork to 26 ng/g in ground beef and 118 ng/g in roast beef. The authors noted that, while levels varied across years, there was no trend, and that:

> “The lack of trend for BPS over the period of 13 years (2008–2020) does not support the speculation that BPS is being used to replace BPA in food packaging, and sources other than food packaging may be possible and should be investigated for BPS.” (Cao et al. 2022a)

Exposure

Human biomonitoring studies indicate that exposure to BPS is widespread. The tables and figure below present data on serum BPS concentrations from recent biomonitoring studies. Table 1.1 presents data from the state's Biomonitoring California program.

Table 1.1 BPS serum concentrations (µg/L) in recent studies of California residents

Study Name[1]	Sampled Group	Geometric mean (µg/L)	50th Percentile	90th Percentile	Number of Participants	Detection Frequency
CARE-LA	Women	0.38 (95% CI: 0.3–0.5)	0.342	2.42	60	76.7%
CARE-2	Adults	NC*	0.233	2.25	151	64.9%
CARE-3	Adults	NC	0.288	2.85	90	64.4%

[1] CARE-LA: California Regional Exposure Study (CARE), Los Angeles County. Sample collection in 2018; CARE-2: Region 2. Sample collection in 2019; CARE-3: Region 3. Sample collection in 2020.
*Geometric mean not calculated (NC) when the chemical was found in <65% of samples; the Limit of Detection was 0.100 µg/L (wet weight). CI: confidence interval.
Data available at https://biomonitoring.ca.gov/ (Biomonitoring California 2020).

In another study conducted in California, urine samples were taken from pregnant women, and analyzed for several chemicals including BPS (Kim et al. 2021). This is the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) study, a high-familial risk autism spectrum disorder cohort, in which 218 pregnant women in California were sampled between 2007–2014. The researchers reported increasing BPS detection in urine across time, from 0% in 2007 to 32% in 2014 (See Table 1.2). The 95th percentile BPS level was 2.8 nanograms per milliliter (ng/mL, equivalent to micrograms per liter (µg/L), the unit reported in other studies). Because the limit of detection (LOD) was lower than for the recent Biomonitoring California studies, frequencies of detections cannot be directly compared across these studies. Also as noted by Kim et al. (2021) the sample sizes are small and therefore the temporal trends and geometric mean concentrations should be interpreted with caution.

Table 1.2 BPS exposure in pregnant women in California during 2007–2014 from a high-familial risk autism spectrum disorder cohort (adapted from Kim et al. 2021)

Year	Number Of Samples	BPS Detection Frequency (%)	BPA Detection Frequency (%)
2007	51	0	67
2008	121	5	63
2009	181	9	58
2010	112	14	59
2011	67	6	55
2012	65	26	62
2013	106	26	62
2014	57	32	42

At the national level, biomonitoring for BPS has been conducted under the US Center for Disease Control and Prevention's NHANES study. Urinary BPS concentration levels were reported in summary form for 1,808 adults and 868 children for the years 2013–2014. Participants were selected using a random selection approach. BPS was detected in 89.4% of urine samples with median levels of 0.37 µg/L for adults and 0.29 µg/L for children. In males, the median BPS level was 0.41 µg/L for adults and 0.28 µg/L for children. Urinary BPS levels in adults varied by race/ethnicity, where non-Hispanic Black and Hispanic adults had significantly higher BPS levels compared to non-Hispanic White adults. Urinary BPS levels in children varied by age (6–11 year olds had higher BPS levels than 12–19 year olds), gender (females had higher BPS levels than males), and race/ethnicity (Hispanic children had higher BPS levels than White children) (Lehmler et al. 2018).

Kim et al. 2021 presented data on temporal trends in BPA and BPS urinary levels for pregnant women in the NHANES and the Markers of Autism Risk in Babies – Learning Early Signs (MARBLES) studies, as well as two additional studies: LIFECODES (pregnant individuals in Massachusetts, 2007–2009), and Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) (pregnant individuals in Puerto Rico, 2011–2016). They also presented NHANES data for non-pregnant women. For NHANES, they selected the ages 20 to 50 stratified by pregnancy status, for the years 2013–2015. Figure 2, adapted from Kim et al. (2021), indicates decreasing BPA levels with concomitant increases in BPS levels measured in urine samples of pregnant and non-pregnant women during the period covered by these studies, 2007–2015.

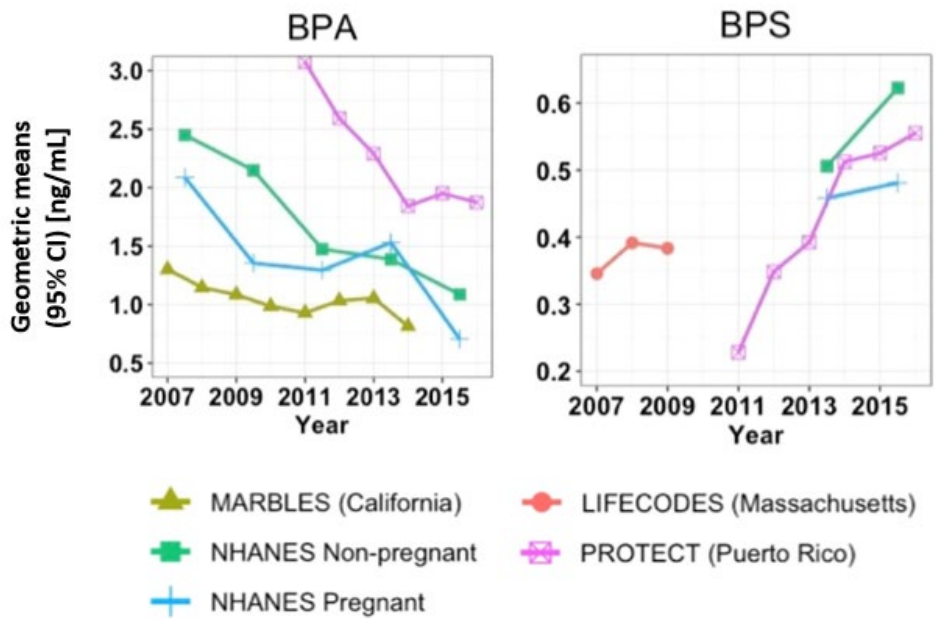

Figure 2 BPS and BPA in pregnant women measured in urine samples collected between 2007 and 2015 in four biomonitoring studies. (Adapted from Kim et al. 2021)

Finally, of note in an online crowdsourced study, BPS was detected above the median method reporting limit in urine at 81% of 726 US participants with values ranging from 0.084–921 ng/mL, and a median of 0.48 ng/mL. Samples were collected between February 2017 and October 2018 (Dodson et al. 2020).

Considerations for exposure assessment in epidemiologic studies

The ability of a study to detect an association if one exists depends on several characteristics related to the assessment and magnitude of exposure. These include the LOD of BPS in the analysis of the samples taken, the percentage of the samples with BPS levels above the LOD, and the median and variability in BPS exposure levels. In the epidemiologic studies presented in this document there was considerable variation in the LODs and, as such, in the ability to detect BPS in the samples. Also, measured BPS levels in the participants across studies differed substantially. Some of the variation in BPS exposure across studies may be explained by study year, as BPS occurrence has changed over time. Other very important considerations are that (i) BPS is rapidly eliminated in urine, with a short half-life (estimates range between 7 to 9 hours for oral exposures; see Section 3. Pharmacokinetics), and (ii) several of the epidemiological studies relied on a single urine sample to classify exposure. There is a growing consensus that in many circumstances a single urine sample may be insufficient to

adequately assess exposure to non-persistent chemicals, such as BPS (Fays et al. 2020; Verner et al. 2020).

1.3 Reviews by Other Health Agencies

The US EPA, the National Institute for Occupational Safety and Health (NIOSH), the FDA, and the National Toxicology Program (NTP) have not reached conclusions or classified BPS as to its potential to cause male reproductive toxicity.

ECHA has identified BPS as a substance meeting the criteria of a reproductive toxicant (toxic for reproduction category 1B, H360FD[2]). ECHA's description for category 1B is as follows (ECHA 2022):

> "Reproductive toxicant category 1B adverse effects on sexual function and fertility or on development or reproductive toxicant category 2 with R60 (May impair fertility) or R61 (May cause harm to the unborn child)."

In the summary of classification and labelling, the hazard statement listed is "May damage fertility. May damage the unborn child." (See: https://echa.europa.eu/substance-information/-/substanceinfo/100.239.213).

ECHA (2022, pp 12–13), in identifying BPS as a substance of "very high concern because of its toxic for reproduction", stated:

> "Based on all available scientific evidence, it can be concluded that BPS fulfils the WHO/ International Programme on Chemical Safety (2002) definition of an endocrine disruptor:
>
> - It shows clear reproductive adverse effect in rodents and fish. The reproductive endocrine system is highly conserved not only between mammals, but also between mammals and other vertebrates like fish.
> - It has endocrine modes of action: clear estrogenic mode of action and alteration of steroidogenesis.
> - The adverse effects, including the recognized EAS [estrogenic, androgenic, and steroidogenic]-mediated effects (e.g., on estrous cycle and sex ratio) and effects sensitive, but not diagnostic of EAS (e.g. fecundity, fertility, implantation sites and number of pups), are a consequence of the endocrine modes of action.

[2] H360FD is the hazard statement code for "May damage fertility. Suspected of damaging the unborn child"

The assessment performed demonstrates that there is scientific evidence of probable serious effects of BPS to the environment and human health due to its endocrine disrupting properties, which give rise to an equivalent level of concern to those of other substances listed in points (a) to I of Article 57 of the REACH [Registration, Evaluation, Authorisation, and Restriction of Chemicals] Regulation." (ECHA 2022)

2. OVERVIEW OF SYSTEMATIC LITERATURE REVIEW APPROACH

Searches of the published scientific literature on the developmental and reproductive toxicity (DART) of BPS were conducted in January 2022 and updates to the original search were conducted in February 2023, July 2023, and January 2024. The goal was to identify peer-reviewed open source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported toxicological and epidemiological information on the DART of this chemical.

The searches were conducted using the following three approaches:

- Primary searches in major biomedical databases, conducted by OEHHA librarian Nancy Firchow, MLS.
- Searches in other data sources, including authoritative reviews and reports, and databases or web resources, conducted by OEHHA scientists.
- Additional focused searches, conducted by OEHHA scientists.

In addition to information identified from these searches, relevant literature was identified in the following:

- One submission received during the data call-in period (March 4 to April 18, 2022) (https://oehha.ca.gov/proposition-65/comments/comment-submissions-request-relevant-information-reproductive-toxicity)

2.1 Primary Search Process

The US EPA Computational Toxicology (CompTox) Chemicals Dashboard (https://comptox.epa.gov/dashboard) was used to identify synonyms for BPS. The PubMed MeSH database (https://www.ncbi.nlm.nih.gov/mesh/) was used to identify subject headings and other index terms related to the chemical, reproduction and development, and adverse effects on reproduction and development.

Preliminary searches were run, and results evaluated to identify additional relevant search terms. The resulting search strategies were then executed in PubMed where searches were divided as follows:

- Human DART studies (including *in vitro* studies)
- Animal DART studies (including *in vitro* studies)
- Absorption, distribution, metabolism, and excretion (ADME) studies

There were no restrictions in the searches on exposure route or duration of exposure, or on publication language. The full DART search strings used in PubMed are included in Appendix A.

The PubMed search strategies were then tailored for use in the additional databases and data sources listed below, according to the search interface and features unique to each resource. For instance, MeSH terms were replaced with Emtree terms for the Embase search strategies.

2.2 Literature Screening Process

The results of the literature searches were uploaded to EndNote libraries and duplicates were removed. In addition to the studies identified through this process, other relevant studies were identified from citations in individual articles, through alert services (e.g., ScienceDirect, Google Scholar, etc.), and through February and July 2023, and January 2024 updates to the searches. A total of 1,496 references were identified in the searches for human and animal DART data and a total of 759 references were identified in the searches for ADME data (see Appendix Table A.7).

The EndNote libraries containing the literature search results (citations) for BPS were uploaded to HAWC (Health Assessment Workspace Collaborative, https://hawcproject.org). HAWC is a tool used for multi-level screening of literature search results.

In Level 1 screening, citations were reviewed independently by OEHHA scientists, based solely on study titles and abstracts, using specific inclusion and exclusion criteria to eliminate studies or articles that did not contain information on DART or other key related topics (e.g., pharmacokinetics, mechanisms of action). This initial screen (Level 1) was intended to identify all studies deemed to have a reasonable possibility of containing information relevant to DART that could be useful for the review process, and to further identify (i.e., tag in HAWC) studies relevant to particular aspects of DART (e.g., male reproductive toxicity, female reproductive toxicity, developmental toxicity).

For purposes of identifying the available evidence on the male reproductive toxicity of BPS, citations identified as having a reasonable possibility of containing information relevant to male reproduction underwent Level 2 screening. In the Level 2 screening of this subset of citations, the full text was obtained. These full papers were screened independently by one OEHHA scientist, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgements about the relevance of the articles because they were reviewing the full text in addition to the title and abstract. Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC. Level 1 and 2 screenings were repeated as search results were updated, and with additional relevant studies identified from citations in individual articles and alert services (e.g., ScienceDirect, Google Scholar) (See Appendix A for additional details).

184 references were cited in this document.

3. PHARMACOKINETICS

This section briefly describes the absorption, distribution, metabolism, and excretion of BPS in humans and laboratory animals. Since humans and laboratory animals share many of the reported metabolic pathways for, and metabolites of, BPS, data from animal studies are included when human data are unavailable or incomplete. Data from animal studies are useful to complement human data and highlight observed route, species, and sex differences.

3.1 Absorption

The primary route of human exposure to BPS is oral, however, exposure may also occur via the inhalation and dermal routes (Kumar et al. 2018). Studies demonstrated that, when exposed orally, BPS is rapidly absorbed with a high bioavailability and likely undergoes enterohepatic circulation in humans (Khmiri et al. 2020; Oh et al. 2018) and in rats and mice (BASF 2019b; Waidyanatha et al. 2018; Waidyanatha et al. 2020). It has been demonstrated that BPS is absorbed in humans via the dermal route, though absorption was slower with lower bioavailability compared to the oral route (Khmiri et al. 2020; Liu and Martin 2019; Oh et al. 2018; Reale et al. 2021). Kinetic parameters derived from rodent studies demonstrated some species and dose dependency but were generally independent of sex in mice (Waidyanatha et al. 2018; Waidyanatha et al. 2020) and rats (BASF 2019b). Despite the absence of sex differences in time to maximum plasma concentration (T_{max}) and in the plasma half-life of total BPS in mice, there were sex differences in some pharmacokinetic parameters in rats. After a single

oral BPS exposure of 110 mg/kg, female rats had a shorter T_{max} for total BPS (0.6 and 1.75 hours in females and males, respectively) and a shorter absorption plasma half-life for total BPS compared to males (0.08 and 0.33 hours, respectively), with a relatively longer elimination half-life compared to males (14.3 vs. 11.2 hours, respectively) (Waidyanatha et al. 2020).

Oral Dosing

BPS was rapidly and well absorbed in humans. In a study of women administered a single oral dose of 0.1 mg/kg (radiolabeled) BPS, the T_{max} for free and glucuronidated BPS was 0.7 and 1.1 hours, respectively, and oral bioavailability was approximately 62% (Khmiri et al. 2020). Small plasma concentration peaks occurred between 4 and 10 hours, indicating possible enterohepatic recirculation (Khmiri et al. 2020). Similarly, in a study of men and women administered a single oral dose of 8.75 µg/kg radiolabeled BPS, serum concentrations of free and total BPS increased rapidly, with T_{max} values of 0.6 hours for free BPS and 0.7 hours for total BPS (Oh et al. 2018).

In one set of studies of male and female rats exposed to a single gavage dose of 30 or 300 mg/kg BPS, there were two T_{max} peaks in plasma at 1 and 4 hours, indicating possible enterohepatic circulation, in both sexes and doses (BASF 2019b). Internal exposure (maximum concentration [C_{max}] and area under the curve) was sex dependent at one hour and increased with dose. In male rats, oral absorption was independent of dose at 93–95% and somewhat dose dependent in females with 87% and 96% absorption in the low- and high-dose groups, respectively (BASF 2019b). In another set of studies in male and female rats exposed to a single BPS dose of 110 mg/kg by gavage, the T_{max} of total BPS was approximately 0.6 hours in female and 1.75 hours in males (Waidyanatha et al. 2020). In male rats treated with 34, 110, or 340 mg/kg BPS by gavage, the T_{max} value of total BPS increased from 0.99 to 2.77 hours with increasing dose. In male mice, T_{max} was 3.08 hours after a single oral exposure by gavage of 340 mg/kg (no T_{max} data reported for female mice) (Waidyanatha et al. 2020). After exposure to a single gavage dose of 110 mg/kg, the calculated oral bioavailability for rats and mice in these studies ranged from 11% to 15% in both sexes and species (Waidyanatha et al. 2020) and was lower compared to that calculated for the BASF 2019b studies in rats. Internal exposure parameters (C_{max} and area under the curve) were dose dependent in males in both species for free and total BPS except for C_{max} on total BPS in mice (Waidyanatha et al. 2020). The discrepancy in bioavailability ranges between the two studies outlined above may have been due to the method of calculating absorption. In the BASF (2019b) study, oral absorption was calculated by adding the radioactivity in bile, urine, cage wash, and carcass, whereas the study by

Waidyanatha et al. (2020) calculated bioavailability using area under the curve and dose data for intravenous and gavage exposures.

As mentioned earlier, in rats administered a single gavage dose of BPS, there were two plasma concentration peaks that occurred between 1 and 4 hours, indicating possible enterohepatic circulation (BASF 2019b). Potential enterohepatic circulation was further supported in bile duct-cannulated male rats administered a single gavage dose of BPS, with 53% of the dose secreted into the bile within 24 hours (Waidyanatha et al. 2018).

The plasma half-life of unconjugated BPS in males following a single oral dose (34, 110, or 340 mg/kg) was estimated to be 2.86–4.21 hours in mice and 5.77–11.9 hours in rats. For total BPS at 110 mg/kg, the absorption half-life was 0.08 and 0.33 in female and male rats, respectively. In mice the absorption half-life was 0.18 hours with no sex differences (Waidyanatha et al. 2020).

Dermal Dosing

Dermal absorption of BPS was demonstrated to be relatively slow, with lower bioavailability in humans and human skin models compared to oral dosing, and highly dependent on vehicle.

In women dosed dermally with 1 mg/kg BPS in phosphate buffer with carboxymethylcellulose for 6 hours, absorption was generally undetectable for most time points, with T_{max} observed in individual participants between 5 and 8 hours (Khmiri et al. 2020).

In an *in vitro* study where 25 μg/cm^2 BPS (dissolved in water) was applied to fresh human skin in diffusion cells for 24 hours, 0.4% of the applied dose was recovered in the receptor fluid (absorbed dose) and 11.4% was recovered in the receptor fluid plus skin including the *stratum corneum* (potentially absorbed dose) compared to 25% and 40%, respectively, of an applied BPA dose using the same dosing regimen. Of note, the *stratum corneum* is not always included in the potentially absorbed dose due to high cellular turnover. The maximum flux (absorption rate) for BPS was 0.006 μg/cm^2/hour compared to 0.67 μg/cm^2/hour for BPA (Reale et al. 2021). In another absorption study using diffusion cells, 20 μg/cm^2 of BPA or BPS in three different vehicles was applied to human skin samples for up to 40 hours. The cumulative absorbed dose of BPS in the receptor fluid at 40 hours post-application was 0.011 (0.06%), 0.041 (0.2%), and 0.256 (1.3%) μg/cm^2 in acetone, water, and artificial sebum vehicles, respectively, compared to 5.7%, 40.9%, and 2.7% for BPA. The potentially absorbed dose of BPS in skin (including the *stratum corneum*) was 20%, 47%, and 27% in acetone, water, and sebum, respectively, and flux at steady state was low in all vehicles, but highest in

artificial sebum, with 0.0003, 0.0010, and 0.0106 µg/cm^2/hour in acetone, water, and sebum, respectively. Comparatively, BPA had a higher flux at steady state in all media (Champmartin et al. 2020). In another human skin *in vitro* model, skin permeability of a 1.5 or 7.7 µg/cm^2 application of isotope-labeled BPA and BPS was assessed. At both dose levels, 6–8% of the BPS dose was recovered in the receiver solution and 16–17% was recovered in the skin compared to 43–46% of the BPA dose in the receiver solution and 13–14% in the skin at 25 hours. This study used a reconstructed human epidermis and applied BPS with BPA as a mixture; these factors likely account for the differences in results with the studies using whole human skin (Liu and Martin 2019).

3.2 Distribution

Once absorbed into circulation, BPS is distributed throughout the body (Khmiri et al. 2020; Oh et al. 2018). BPS has been detected in human cord blood (Liu et al. 2017), amniotic fluid (Tuzimski et al. 2023), and seminal plasma (Buck Louis et al. 2018). BPS has been also detected in human breast milk samples (Iribarne-Durán et al. 2022; Luo et al. 2021; Niu et al. 2021). In men and women administered a single oral dose of 8.75 µg/kg BPS, the estimated volume of distribution was 205 L, indicating extensive distribution to peripheral tissues (Oh et al. 2018).

Single-dose gavage studies in rats reported extensive biliary excretion within the first two hours of dosing, with significant reuptake in the gut (i.e., enterohepatic recirculation) (BASF 2019b; Waidyanatha et al. 2018). In rats after a single gavage administration, BPS has been detected in gastrointestinal tissues, liver, kidney, heart, spleen, lung, and muscle (Mao et al. 2022). In rats, the BASF (2019b) study reported the highest tissue concentrations in the gastrointestinal tract after 1 hour in the 300 mg/kg dose group. In male rats, after the gastrointestinal tract, the highest residues in the 300 mg/kg dose group were found in the kidney, liver, and plasma. Similar results were observed in male and female mice after a single BPS dose by gavage of 150 mg/kg (Waidyanatha et al. 2018) and in rats continuously administered a gavage dose of 500 µg/kg/day BPS for 31 days (Mao et al. 2022). BPS did not accumulate in tissues or blood over time in rats and mice administered BPS as a single dose (Mao et al. 2022; BASF 2019b; Waidyanatha et al. 2018).

Placental Transfer

There is evidence that BPS can cross the placenta in humans and animals. BPS has been detected in human placental tissue, amniotic fluid samples, and cord blood (Abrantes-Soares et al. 2022; Liu et al. 2017; Pan et al. 2020; Zhang et al. 2020). BPS was also detected in amniotic fluid and fetal plasma in samples collected from pregnant ewes administered BPS via injection (Gingrich et al. 2019; Grandin et al. 2018).

Semen Transfer

The presence of a chemical in seminal plasma may better reflect exposure of male reproductive organs than blood or urine levels. BPS has been detected in seminal plasma (Buck Louis et al. 2018; Jeseta et al. 2022). BPS in seminal plasma most likely indicates a direct contribution of blood BPS (unconjugated) to the accessory glands (e.g., seminal vesicles, prostate gland) with a small contribution of testicular BPS (Jeseta et al. 2022).

3.3 Metabolism

BPS undergoes metabolism via sulfation, glucuronidation, and hydroxylation to form BPS-sulfate, BPS-glucuronide, and hydroxylated BPS (Waidyanatha et al. 2018). Figure 3, adapted from Waidyanatha et al. (2018), depicts the main metabolic pathways and metabolites for BPS in humans, rats, and mice (Oh et al. 2018; Sonker et al. 2021; Waidyanatha et al. 2018). Studies indicate that BPS-glucuronide is the primary metabolite of BPS formed in humans (Khmiri et al. 2020; Oh et al. 2018), rats (Mao et al. 2022; Waidyanatha et al. 2018), and mice (Waidyanatha et al. 2018). In an *in vitro* study in HepaRG cells, a human hepatic cell line, 85.8% of BPS was metabolized to BPS-glucuronide and 10.5% to BPS-sulfate (Oh et al. 2018). One study found that in isolated hepatocytes from humans, rats, and mice, BPS was metabolized to hydroxylated BPS (humans, rats, mice), BPS-sulfate (humans, rats), BPS-glucuronide (humans, rats), and the sulfate conjugate of hydroxylated BPS (rats) (Waidyanatha et al. 2018).

Variability in metabolic capabilities has been reported among different human cell lines (Le Fol et al. 2015), and considerable interindividual variation in the metabolism of BPS was observed in a study conducted in women, with the percentage of a single administered dose recovered in urine as BPS-glucuronide over 72 hours ranging from 37 to 72% (Khmiri et al. 2020). Another single-dose study conducted in adult men and women reported that the percentage of the dose recovered in urine as total BPS over 48 hours was lower in women (59–77%) compared to men (67–104%) (Oh et al. 2018).

Comparative metabolism studies in male and female rats and mice administered a single gavage dose of BPS reported that BPS-glucuronide accounted for approximately 40–50% of the administered dose in both rats and mice, while BPS-sulfate accounted for approximately 18–21% of the administered dose in mice and 4% in rats at 150 mg/kg (Waidyanatha et al. 2018).

In a dermal absorption *in vitro* model using human skin, low metabolism was observed when a 20 μg/cm^2 dose of BPS in acetone, water, or artificial sebum was applied to

human skin diffusion cells for 40 hours. The majority (85–93%) of the BPS in the receptor fluid was unmetabolized, indicating minimal metabolism within the skin compartment (Champmartin et al. 2020).

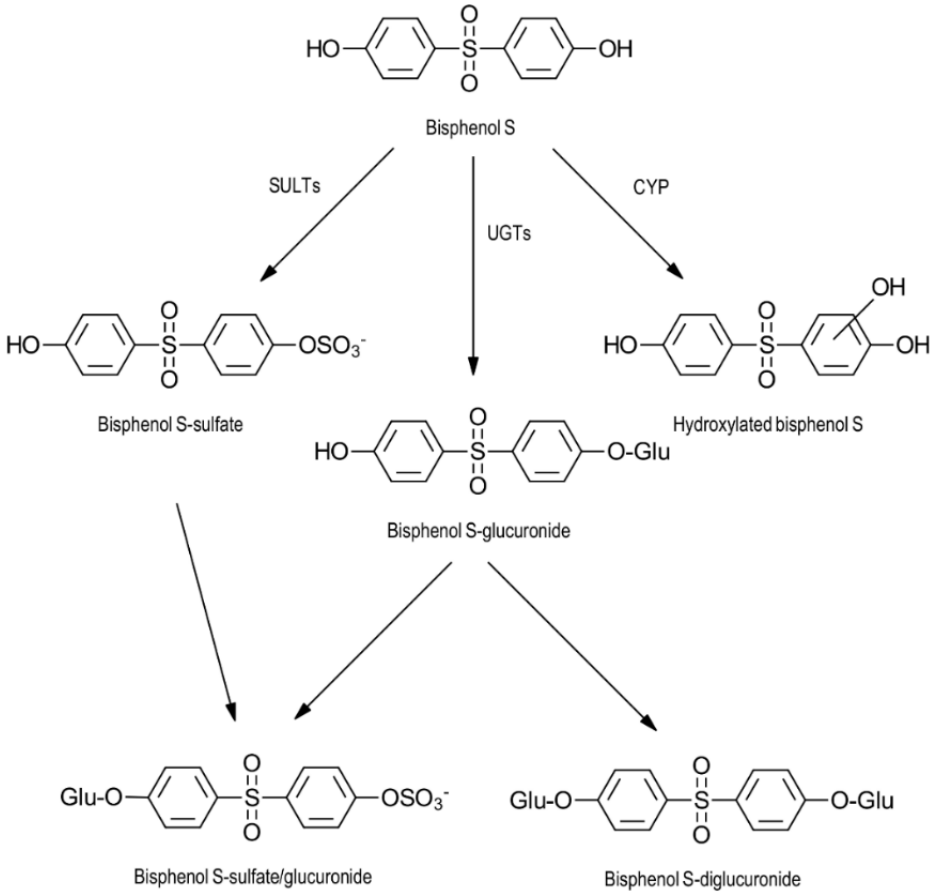

Figure 3 Proposed metabolism of BPS in rodents
Abbreviations: SULTs sulfotransferases; UGTs = uridine 5′-diphospho-glucuronosyl-transferase; CYP= cytochrome P450 enzyme (Adapted from Waidyanatha et al. (2018)).

3.4 Excretion

Studies in humans and animals indicate that BPS is excreted in the urine, bile, feces, breast milk, and, to a minor extent, in expired air after oral exposure. Free and total BPS were detected in the urine of men who handled BPS-containing paper followed by hand washing at 2 hours post-exposure (Liu and Martin 2019). In humans, BPS is excreted primarily via urine as BPS-glucuronide. In one study, urinary excretion of a single oral dose of total BPS was 92% in men and 70% in women after 48 hours, with an estimated half-life of elimination of 6.9 hours, representing metabolism and excretion (Oh et al. 2018). Another study in women estimated a half-life of elimination for a single oral dose

of BPS of around 8 to 9 hours for free and BPS-glucuronide respectively and reported that urinary excretion was almost complete after 72 hours (Khmiri et al. 2020). In the dermal exposure study in men, 78% of urine samples had detectable total BPS after two days (Liu and Martin 2019). BPS is also primarily excreted via urine in mice, rats, and sheep. No substantial species or sex differences in urinary BPS excretion were identified in studies of male and female rats and mice administered a single gavage dose of BPS, with approximately 67–70% of an administered dose of 150 mg/kg excreted within 72 hours (Waidyanatha et al. 2018). Greater variability was reported for fecal excretion, however, with 22, 16, 12, and 17 percent of the administered dose excreted by male rats, female rats, male mice, and female mice, respectively, within 72 hours (Waidyanatha et al. 2018).

In male and female rats administered a single gavage dose of 30 or 300 mg/kg with radiolabeled BPS, urinary and fecal excretion accounted for approximately 60% and 43%, respectively, in males in the 30 mg/kg group; 48% and 44%, respectively, in males in the 300 mg/kg group; 51% and 41%, respectively, in females in the 30 mg/kg group; and 39% and 56%, respectively, in females in the 300 mg/kg group. In this study, high biliary excretion was demonstrated in bile duct-cannulated rats administered the same single doses of BPS, accounting for approximately 44–56% and 38–46% of the dose in males and females, respectively. In addition, less than 2% of the 30 mg/kg oral dose of BPS was detected in exhaled air (BASF 2019b).

In plasma the elimination half-life in rats was 4.11 hours in females and 8.06 hours for males after a single oral exposure to free BPS at 110 mg/kg. The elimination half-life in male mice was 2.86 hours at 34 mg/kg and 4.21 hours at 340 mg/kg, and in male rats was 5.77 hours at 34 mg/kg and 11.9 hours at 340 mg/kg after a single gavage administration of free BPS (Waidyanatha et al. 2020).

4. MALE REPRODUCTIVE TOXICITY

Male reproductive function is supported and regulated by the hypothalamic-pituitary-gonadal axis and the adrenal and thyroid glands by producing hormones and hormone precursors. Endocrine regulation and autocrine/paracrine signaling in the testicular microenvironment facilitate the production of male reproductive cells (spermatozoa or sperm) from male germ stem cells (spermatogonia) during spermatogenesis. The development, production, and functionalization of spermatozoa is a tightly coordinated process. Spermatozoa develop in the seminiferous tubules of the testis and are stored in the epididymis, where they gain motility and fertilization ability before transport through the ejaculatory ducts. At this point, spermatozoa are combined with seminal and

prostatic fluid secreted by the seminal vesicles and prostate. These fluids offer nourishment and protection to move through and survive in the female reproductive tract. Semen, or ejaculate, is comprised of spermatozoa, seminal and prostatic fluid, and other glandular secretions and fluids.

During early embryogenesis, spermatogonia develop from primordial germ cells in the genital ridge, the precursor to the testes. Spermatogenesis occurs in the seminiferous tubule epithelium and is facilitated by Leydig cells (testicular interstitial cells) which provide autocrine/paracrine signals (testosterone) for sperm development, and Sertoli cells which provide structural, metabolic, and nutritive support. Spermatogonia mitotically divide and the daughter cells either continue to divide, or mature and differentiate into primary spermatocytes which undergo meiotic division I to generate secondary spermatocytes, which in turn undergo meiotic division II to generate spermatids. Spermatids differentiate into spermatozoa during spermiogenesis, a process involving polarization, nuclear condensation, and elongation. Immature spermatozoa are released into the seminiferous tubule lumen during spermiation and are transported to the epididymis for further maturation and storage.

Male reproductive function, especially during spermatogenesis, is highly sensitive to toxicant exposures. This is due in part to the complex cellular and molecular regulatory networks involved in maintaining and supporting a functional reproductive system. Exposure to endocrine-disrupting chemicals such as bisphenols can interfere with processes necessary to regulate and support male reproductive function.

In this section, the available evidence on the effects of BPS on male reproduction is presented in the following order: evidence from human epidemiologic studies, evidence from whole animal *in vivo* mammalian and non-mammalian studies, and evidence from studies most often employing *in vitro* experimental designs, which help inform consideration of mechanisms of action.

4.1 Studies in Humans

OEHHA identified fourteen epidemiologic studies of possible effects of BPS on the male reproductive system. Eleven of the identified studies, two prospective cohort and nine cross-sectional studies, were included and they assessed the following reproductive function outcomes: parameters of semen quality, reproductive hormones and sex hormone-binding globulin (SHBG), time to pregnancy, and male offspring reproductive development. These studies are summarized below, ordered by outcome, and the findings cited are statistically significant unless otherwise noted. Key elements of each study are presented in Table 4.1.1, with statistically significant results shown in **bold**, along with the p-values and confidence intervals.

Three studies were not included for further consideration. A study by Kolatorova et al. (2018) examined transplacental transport of BPS and fetal steroidogenesis. However, BPS was only detected in one of 27 participants. Another study by Zufferey et al. (2020) focused on the association between endocannabinoids and sperm quality; BPS was only considered as a covariate and only detected in 19 of 200 participants. The third study by Komarowska et al. (2021) examined cryptorchidism; however, no multivariable methods were reported.

Several characteristics for each study, including the limit of detection (LOD), percentage of the sample with BPS levels above the LOD, and the median BPS exposure levels, influence the study's ability to detect an association if one exists. Looking across studies, there was considerable variation in the LODs and thus, in the ability to detect BPS in the samples (Appendix B Table B.1). BPS levels in the participants across studies differed substantially, although the variability in BPS concentrations within studies was limited. Limited use of BPS during the time of the studies with earlier collection dates and potential differences by country might explain some of this variation. Additionally, it is important to recognize that many studies relied on a single urine sample to classify exposure. BPS levels in urine are highly variable, rapidly eliminated, but ubiquitously present, and there is a growing consensus that just a single urine sample may be insufficient to adequately assess exposure to non-persistent chemicals in many circumstances (Fays et al. 2020; Verner et al. 2020). Of the eight studies in this section which used urine as a biomarker for exposure, only three collected repeated urine samples (Blaauwendraad et al. 2022; Chen et al. 2022; Zeng et al. 2022). Chen et al. and Zeng et al. analyzed data from two spot urine samples that were collected two hours apart and averaged. Blaauwendraad et al. (2022) analyzed data from three spot urine samples, one collected in each trimester of pregnancy, and averaged. Only Chen et al. reported the intraclass correlation coefficient (ICC) for BPS samples and reproducibility was low (ICC = 0.01).

4.1.1 Semen Quality

Various measures of sperm quality were the focus of five of the studies (Benson et al. 2021; Chen et al. 2022; Ghayda et al. 2019; Jeseta et al. 2024; Smarr et al. 2018). These measures included sperm concentration, sperm count, ejaculate volume, sperm motility, sperm morphology, and DNA fragmentation. As shown in Table 4.1.1, four of these studies, all cross-sectional in design, observed some significant associations (Benson et al. 2021; Chen et al. 2022; Ghayda et al. 2019; Jeseta et al. 2024).

Sperm concentration and count were examined in all five studies, with two studies reporting significant associations (Ghayda et al. 2019; Jeseta et al. 2024). In a study

from the Czech Republic, Jeseta et al. (2024) reported lower sperm concentration (beta coefficient (β) = -0.18 million/milliliter (mil/mL), p = 0.012) when comparing individuals with seminal plasma BPS concentrations above and below the limit of quantification. In a US study, Ghayda et al. (2019) reported lower sperm concentrations (30.7 vs. 38.3 mil/mL, p = 0.03) when comparing men with detectable urine BPS to men with non-detectable BPS, with a larger difference observed among men with body mass index (BMI) ≥ 25 kilograms per meter squared (kg/m^2) (26.7 vs. 42.3 mil/mL, p = 0.007). Both studies also reported associations with lower total sperm count. In the Jeseta et al. (2024) study, a ten-fold increase in seminal plasma BPS was associated with a mean relative decrease in sperm count (relative risk (RR): 0.85, 95% CI: 0.74, 0.97) and individuals with BPS concentrations above versus below the limit of quantification had lower total sperm counts (β = -0.22 mil, p = 0.004). Comparing men with detectable versus non-detectable urine BPS concentrations, Ghayda et al. (2019) observed decreased total sperm count (66.3 vs. 98.7 mil, p = 0.02) only in men with BMI ≥ 25 kg/m^2. In that study, there were significant interactions with BMI for both sperm concentration and total count. In all other studies, BMI was included as a covariate, but analyses did not include stratification by BMI or a test for interaction.

Three studies reported null associations for BPS and sperm concentration and count (Benson et al. 2021; Chen et al. 2022; Smarr et al. 2018). BPS exposure levels in the Benson study were extremely low (median urine 0.06 ng/mL, interquartile range (IQR) 0.03, 0.17; 95^{th} percentile 1.12) in comparison to those seen in the Ghayda study (median urine 0.30 ng/mL, IQR 0.20, 0.90). The study by Smarr et al. (2018) measured BPS in seminal plasma with BPS levels (median (IQR), 0.11 ng/mL (0.02, 0.28)) that were higher than those seen by Jeseta et al. (2024) (median (IQR), 0.02 ng/mL (0.01, 0.07)), though 35% of participants had BPS levels below the limit of quantification and there was no mention of how values below the limit of quantification were considered in analysis. In a study of 16 men, BPS levels were shown to be much lower in seminal plasma compared to urine in both normozoospermic and vasectomized men (mean of the ratios of urinary BPS to seminal plasma BPS = 6.11 and 3.39, respectively) (Jeseta et al. 2024).

Four studies examined ejaculate volume and three reported significant associations. Ghayda et al. (2019) reported significantly lower volume among men with detectable urine BPS concentrations compared to those with non-detectable concentrations (2.66 vs. 2.91 mL, p = 0.03). Benson et al. (2021) reported a 13% significant decrease in ejaculate volume in the third quartile versus the first quartile of urine BPS (95% CI: -23%, -1%), with no associations observed in the second or fourth quartiles. Jeseta et al. (2024) reported that a ten-fold increase in seminal plasma BPS levels was associated with a mean relative decrease of ejaculate volume by 9% (RR: 0.91, 95% CI: 0.85,

0.98). A fourth study by Smarr et al. (2018) reported associations with semen volume in the same direction that were not statistically significant. Increases in BPS were associated with lower semen volume (β: -0.05 mL, 95% CI: -0.12, 0.02) and lower odds (odds ratio (OR): 0.80, 95% CI: 0.60, 1.07) of having semen volume greater than 1.5 mL.

Sperm motility was examined in all five studies (Benson et al. 2021; Chen et al. 2022; Ghayda et al. 2019; Jeseta et al. 2024; Smarr et al. 2018). Lower progressive and total motility were observed by Chen et al. (2022), in which higher BPS exposure was related to increased odds of having below-reference progressive motility (OR: 1.62, 95% CI: 1.07, 2.43) (P trend = 0.02) and total motility (OR: 1.57, 95% CI: 1.06, 2.33) (P trend = 0.02). Based on Bayesian kernel machine regression models with bisphenol A (BPA) and bisphenol F (BPF) exposures held at the 25th percentile, an IQR increase in BPS exposure was associated with significant decreases in sperm progressive motility and total motility. Additionally, inverted U-shaped curves were observed for continuous progressive motility and total motility with BPS exposure when fixing BPA and BPF at their median levels (Chen et al. 2022). Ghayda et al. (2019) reported borderline lower total motility in men with detectable versus undetectable urinary BPS concentrations (43.7% vs. 47.0%, p = 0.06), with stronger differences observed in men with a BMI ≥ 25 kg/m^2 (41.9% vs. 46.9%, p = 0.02). Benson et al. (2021) reported no association with sperm motility, although as mentioned above, BPS exposure levels were extremely low. Jeseta et al. (2024) and Smarr et al. (2018) also reported no significant associations, though in Smarr et al., the direction of associations for all measures of sperm motility examined (average path velocity, straight-line velocity, curvilinear velocity, amplitude of lateral head, beat cross frequency, straightness, linearity, and percent motility) were in the direction of reduced motility. Chen et al. (2022) also examined the motion parameters of straight-line velocity, curvilinear velocity, and linearity and there were no significant associations reported.

Sperm morphology was also examined in all five studies (Benson et al. 2021; Chen et al. 2022; Ghayda et al. 2019; Jeseta et al. 2024; Smarr et al. 2018); none observed significant findings. Two studies measured viability and DNA fragmentation and reported no significant results (Jeseta et al. 2024; Smarr et al. 2018).

4.1.2 Reproductive Hormones

Reproductive hormones were examined in four cross-sectional studies, three of which used National Health and Nutrition Examination Survey (NHANES) data.

As shown in Table 4.1.1, two of the three studies (Hu et al. 2022; Wang et al. 2021) that analyzed NHANES data used the same survey cycles (2013–2014, 2015–2016) to

examine the association of BPS exposure with reproductive hormones in children and adolescents. All significant results in these two studies were in male children (ages 6–11); neither study observed any significant associations in male adolescents (ages 12–19), or by pubertal status. Both studies observed lower estradiol (E2) levels associated with exposure, (Hu et al., E2: β: -0.03, 95% CI: -0.05, -0.01 ($p < 0.05$)), (Wang et al., E2: β: -0.045, 95% CI: -0.082, -0.007 ($p < 0.05$)), as well as a negative trend for E2 by quartile in Hu et al. Wang et al. (2021) reported that the detection frequency of E2 was insufficient to analyze by quartiles. Hu et al. (2022) also observed negative associations with total testosterone (TT), free androgen index (FAI), TT/E2, and a positive association with SHBG in continuous analyses, with the trends being significant for each. Wang et al. (2021) presented supplemental data suggestive of a negative association with FAI and TT (Quartile (Q) 4 vs. Q1) although this was not noted in the main text. Many comparisons were conducted in these two studies. Hu et al. (2022) adjusted for multiple comparisons using the Benjamini-Hochberg procedure; no adjustment was mentioned in Wang et al. (2021)

The third study, by Zhang C et al. (2022), used two NHANES survey cycles (2013–2014, 2015–2016) to examine these associations in adult males. This study reported a positive association with SHBG (β: 0.25, 95% CI: 0.071, 0.429) ($p = 0.006$) and a negative association with free testosterone (FT) (β: -0.01%, $p = 0.0258$). No significant association was observed with TT/E2. In analyses stratified by BMI, BPS was not significantly associated with E2 by quartile, although a significant interaction was seen. The association was negative for normal and overweight men, and positive for obese men (Zhang C et al. 2022).

The fourth study, by Zeng et al. (2022), included men attending an infertility clinic in China. Higher BPS exposure was associated with lower E2 levels and E2/T ratios. Men in the highest exposure quartile, compared to those in the lowest quartile, had an 11.47% reduction in E2 (95% CI: -17.98, -4.45) (P trend = 0.002), while men in the third quartile compared with those in the lowest quartile had a 13.32% reduction in E2/T ratio (95% CI: -20.88, -5.03) (P trend = 0.02). Higher BPS exposure was also significantly associated with lower SHBG levels (Q4 vs. Q1, -10.52% (95% CI: -19.46, -0.59)) (P trend = 0.09). In stratified analysis, an inverse association between exposure and follicle-stimulating hormone (FSH) levels was only evident among men with BMI ≥ 24 kg/m^2 (P trend and interaction = 0.03). Additionally, when stratified by age, compared to men aged ≤ 30 years, men aged > 30 years with higher BPS exposure had significantly lower E2 levels (Q3 vs. Q1, -12.74% (95% CI: -21.13, -3.46) (Q4 vs. Q1, -13.52% (95% CI: -22.09, -4.01)) (P trend = 0.003) and the association with lower E2/T ratio was more pronounced (Q3 vs. Q1, -16.05% (95% CI: -25.07, -5.96)) (Q4 vs Q1, -8.82% (95% CI: -18.91, 2.52)) (P trend = 0.02), while the association with lower SHBG levels was more

pronounced in men aged ≤ 30 years (Q4 vs. Q1, -16.09% (95% CI: -28.78, -1.15)) (P trend = 0.08). However, these interaction terms were not statistically significant.

4.1.3 Time to Pregnancy

In a prospective cohort study of time to pregnancy (Buck Louis et al. 2018), BPS was associated with lower fecundability odds ratios in both male-adjusted (OR: 0.91, 95% CI: 0.78, 1.05) and couples-adjusted models (OR: 0.92, 95% CI: 0.79, 1.08) however, these were not statistically significant. As the authors suggested, the lower BPS concentrations measured in semen (median (IQR) 0.11 ng/mL (0.02, 0.28)) may require a larger sample size (n=339) to assess couple fecundability. The Longitudinal Investigation of Fertility and the Environment Study served as the study population for both this study and for the study by Smarr et al. (2018).

4.1.4 Male Offspring Reproductive Development

Data from the Generation R prospective cohort study was used to analyze associations between maternal BPS exposure and male reproductive tract abnormalities in infancy, testicular volume at age 10, and pubertal development at age 13. Maternal BPS, averaged from three urine samples taken across gestation, was not associated with any male offspring outcomes (Blaauwendraad et al. 2022).

Table 4.1.1 BPS: Epidemiologic studies of male reproductive toxicity.[3]

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Semen Quality					
Benson et al. 2021 Denmark 2017–2019 Cross-sectional Sample drawn from the Danish National Birth Cohort, sons of mothers enrolled from 1996–1999. Males 18–20 years old N = 5,697 invited to participate n = 1,057 participated in clinical exam n = 556 samples analyzed Inclusion criteria: Male children > 18 years and 9 months, no previous history of sterilization or chemotherapy, and descended testicles.	Urine (creatinine-adjusted): Percentiles: 5th: < limit of detection (LOD) 25th: 0.03 50th: 0.06 75th: 0.17 95th: 1.12 LOD: 0.03 % < LOD = 28 Imputation for values below LOD: not mentioned.	Semen quality: Ejaculate volume (milliliters [mL]) Total sperm count (millions [mil.]) Sperm concentration (mil/mL) Sperm motility (% progressive and non-progressive) Sperm morphology (% normal)	Participants in the third quartile (Q3) compared with those in the lowest quartile (Q1): Ejaculate volume: **-13% (95% CI: -23%, -1%)** (Reported in Table 4 as adjusted ratio: **0.87 (95% CI: 0.77, 0.99))** No associations observed for any of the other outcomes either modeled as quartiles or continuous variables.	Models adjusted for smoking, alcohol intake, body mass index (BMI) (all assessed at clinical exam of participant), fever within three months prior to sampling, sexual abstinence time, maternal first trimester smoking, maternal pre-pregnancy BMI, and highest parental education. Analyses of motility were also adjusted for the number of minutes from sample collection to analysis. Samples where spillage was reported were excluded from analyses of ejaculate volume and total sperm count. Other bisphenols measured: bisphenol A (BPA), bisphenol F (BPF).	BPS levels were very low. Q3 BPA and BPF were associated with increased progressive and non-progressive motility compared to Q1 exposures. The proportion of participants providing urine samples was low, though authors report "All subjects were most likely unaware of their level of bisphenol exposure which…minimises the risk of selection bias". Also, selection weights for non-participation were included in analyses. Table 3 reports Q3 BPS as 0.06–0.17 ng/mL and Q4 BPS as >0.017 ng/mL. The value for Q4 is assumed to be a typo.

[3] Studies are ordered by outcomes (semen quality; reproductive hormones; time to pregnancy; offspring reproductive development), and within outcomes, by author. Statistically significant results are in **bold** type.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Chen et al. 2022 China 2013 Cross-sectional N = 1,247 male partners of couples at an infertility clinic n = 984 Mean age (standard deviation (SD)): 32 (5.4) years. Exclusion criteria: Missing or insufficient urine samples, had endocrine or reproductive-related diseases, or reported occupational exposure to synthetic materials.	Two spot urine samples (at least 2 hours apart): Unadjusted values: Median (Interquartile range (IQR)): First urine: 0.35 (0.16, 0.80) Second urine: 0.30 (0.12, 0.71) Average: 0.38 (0.17, 0.81) Creatinine adjusted values (µg/g): Median (IQR): First urine: 0.25 (0.11, 0.62) Second urine: 0.26 (0.11, 0.64) Average: 0.29 (0.13, 0.69) LOD = 0.02 % < LOD = 4.17 (first urine), 4.98 (second urine) Values below the LOD were calculated as LOD÷√2. Natural logarithm transformed urinary creatinine-adjusted BPS concentrations in	Semen quality: Total sperm count (mil) Sperm concentration (mil/mL) Progressive and total motility (%) Motion parameters: straight-line velocity (VSL), curvilinear velocity (VCL), and linearity (VSL/VSC x 100). Sperm morphology: % abnormal head, % normal morphology. Outcomes were analyzed as continuous and binary variables (using World Health Organization (WHO) reference values. For binary outcomes, the comparison group	<u>Semen quality modeled as binary outcomes:</u> Higher BPS exposure was related to an increased odds of having below-reference progressive motility (< 32%) and total motility (< 40%). Below-reference progressive motility and total motility comparing Q4 to Q1: **Progressive motility, odds ratio (OR): 1.62 (95% CI: 1.07, 2.43) (*P* trend 0.02)** **Total motility, OR: 1.57 (95% CI: 1.06, 2.33) (*P* trend 0.02)** No associations were found between below-reference sperm concentration, total sperm count, VSL, VCL, linearity, or sperm morphology and BPS in quartiles. <u>Semen quality modeled as continuous outcomes:</u> Progressive motility, -3.15%; (95% CI: -6.32%, 0.02%) (*P* trend 0.06) **Total motility, -3.86% (95% CI: -7.46%, -0.25%) (*P* trend 0.04)** No associations were found between sperm concentration, total sperm count, VSL, VCL, linearity, percent abnormal head, percent normal morphology and continuous BPS.	Models adjusted for age, BMI, education, having ever fathered a pregnancy, abstinence time, smoking status, alcohol use. Other bisphenols measured: BPA, BPF.	Low reproducibility of urinary creatinine-adjusted BPS for repeated urine measurements (intraclass correlation coefficient = 0.01). Higher BPA exposure was associated with increased ORs of having below-reference sperm concentration, total sperm count, progressive motility, and total motility (all *P* trends < 0.05). BPA was also inversely associated with sperm linearity. BPF was associated with higher motility and higher % of sperm with abnormal head.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
	two urine samples were averaged to reduce misclassification bias.	was participants with four semen quality parameters ≥ the WHO reference values for sperm concentration (15 × 10^6 mL), total sperm count (39 × 10^6), progressive motility (32%), and total motility (40%) (n=621)	Mixture models: Bayesian kernel machine regression (BKMR) models with BPA and BPF held at 25th percentile: **An IQR increase in BPS exposure was associated with significant decreases in sperm progressive motility and total motility** (values not given). BMKR models with BPA and BPF held at their median levels: **Inverted U-shaped curves were observed for continuous progressive motility and total motility** (values not given).		
Ghayda et al. 2019 Boston 2011–2017 Cross-sectional Environment and Reproductive Health (EARTH) (2004–2017) In the original cohort, BPS was first evaluated starting in 2011; thus 382 men were excluded due to lack of measured urinary BPS concentration data in the earlier years.	Urine spot sample (corrected for specific gravity) Geometric mean (SD): 0.37 (0.03) Percentiles: 25th: 0.20 50th: 0.30 75th: 0.90 LOD: 0.1 % < LOD = 24 Values below the LOD were calculated as LOD÷√2.	Semen quality: Ejaculate volume (mL) Total sperm count (mil/ejaculate) Sperm concentration (mil/mL) Sperm motility, total motile count (%) Sperm morphology, normal morphology count Semen and urine samples were	BPS categorized as detectable (> LOD) vs. non detectable urinary concentrations (< LOD), adjusted means: Semen volume: **2.66 (95% CI: 2.39, 2.92) vs. 2.91 (95% CI: 2.69, 3.12) (p = 0.03)** Sperm concentration: **30.7 (95% CI: 22.4, 42.0) vs. 38.3 (95% CI: 28.0, 52.4) (p = 0.03)** Total sperm count: 76.8 (95% CI: 56.2, 105) vs. 90.0 (95% CI: 65.7, 123) (p = 0.09) Total motility: 43.7 (95% CI: 40.0, 47.4) vs. 47.0 (95% CI: 42.6, 41.3) (p = 0.06)	Models adjusted for abstinence time, age, BMI, year of sample collection, BPA. Other bisphenols measured: BPA, BPF.* *BPF excluded due to low detection rate (25%).	Response rate was about 40% of those contacted from the prospective EARTH study. 56% of the participants contributed more than one urine sample, which would partially reduce exposure misclassification. Male factor infertility was diagnosed at enrollment in 36 men (23%). More than one-third of semen samples (39%) were below the WHO 2010 lower reference

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
n = 158 men, ages 18–56 years Mean age (IQR): 35.6 (32.6–39.6) years Inclusion criteria: male partners of couples seeking fertility treatment and enrolled in the prospective cohort study, without history of a vasectomy, complete BPS urinary concentrations within cohort enrollment.		collected at the same time. Outcomes were analyzed as continuous and binary (below WHO reference values) variables.	<u>BPS analyzed by quartiles, adjusted means:</u> Semen volume: **Higher volume in Q2 (3.00, 95% CI: 2.75, 3.25) vs. Q1 (2.68, 95% CI: 2.41, 2.95) (p < 0.05).** Sperm concentration: **Lower concentration in Q3 (29.2, 95% CI: 20.6, 41.5) vs. Q1 (38.4, 95% CI: 28.0, 52.7) (p = 0.03).** <u>Analyses stratified by BMI:</u> Some associations were only found among men with BMI ≥ 25 kg/m^2 (n=106), when comparing men with detectable BPS concentrations to men with non-detectable levels (no 95% CIs provided in figure 1): Sperm concentration: **26.7 vs. 42.3 mil/mL (p = 0.007)** Total count: **66.3 vs. 98.7 mil (p = 0.02)** Total motility: **41.9 vs. 46.9% (p = 0.02)** P-interactions with BMI**:** **sperm concentration = 0.001** **total sperm count = 0.05** total motility = 0.15		limit for progressive sperm motility. Men who had higher BPS concentrations had significantly higher BMI. Authors noted that self-reported fabric softener and paint/solvent use, as well as intake of beef and cheese within 24 hours before urine collection were positively associated with BPS concentrations. Spearman correlation between BPA and BPS: 0.45.
Jeseta et al. 2024 Czech Republic 2019–2021 Cross-sectional Men attending the Centre of Reproductive	Seminal plasma Percentiles: 25th: 0.01 50th: 0.024 75th: 0.07	Semen quality: Ejaculate volume (mL) Sperm concentration (mil/mL)	<u>Associations per 10-fold increase in continuous BPS:</u> Ejaculate volume: **β= -0.09 (p = 0.013)** **RR = 0.91 (95% CI: 0.85, 0.98)**	Models adjusted for age, BMI, ejaculation abstinence, smoking, reactive oxygen species. Other bisphenols measured: BPA, BPF, bisphenol AF.	BPA was detected in 76.5% of samples and was associated with lower ejaculate volume, lower sperm count, lower progressive

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Medicine of University Hospital Brno. n = 306 Median age (IQR): 32.0 (9.0) years. Exclusion criteria: vasectomy, use of antidepressants, other medications or disease with potential effects on spermiogram, fever in last month, ongoing chemotherapy, radiological exposure, application of testosterone, seminal vesicles inflammation, work with elemental sulfur, potential mismatch of samples during laboratory analysis.	(25^{th} and 75^{th} percentiles estimated from Figure 2). LOD = 0.002 % < LOD = 22.6 Values < limit of quantification (LOQ) were imputed with 1000 repeated imputations. Value for LOQ was not given.	Progressive motility (%) Sperm morphology (%) Total sperm count (mil) DNA fragmentation index % = (fragmented spermatozoa + degenerated spermatozoa/total spermatozoa counted) x 100.	Total sperm count: **β = -0.17 (p = 0.018)** **RR = 0.85 (95 % CI: 0.74, 0.97)** No significant associations were observed for sperm concentration, sperm progressive motility, sperm morphology, or DNA Integrity. <u>Associations with categorical BPS, above versus below LOQ:</u> Total sperm count: **-0.22 (p = 0.004)** Sperm concentration: **-0.18 (p = 0.012)** No significant associations were reported for ejaculate volume, sperm progressive motility, sperm morphology, and DNA integrity. The direction of association for all parameters was negative.		motility and lower % with normal morphology. BPF was detected in 14.4% of samples and was associated with decreased fragmented sperm. bisphenol AF was detected in 23.9% of samples and was not associated with any outcome. Models adjusted for reactive oxygen species, which may be on the casual pathway from BPS to semen quality.
Smarr et al. 2018 Michigan and Texas 2005–2009 Cross-sectional study nested within an ongoing cohort. Longitudinal Investigation of Fertility and the Environment (LIFE) Study	Seminal plasma Geometric mean (95% CI): 0.14 (0.12, 0.17) Percentiles: 25^{th}: 0.02: 50^{th}: 0.11: 75^{th}: 0.28: 95^{th}: 1.72 LOD: 0.018 (from Buck Louis et al. 2018	Semen quality: Sperm concentration million/mL (mil/mL) Semen volume (mL) Total sperm count million/ejaculate Sperm viability	No statistically significant associations with BPS. There was an association with lower odds of having normal sperm quality per WHO reference levels (OR 0.77, 95% CI: 0.60, 0.98), though the false discovery rate adjusted p-value for multiple comparisons was 0.76. Non statistically significant associations with lower semen volume:	Models adjusted for age, BMI, household income, race, serum cotinine, research site. Other bisphenols measured: BPA, BPF.	Authors describe this study as a “novel exploratory investigation.” To account for performing multiple hypothesis tests, false discovery rate adjusted p-values were reported for all analyses.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
N = 501 reproductive aged couples. n = 339 Mean age (SD): 31.8 (4.9) years Inclusion criteria: Sample with sufficient residual semen volume (> 1.5 mL), ≥18 years old, no physician-diagnosed infertility or sterility, in a committed relationship, ability to communicate in English or Spanish.	but not stated in this paper) % < LOQ = 35 No mention of substitution for values < LOQ	Sperm motility DNA fragmentation Sperm morphology Two semen samples were collected at home, one at baseline and a second one month later.	Semen volume > 1.5 mL: OR 0.80 (95% CI: 0.60, 1.07), False discovery rate p = 0.47 Semen volume analyzed continuously: β = -0.05 mL (95% CI: -0.12, 0.02)		Seminal plasma BPA was associated with lower semen volume. BPS was measured in the second semen sample whereas sperm quality markers were measured in both first (all semen quality parameters) and second (concentration volume, motility, sperm head morphology) samples.
Reproductive Hormones					
Hu et al. 2022 United States National Health and Nutrition Examination Survey (NHANES) 2013–2016 Cross-sectional Children 6–19 years N = 1,179 females and males Mean age: 12.31 years 6–11 year-olds were classified as children (males n = 270)	These values are for males and females combined for 6–19 year-olds (no measures by sex were provided) Spot urine (creatinine-adjusted): Geometric mean: 0.52 Percentiles*: 25th: 0.19 50th: 0.37 75th: 0.74 95th: 2.91 LOD = 0.1	Serum reproductive hormones and related proteins: Total testosterone (TT, ng/dL) Estradiol (E2, pg/mL) Sex hormone-binding globulin (SHBG, nmol/L) Free androgen index (FAI = TT*100/SHBG) TT/E2	<u>In boys 6 to 11 (regardless of puberty status)</u> (Table S4) linear regression analysis: **E2: -0.03 (95% CI: -0.05, -0.01) (p < 0.05)** **TT: -0.10 (95% CI: -0.17, -0.04) (p < 0.05)** **FAI: -0.12 (95% CI: -0.19, -0.05) (p < 0.05)** **TT/E2: -0.07 (95% CI: -0.13, -0.02) (p < 0.05)** **SHBG: 0.02 (95% CI: 0.001, 0.04) (p < 0.05)** When BPS was analyzed by quartiles, there was a significant trend with lower E2	Models adjusted for age, race, BMI, serum cotinine concentrations, family income to poverty ratio, six-month time period when surveyed, time of day and season of blood sample collection, NHANES cycles. Other bisphenols measured: BPA.	Generalized linear regression models were adjusted for multiple comparisons using Benjamini-Hochberg correction to control false discovery rate at < 5%. P-values that were statistically significant in the primary analysis remained significant after adjusting for multiple comparisons. Additional analyses: Bayesian kernel machine regression and

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
12–19 year-olds were classified as adolescents (males n = 335) Puberty was defined as TT ≥ 50 ng/dL in boys. Inclusion criteria: systematic sample of US population, ages 6–19, urinary bisphenols measured, complete data on covariates, no history of sex hormone medication.	% < LOD = 11.7 Values below the LOD were assigned a value equal to the LOD÷√2		(p-trend = 0.001), lower TT (p-trend = 0.002), higher SHBG (p-trend = 0.034), lower FAI (p-trend = 0.001), and lower TT/E2 (p-trend = 0.009) (Figure S8). No significant associations in boys 12–19. No significant results were seen in analyses stratified by puberty status.		weighted quantile sum regression models were conducted. Results for BPA were mostly similar to BPS. Spearman correlation between BPS and BPA = 0.33. *Authors reported an error in the paper interchanging the BPA and BPS descriptive statistics, corrected BPS values are given. Results reported here are for males only.
Wang et al. 2021 United States NHANES 2013–2016 Cross-sectional N = 1,317 participants, males and females 6–19-year-olds mean age: 13 years 6–11-year-olds were classified as children (males n = 271) 12–19-year-olds were classified as adolescents (males n = 391)	Spot urine (creatinine-adjusted in statistical analyses) Median (IQR): Total population: 0.3 (0.6) Male children: 0.3 (0.6) Male adolescents: 0.3 (0.5) LOD = 0.1 % < LOD: 10.7 (male children) 10.0 (male adolescents)	Serum reproductive hormones and related proteins: TT (ng/dL) E2 (pg/mL) SHBG (nmol/L) FAI calculated as the value of TT (ng/dL) × 100 divided by SHBG (nmol/L), to assess the approximate amount of free testosterone.	Negative association between continuous measures of BPS and E2 in male children: **-0.045 (95% CI: -0.082, -0.007) (p < 0.05)** **TT and FAI appear significantly lower in Q4 vs. Q1 in male children** with a possible decreasing trend across quartiles (adjusted for BPA, BPF (Fig 2 and Supplemental (S) Fig S6). Results were not materially altered after mutual adjustment for BPA and BPF (S6). E2 appears significantly higher in Q2 vs. Q1 in male adolescents (Fig 2). Suggestion of non-linear association with FAI (p for non-linearity = 0.05) in male adolescents, (Fig S3).	Models adjusted for age, race/ethnicity, poverty income ratio, BMI, urinary creatinine, serum cotinine, time of sample collection, survey cycle. Other bisphenols measured: BPA, BPF.	Quartile analyses for E2 and TT/E2 were not performed in male children 6–11 years old, as the detection frequency of E2 was < 50% in that group. No mention of adjustment for multiple comparisons. Exposure-response relationships were examined via restricted cubic splines. Spearman correlation between BPS and BPA

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Inclusion criteria: systematic sample of US population, ages 6–19, complete data on exposure and covariates.	Values below the LOD were imputed as the value of LOD÷√2	TT/E2 used as an indirect measure of aromatase activity because aromatase catalyzes the conversion of testosterone to estradiol.			= 0.33, and between BPS and BPF = 0.15.
Zeng et al. 2022 China 2013 Cross-sectional N = 1,247 men attending an infertility clinic were recruited 207 men were excluded n = 462 men with blood and sufficient urine samples mean age = 32.2 (±5.2) years Inclusion/exclusion criteria: included in study if had bisphenol urine and serum reproductive hormone measurements, excluded if had occupational exposure	Average of two urine samples at least two hours apart: Median (IQR) (crude): 0.39 (0.18, 0.87) Median (IQR) (creatinine-adjusted): 0.33 (0.18, 0.80) ug/g LOD = "ranged from 0.02 – 0.10" for BPA, BPF, BPS % < LOD = First urine sample: 4.55, second urine sample: 4.98 Values below the LOD were assigned a value equal to the LOD÷√2	Serum reproductive hormones and related protein: TT (ng/dL) Free Testosterone (FT) (ng/dL) E2 (pg/mL) Follicle-stimulating Hormone (FSH, mIU/mL) Luteinizing hormone (LH, mIU/mL) SHBG (nmol/L) E2/T ratio LH/T ratio	BPS exposure associated with (percent change): <u>E2:</u> **Q1 to Q3: -7.81% (95% CI: -14.52, -0.57)** **Q1 to Q4: -11.47% (95% CI: -17.98, -4.45) (*P* trend = 0.002)** <u>E2/T:</u> **Q1 to Q3: -13.32% (95% CI: -20.88, -5.03) (*P* trend = 0.02)** <u>SHBG:</u> **Q1 to Q4: -10.52% (95% CI: -19.46, -0.59)** (*P* trend = 0.09) <u>FAI:</u> **Q1 to Q3: 10.98% (95% CI: 1.33, 21.55)** (*P* trend = 0.10) <u>Free T:</u> **Q1 to Q3: 9.94% (95% CI: 1.17, 19.47)** (*P* trend 0.42) Restricted cubic spline models: **E2 inverse linear association (*P* = 0.007)** SHBG suggestive decreasing trend (*P* trend = 0.07)	Models adjusted for age, BMI, education, alcohol use, household income, ever fathered a child, smoking status. Other bisphenols measured: BPA, BPF.	Blood samples were drawn between 8:30 am and 11:30 am "to reduce the diurnal variability." As noted by the authors, the decreasing trend in SHBG levels associated with increasing BPS exposure may be a parallel of decreased E2 levels since E2 promotes SHBG expression. BPF was negatively associated with E2 and E2/T. BPA was negatively associated with TT and SHBG.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
or endocrine/reproductive disorders.			Stratified analysis adjusted (percent change): E2: among men aged > 30 years: **Q1 to Q3: -12.74% (95% CI: -21.13, -3.46)** **Q1 to Q4: -13.52% (95% CI: -22.09, -4.01) (*P* trend = 0.003)** E2/T ratio among men aged > 30 years: **Q1 to Q3: -16.05% (95% CI: -25.07, -5.96)** Q1 to Q4: -8.82% (95% CI: -18.91, 2.52) **(*P* trend = 0.02)** SHBG among men aged ≤ 30 years: **Q1 to Q4: -16.09% (95% CI: -28.78, -1.15)** (*P* trend = 0.08) FSH among men with BMI ≥ 24 kg/m^2: **Q1 to Q4: -20.88% (95% CI: -36.81, -0.93)** **(*P* trend =: 0.03, *P* interaction = 0.03)** LH among men with BMI ≥ 24 kg/m^2: **Q1 to Q2: -18.48% (95% CI: -32.02, -2.25)** (*P* trend = 0.22) SHBG among men with BMI < 24 kg/m^2: **Q1 to Q4: -13.05% (95% CI: -23.95, -0.6)** (*P* trend = 0.18)		
Zhang C et al. 2022 United States NHANES 2013–2016 Cross-sectional Adult males	Spot urine (creatinine-adjusted in the statistical analyses) Geometric mean = 0.55 (95% CI: 0.51, 0.58)	Serum reproductive hormones and related proteins: TT (ng/dL) FT (nmol/L)	Linear regression analysis: SHBG: **0.250 (95% CI: 0.071, 0.429) (p = 0.006)** FT: **-0.0001 (95% CI: -0.0001, 0.0000)** (**p = 0.0258)** (Reported in text as -0.01%).	Models adjusted for age, race poverty income ratio, BMI, smoking status, urinary creatinine, time of sample collection (morning, afternoon, evening), season of	About 74% of participants were overweight or obese. In previous studies, BPS levels were higher in these individuals.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
N = 1,575 Mean age = 49.15 (±17.59) years Inclusion criteria: systematic sample of US population, ages ≥20 years, patients excluded if they were taking medication (testosterone, estrogen, progesterone, or "other sex hormones".	Median (IQR): 0.50 (0.20,1.10) LOD = 0.1 % < LOD = 7.68 Values below the LOD were assigned a value equal to the LOD÷√2	E2 (pg/mL) SHBG (nmol/L) TT/E2	No association with TT/E2 in continuous or categorical analyses. In analyses stratified by BMI, BPS was not significantly associated with E2 by quartile, although a significant interaction was seen. The association was negative for normal and overweight men, and positive for obese men. ***P* for interaction = 0.007.**	sample collection (two six-month periods). Other bisphenols measured: BPA, BPF.	BPA was negatively associated with FT in a continuous model and positively associated with SHBG. BPF was positively associated with TT and FT.
Time to Pregnancy					
Buck Louis et al. 2018 Michigan and Texas 2005–2009 Prospective cohort from the Longitudinal Investigation of Fertility and the Environment (LIFE) Study n = 501 couples recruited prior to conception n = 339 male partners of couples with an observed time-to-pregnancy, and sufficient residual semen volume for sampling	Two seminal plasma samples: Median (IQR) 0.11 (0.02, 0.28) LOD: 0.018 % < LOD = 25 Values below LOD or LOQ were not substituted. The first semen sample was obtained the day following the interview and the second sample approximately one month later, both at home.	Couple fecundity measured by time-to-pregnancy. Time-to-pregnancy denoted by the number of menstrual cycles required for couples to become pregnant.	No statistically significant association with time-to-pregnancy. Fecundability odds ratios (95% CI): Male adjusted: 0.91 (0.78, 1.05) Couple adjusted: 0.92 (0.79, 1.08)	Male model adjusted for male age, male BMI, male serum cotinine, research site. Couple model adjusted for: female age, difference in couples' ages, both partners' BMI, both partners' serum cotinine concentrations, and research site. Other bisphenols measured: BPA, BPF.	Authors used seminal fluid to measure BPS exposure as "it is assumed to provide a more direct measure of within testes exposure." Couples were followed daily until pregnancy or 12 months of trying without pregnancy. Reduced statistical power in that 32% of men participating in the LIFE Study did not have residual semen samples available for analysis. This study also examined 5 benzophenones, 15

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Mean age: 31 years Inclusion criteria: ≥18 years, able to communicate in English or Spanish, and no history of clinical infertility diagnosis.	BPS was measured in the second semen sample.				phthalate metabolites, and 9 phthalate diesters. None of these substances, including the other bisphenols were associated with time-to-pregnancy.
Offspring Reproductive Development					
Blaauwendraad et al. (2022) Rotterdam, Netherlands 2004–2005 Prospective cohort study Generation R Study N = 696 singleton life-born boys n = 639 boys with data on infant reproductive tract abnormalities n = 355 boys with data on testicular volume n = 320 with data on pubertal characteristics. Mean (SD) maternal age in years: 30.7 (4.8) Inclusion criteria: resident in study area	Three urine spot samples, averaged Adjusted with creatinine LOD: 0.15 % < LOD (per trimester sampling time): 31.5, 70.3, 80.0 Values below the LOD were imputed as LOD÷√2 Percentiles (per trimester sampling time): 25th: <LOD, < LOD, < LOD 50th: 0.2, <LOD, <LOD 75th: 0.7, <LOD, <LOD Timing of samples (median (IQR)): 12.9 (12.1–14.4) weeks, 20.4 (19.9–	Reproductive development: cryptorchidism, hypospadias at infancy Testicular volume by magnetic resonance imaging at 10 years. Pubertal development using Tanner staging at 13 years.	No associations with cryptorchidism or hypospadias. Non-significant increase in testicular volume at age 10 (standard deviation score difference: 0.04, 95% CI: -0.04, 0.12) (p = 0.334). No associations with genital development or pubic hair development at age 13.	Models adjusted for maternal age, ethnicity, pre-pregnancy BMI, education, parity, energy intake, smoking and alcohol use, breastfeeding, child's gestational age-adjusted birthweight, age at visit, and child's age-adjusted BMI at time of measurement. Other bisphenols measured: BPA, BPF.	Exposure concentrations were reported in nmol/L. Converted to ng/mL here. 2nd trimester BPF was not analyzed due to ≤ 20% of samples above LOD. Correlations between BPS and other bisphenols: BPA range 0.03–0.06 by trimester, BPF range 0.04–0.28 by trimester. Higher average total bisphenol concentrations were associated with larger testicular volume at age 10 and reduced odds of having lower than expected pubic hair development at age 13. Specific birth years were not reported in this

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
at delivery date, bisphenol measurement in each trimester of pregnancy, singleton, live birth.	20.9) weeks, 30.2 (29.9–30.8) weeks.				study. Other published studies reported that Generation R measured urine BPS in 2004–2005.

4.2 Studies in Animals

4.2.1 Overview

The male reproductive toxicity of BPS has been evaluated in various animal models, including 14 studies in mice, 20 studies in rats, one study in hamsters, one study in gerbils, four studies in zebrafish, and one study in guppies. All studies in mice were reported as peer-reviewed publications and the strains included 129S1/SvImJ (one study), CD-1 (four studies), C57BL/6 (four studies), CF-1 (one study), ICR (three studies), and Parkes (one study). Of the 20 rat studies summarized here, 14 were peer-reviewed publications and six were available as laboratory reports (BASF) or reviews by other agencies (ECHA). Rat strains used in these studies included Sprague Dawley (SD, 15 studies), Wistar (four studies), and Long-Evans (one study).

The key features of each study noted in this section include the species and strains of animals used, exposure regimen (dose, route, duration), timing of exposure (e.g., gestational, lactational, pubertal, and adult), and timing of outcome evaluation. In brief, most studies were conducted in laboratory mice and rats, of which multiple strains were used. Most mammals were exposed to BPS orally by gavage, diet, or drinking water, although one study in rats and three in mice used subcutaneous injections, and zebrafish and guppies were exposed in water. Daily exposure ranged from 0.001 µg/kg to 1,000 mg/kg in mammals, and 0.1 µg/L to 200 µg/mL in fish.

BPS-related male reproductive toxicity outcomes reported in the reviewed studies included effects on organ weight or histology (of testis, epididymis, seminal vesicle, and the prostate, adrenal, pituitary, and thyroid glands), effects on sperm, the endocrine system, mammary gland development, and reproductive performance. All results presented in this section were reported as statistically significant by pairwise comparison with controls ($p \leq 0.05$) unless otherwise noted (e.g., $p \leq 0.05$ by trend test or non-significant).

Summaries of these studies are presented in Table 4.2.1 (mammals) and Table 4.2.2 (zebrafish), and the evidence of BPS effects on male reproduction from these studies is discussed below. Experiments that evaluated BPS as a component of a chemical mixture are not included in this review.

4.2.2 Organ Weight and Histopathology

Changes in male reproductive/endocrine organ weights may reflect underlying physiological alterations. Modulating factors may include hormonal or fluid imbalance, hemodynamic dysregulation, inflammation, cellular and tissue damage, or altered

spermatogenesis (Creasy and Chapin 2013). The effects of BPS on organ weight and histoarchitecture for testis, epididymis, prostate, seminal vesicle, adrenal gland, pituitary gland, and thyroid gland are described below. Unless otherwise noted, organ weights are reported as relative to body weight.

Testis

Weight

The effects of BPS on testicular weight were assessed in five mouse studies, 12 rat studies, and one study each for gerbils and hamsters, with mixed results.

Mouse

Two studies considered the effects of BPS after gestational exposure in CD-1 mice. The first study reported an increase in relative testicular weight and decreased body weight in the male great-grandoffspring (F3) of F0 dams treated during gestation with 0.5 µg/kg-day BPS (Shi et al. 2019). In the second study, no effects on testicular weight were observed in the male offspring of dams treated during gestation with up to 50 µg/kg-day BPS (Shi et al. 2018). Another study of CD-1 mice exposed from postnatal day (PND) 0 to 60 by subcutaneous dosing with up to 10 mg/kg BPS every 3 days reported no effects on testes weight (Shi et al. 2017).

Adult male Parkes mice treated daily with 150 mg/kg-day BPS for 28 days had decreased relative testicular weight and decreased body weight (Sahu and Verma 2023), while no effect on testicular weight was observed in adult male C57BL/6 mice treated with up to 200 mg/kg-day BPS for 28 days (Dai et al. 2021).

Rat

Twelve studies in rats reported on testis weight, with mixed findings.

BPS exposure during gestation increased testicular weight in Wistar rats treated with up to 40 µg/kg-day BPS from gestational day (GD) 4 to 21 (Molangiri et al. 2022), while no effects on offspring testicular weight were observed in three studies in SD rats treated during gestation (BASF 2019a; Kaimal et al. 2021; Ullah et al. 2019b). One of the studies in SD rats was an extended-one-generation reproductive toxicity (EOGRT) study, where F0 male and female SD rats were treated from premating, through mating, and post-mating and F1 rats were exposed during gestation, lactation, and postnatally; no effects on testis weight were observed in F0 or F1 males (BASF 2019a).

Exposure to BPS in postnatal and juvenile rats resulted in a non-significant decrease (-64 to -68%) in testicular weight in SD rats treated with up to 200 µg/kg-day BPS from PND 1 to 27 (John et al. 2019), while no effects on testicular weight were observed in

juvenile male Wistar rats treated with 50 µg/L from PND 21 for 10 weeks (Darghouthi et al. 2022), or SD rats (Ullah et al. 2018b; Ullah et al. 2021) treated with up to 50 µg/L BPS.

Increased relative testicular weight in adult SD rats at 1000 mg/kg-day was reported in Anonymous Study 16 as described in ECHA (2019), while decreased absolute testicular weight was observed in adult male Wistar rats dosed at 300 and 1,000/[600 from treatment day 70 onwards] mg/kg-day for 90 days, and increased relative testis weight was observed at 1,000/[600] mg/kg-day (BASF 2014). No effects on testicular weight were observed in adult SD rats treated with up to 300 mg/kg-day for up to 12 weeks in Anonymous Studies 12 and 14 as described in ECHA (2019).

Gerbil

No effects of BPS treatment on testis weight were reported in a study in adult gerbils treated with 40 µg/kg-day for 28 days (Silva et al. 2019).

Hamster

One study in adult male golden (Syrian) hamsters reported decreased relative testis weight with 75 mg/kg-day BPS exposure for 28 days (Kumar et al. 2020).

Histology

Histopathologic effects in testicular tissue were assessed in four mouse studies, 11 rat studies, and one hamster study.

This section and a later section on germ cell effects considers and evaluates the testicular tissues and cells involved in spermatogenesis.

The stages of spermatogenesis, known as the spermatogenic or seminiferous epithelial cycle are made up of specific patterns of germ cell types that provide sequential signatures of associations. Each stage of the epithelial cycle features a unique combination and proportion of germ cell types and cellular events. The number of stages differ by species with 14 stages in rats, 12 stages in mice, and six stages in humans. Stages I–VIII in rats and mice represent the early stages of spermatogenesis (e.g., spermatogonia, pachytene spermatocytes, round spermatids, and elongated spermatids) while Stages IX–XIV in rats and Stages IX–XII in mice represents late spermatogenesis (e.g., dividing spermatocytes, pachytene spermatocytes, and elongating spermatids). The appearance of the seminiferous tubule lumen, diameter, and epithelial height will also vary with stage. Stage identification in histologic examination is necessary to properly analyze whether sperm cell types and cell patterns in the seminiferous tubules are aberrantly absent or present (Creasy and Chapin 2013).

Mouse

All four mouse studies observed histopathological effects in the testis with BPS exposure. One lactational study in ICR mice reported a decrease in the height of seminiferous epithelia in middle-stage tubules at 21.6 µg/kg-day BPS in PND 90 male offspring of dams treated from PND 0-15 (Fenclová et al. 2022a).

Three studies in adult mice also reported histopathologic effects of BPS exposure in the testis. In male ICR mice treated for eight weeks, there was an increased incidence of testicular abnormalities as indicated by increased atypical residual bodies, increased (non-significant) vacuolization of germ layer cells, and increased (non-significant) incidence of enlarged multi-nuclear germ cells at 100 µg/kg-day, and decreased mature spermatozoa in the germ cell layer at 1 µg/kg-day BPS (Řimnáčová et al. 2020). In male C57BL/6 mice treated for 28 days, there were several histopathological findings in testicular tissue including irregular spermatogenic cell arrangements, more scattered spermatocytes, decreased epithelial height, and swollen and vacuolated mitochondria at all doses (range: 2 – 200 mg/kg-day); and autophagic vacuoles and condensed and marginated chromatin at 20 and 200 mg/kg-day (Dai et al. 2021). A study in male Parkes mice treated with 150 mg/kg-day BPS reported the presence of vacuoles, reduced germ cell count, germ cell loosening, lumen without sperm, decreased germinal epithelial height, seminiferous tubule diameter and area (Sahu and Verma 2023), however in this study, the seminiferous tubules were not appropriately staged for comparison, limiting interpretation of the histologic data.

Rat

Two gestational exposure studies and one early postnatal exposure study in rats reported BPS effects on testis histomorphology. In PND 90 male offspring of pregnant Wistar rats treated from GD 4 to 21, histopathological evaluation found widely spaced seminiferous tubules surrounded by fewer Leydig cells and congested interstitial arteries at 0.4 µg/kg-day. There were larger interstitial gaps in the seminiferous tubules with partly damaged interstitial Leydig cells at 4.0 µg/kg-day, and decreased diameter of seminiferous tubules at 0.4 and 4.0 µg/kg-day (Molangiri et al. 2022). In PND 80 male offspring of SD rats treated from GD 1 to 21, there was a decrease in seminiferous tubule area at 25 and 50 µg/L. Seminiferous tubule epithelial height was increased at 50 µg/L, and there were decreases in interstitial space, lumen, and seminiferous tubule diameter at 50 µg/L (Ullah et al. 2019b). However, the seminiferous tubules were not appropriately staged for comparison, limiting interpretation of the morphometric data. In PND 27 male SD rats administered a subcutaneous dose of BPS from PND 1 to 27, histopathological changes included altered seminiferous tubule appearance at 2 µg/kg-day and decreased tubular lumen diameter and epithelial height at 2 and 200 µg/kg-day. However, based on the histopathology images, data were not analyzed according to the

specific stages of the seminiferous tubules, and testicular tissues appear to be poorly processed for morphometric analysis (John et al. 2019). A prior review of the literature excluded this study, noting that these histological data were “poorly informative” (Beausoleil et al. 2022).

No histopathological effects were reported in F0 and F1 males in the EOGRT study described earlier, where F0 SD rats were treated from premating, through mating, and post-mating and F1 rats were exposed during gestation, lactation, and postnatally (BASF 2019a).

Three studies reported effects of BPS on testis histomorphology in rats first exposed as juveniles. One study in juvenile male SD rats treated from PND 23 for 48 weeks observed relatively alternated tubules with larger interstitial spaces, emptier lumen, and decreased epithelial height at 5 and 50 µg/L, as well as decreased seminiferous tubule area and diameter and increased interstitial area at 50 µg/L (Ullah et al. 2021). Another study by Ullah et al. (2018b) with a similar study design in juvenile male SD rats noted smaller seminiferous tubules with larger interstitial spaces and emptier lumen at 25 and 50 µg/L. Seminiferous epithelial height was decreased at 50 µg/L. While histological images indicate proper fixation of testicular tissues, seminiferous tubules were not appropriately staged for reliable comparison in this study, therefore caution should be taken in the interpretation of the histopathological findings (Ullah et al. 2018b). In the third study, juvenile male Wistar rats treated from PND 21 for 10 weeks exhibited altered testicular histoarchitecture, including clearer seminiferous tubule lumen (Darghouthi et al. 2022). However, testicular tissues appeared to be poorly fixed according to the histology images.

Three studies in adult male SD rats reported effects of BPS on testis histomorphology. In two studies in rats treated with up to 50 mg/kg-day BPS for 28 days (Ullah et al. 2018a; Ullah et al. 2016), the seminiferous epithelium appeared thin at all doses (Ullah et al. 2016), and testicular epithelial height was decreased at 25 and 50 µg/kg-day (Ullah et al. 2016) and at 50 mg/kg-day (Ullah et al. 2018a). Sperm was absent in the tubular lumen at 50 mg/kg-day (Ullah et al. 2018a). Ullah et al. (2016) did not report whether seminiferous tubules of the same stage were compared among the groups. Morphometric analysis was normal and there were no effects on the number of different cell types in the seminiferous tubules (Ullah et al. 2016). In Ullah et al. (2018a), the published histology images indicate that seminiferous tubules were not appropriately staged for comparison among the groups, and testicular tissues appeared to be poorly fixed and processed (Ullah et al. 2018a), poor informativeness of the histological data was also noted in a review (Beausoleil et al. 2022). In the third study in adult rats treated with 50 and 100 mg/kg-day BPS for 30 days (Wu et al. 2021), the transport of elongated spermatids and phagosomes across the epithelium was disrupted in stage

VIII-IX seminiferous tubules. Specifically, spermatids were deeply embedded in the epithelium instead of lining the tubular lumen to be released at stage VIII and exhibited loss of polarity; their heads were shifted ~90°–180° away from the basement membrane. Meanwhile, phagosomes were found near the tubular lumen instead of being transported to the basal epithelium for lysosomal degradation at stage IX (Wu et al. 2021). No histopathological findings were reported in adult male (PND 42) Wistar rats treated with up to 1000/[600] mg/kg-day BPS for 90 days (BASF 2014).

Hamster

One study in adult golden (Syrian) hamsters treated with 75 mg/kg-day BPS for 28 days reported marked testicular degeneration characterized by the presence of vacuoles, giant cells, Leydig cell atrophy, and empty lumen. There were also decreases in germinal epithelium height and seminiferous tubule diameter and area. However, the seminiferous tubules were not staged for reliable morphometric analysis and comparison. These effects did not occur in a group co-treated with a 10 mg/kg-alternate day melatonin injection (Kumar et al. 2020).

Epididymis

The epididymis is the site for spermatozoa maturation (e.g., gaining progressive motility and fertilization ability), storage, and transport. The early and late stages of sperm maturation, and the storage of functionally mature sperm occur in the caput, corpus, and caudal regions of the epididymis, respectively (Cornwall 2009). Eleven studies in rats assessed the effects of BPS on epididymis weight and/or histopathology.

Weight

BPS-mediated effects on epididymal weights were assessed in nine rat studies. Four studies in rats reported decreases in epididymis weight. In juvenile male SD rats, decreased relative epididymis weight was observed at 50 µg/L BPS in two studies (Ullah et al. 2018b; Ullah et al. 2021), while no effects on epididymis weight were observed in juvenile male Wistar rats treated with 50 µg/L BPS from PND 21 for 10 weeks (Darghouthi et al. 2022). A study in adult male Wistar rats observed decreased absolute and relative epididymis weight at 1,000/[600] mg/kg-day (BASF 2014). One study in adult male SD rats treated for 28 days reported a decrease in absolute epididymal weight with a decrease in body weight at 600 mg/kg-day (BASF 2020), while other studies in adult SD rats did not observe effects on epididymis weight. Specifically, no effects were reported in adult male SD rats treated with up to 100 mg/kg BPS for 28 days (Ullah et al. 2016) or 30 days (Wu et al. 2021), or with 10 to 300 mg/kg-day BPS for 45 days in Anonymous Study 12 as described in (ECHA 2019). In the EOGRT study, no effects on epididymal weight were observed in F0 adult male SD rats treated with up to 180 mg/kg-day BPS or in their male progeny (assessed at 13 or 19-25 weeks of age)

exposed during gestation, lactation, and the postnatal period. In addition, vas deferens weights were measured in F0 males, and in F1 males at 13 weeks of age, and no effects were observed in either group (BASF 2019a).

Histology

Six studies in rats assessed the effects of BPS on epididymis histomorphology and one study in juvenile male SD rats treated from PND 23 for 48 weeks reported a decrease in the tubular diameter of the caput region of the epididymis at 50 µg/L BPS (Ullah et al. 2021). One study in adult male SD rats treated for 28 days observed some empty lumen in epididymal sections at 25 and 50 µg/kg-day (Ullah et al. 2016). No histopathological effects were reported in PND 80 male offspring of SD rats administered 5 to 50 µg/L BPS from GD 1 to 21 (Ullah et al. 2019b), in F0 and F1 males in the EOGRT study (BASF 2019a), in adult male SD rats treated with 5 to 50 mg/kg-day BPS for 28 days (Ullah et al. 2018a), and in adult male Wistar rats treated with up to 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).

Seminal vesicle

The seminal vesicles and prostate are accessory sex glands in males. Secretions from these glands support sperm motility and viability. Semen volume is largely made up of seminal and prostatic fluid.

Weight

BPS-mediated effects on seminal vesicle weights were assessed in 11 studies in rats. Decreases in seminal vesicle weight were reported in six rat studies. In male SD rats exposed to BPS during gestation (GD 1-21) there was a decrease in absolute seminal vesicle weight with 50 µg/L BPS at PND 80 but not at PND 16 (Ullah et al. 2019b). In early postnatal SD rats treated with a subcutaneous injection of BPS from PND 1 to 27, there was a non-significant decrease (-74 and -94%) in absolute seminal vesicle weight at 2 and 200 µg/kg-day (John et al. 2019). There were no effects in the male offspring (16-24 weeks of age) of pregnant SD rats treated with 5 µg/kg-day BPS from GD 6-21 (Kaimal et al. 2021), and F1 male SD rats (13 or 19-25 weeks of age) exposed during gestation, lactation, and the postnatal period in the EOGRT study (BASF 2019a).

In juvenile male SD rats treated with 5 to 50 µg/L BPS from PND 23 for 48 weeks, there was a decrease in absolute and relative seminal vesicle weight (Ullah et al. 2018b; Ullah et al. 2021). No effects were reported in juvenile male Wistar rats treated with 50 µg/L BPS from PND 21 for 10 weeks (Darghouthi et al. 2022).

In adult male SD rats, there was a decrease in absolute seminal vesicle weight with no effect on body weight at 300 mg/kg-day BPS for 45 days in Anonymous Study 12 as

described (ECHA 2019), and at 600 mg/kg-day BPS for 28 days with decreased body weight (BASF 2020). No effects were reported in adult male SD rats treated with 30 to 300 mg/kg-day BPS for up to 12 weeks in Anonymous Study 14 as described in (ECHA 2019), in F0 male SD rats in the EOGRT study (BASF 2019a), and in adult male Wistar rats treated with up to 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).

Histology

There were no histopathological findings in seminal vesicles in the two rat studies that assessed histology. There were no findings in either the F0 or F1 males in the EOGRT study (BASF 2019a), or in adult male Wistar rats (PND 42) orally dosed with 100 to 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).

Prostate

The effects of BPS treatment on prostate weight were assessed in one mouse study, ten rat studies, and one study in gerbils.

Weight

Mouse

One study in adult male C57BL/6 mice treated for two months via a subcutaneous implant of 25 mg BPS with or without a co-implant of 25 mg testosterone reported increases in hemi-prostate, ventral prostate, and dorsolateral prostate weights (assumed absolute) in the BPS + testosterone group with no effect on body weight (Nguyen JL et al. 2022).

Rat

Four studies in rats reported decreases in prostate weight. In the EOGRT study, decreased absolute prostate weights at 60 and 180 mg/kg-day were reported in 13-week-old F1 male SD rat progeny, while no effects on prostate weight were reported for F1 male progeny at 19-25 weeks of age (BASF 2019a). In a second study, in male SD rats treated from PND 1 to 27 there was a non-significant decrease (-50% each) in absolute prostate weight at 2 µg/kg-day and 200 µg/kg-day on PND 27 (John et al. 2019). No effects on prostate weight were reported in SD rats exposed during gestation with 5 µg/kg-day BPS (Kaimal et al. 2021) or in PND 80 male offspring from dams exposed with up to 50 µg/L BPS (Ullah et al. 2019b).

Another study reported decreased absolute and relative prostate weights in juvenile Wistar males treated with BPS at 50 µg/L from PND 21 for 10 weeks (Darghouthi et al. 2022). No effects were reported in juvenile male SD rats treated from 0.5 to 50 µg/L BPS and from PND 23 for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021). Decreased

absolute prostate weight, accompanied by a decrease in body weight was reported at 600 mg/kg-day in adult male SD treated with BPS for 28 days (BASF 2020). In the EOGRT study, no effects on prostate weight were reported for the F0 SD males (BASF 2019a).

No effects on prostate weight were observed in adult SD rats treated with up to 300 mg/kg-day for up to 12 weeks in Anonymous Studies 12 and 14 as described in ECHA (2019).

Gerbil

In a study in adult gerbils, no effects of BPS treatment on prostate weight were reported in males treated with 40 µg/kg-day BPS for 28 days. (Silva et al. 2019).

Histology

Gerbil

In the Silva et al. (2019) study in adult male gerbils, there were structural and histopathologic findings in the prostatic complex (urethral segment and ventral, dorsolateral and dorsal prostate lobes) in the treated group. In the ventral prostate, there was a decreased tissue relative frequency of the lumen and increased tissue relative frequency of epithelium, muscular and non-muscular stroma, and hyperplasia (Silva et al. 2019).

Adrenal

The adrenal cortex produces steroid hormones that are essential for male reproductive development and function including dehydroepiandrosterone, androstenedione, testosterone, and glucocorticoids.

Weight

Five studies in rats assessed the effects of BPS treatment on adrenal gland weight, and four of the studies reported increases. In the EOGRT study by BASF (2019a), increased adrenal gland weights were found in F0 and F1 males. In F0 males, both absolute and relative adrenal gland weights were increased at 60 and 180 mg/kg-day, and F1 males had increased absolute and/or relative adrenal gland weights at 60 or 180 mg/kg-day (BASF 2019a). Increased absolute and relative adrenal gland weights were observed in adult male Wistar rats at 1,000/[600] mg/kg-day (BASF 2014), and in adult male SD rats at 1,000 mg/kg-day in Anonymous Study 16, as described in ECHA (2019). The fourth study in adult male SD rats treated with BPS for 28 days reported non-significant increases (+18 and +39%) in relative adrenal weights at 300 and 600 mg/kg-day (BASF 2020).

No effects on adrenal weight were observed in adult SD rats treated with up to 300 mg/kg-day for up to 12 weeks in Anonymous Study 14, as described in ECHA (2019).

Histology

Histopathological effects in the adrenal gland were assessed in four rat studies and adrenal hypertrophy and/or hyperplasia were observed in three, at doses of 1,000/[600] mg/kg-day (BASF 2014), 600 mg/kg-day (BASF 2020); and 1,000 mg/kg-day in Anonymous Study 16, as described in ECHA (2019). No effects were reported in F0 and F1 males dosed with 20 to 180 mg/kg-day BPS in the EOGRT study (BASF 2019a).

Pituitary

Male reproductive function is regulated by the hypothalamus-pituitary-gonadal axis. The pituitary gland, in response to hypothalamic signals, produces luteinizing hormone (LH), which stimulates Leydig cells to produce testosterone and other androgens, and follicle stimulating hormone (FSH), which acts on Sertoli cells to facilitate and support spermatogenesis.

Weight

Two rat studies assessed the effects of BPS on pituitary weight. One study reported an increase in relative pituitary weight at 300 mg/kg-day BPS in adult male SD rats treated with BPS for 45 days in Anonymous Study 12, as described in ECHA (2019), while the EOGRT study found no effects on absolute or relative pituitary weight in F0 and F1 males dosed with 20 to 180 mg/kg-day BPS (BASF 2019a).

Histology

The EOGRT study found no histopathological effects in F0 and F1 males dosed with 20 to 180 mg/kg-day BPS (BASF 2019a). The second rat study that assessed histology (weight not evaluated) in the pituitary also did not report findings in adult male Wistar rats treated with up to 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).

Thyroid gland

Thyroid hormones have an important role in testicular development, spermatogenesis, and sex hormone metabolism. Dysregulated thyroid gland function has been associated with altered sperm parameters, including concentration, motility, and morphology.

Weight

Three studies in rats assessed BPS effects on thyroid gland weight. One study in adult male Wistar rats reported increased relative thyroid gland weight in the 1,000/[600] mg/kg-day dose group after a 90-day treatment (BASF 2014), and in the other two

studies, no effects were reported in F0 and F1 males in the EOGRT study (BASF 2019a), or in adult male SD rats treated with up to 600 mg/kg-day BPS for 28 days (BASF 2020).

Histology

Two studies in mice assessed and reported histopathological effects of BPS on thyroid gland histology. In adult male C57BL/6 mice treated with BPS for five weeks, there was an increase in the height of the thyroid follicular epithelium at 20 mg/kg-day (Hu et al. 2023). In adult Parkes mice, there was disordered histoarchitecture in the thyroid gland with epithelial cell thinning, presence of vacuoles, and decreased follicular epithelial cell height after exposure to 150 mg/kg-day BPS for 28 days (Sahu and Verma 2023).

Three studies in rats assessed and reported no histopathological effects of BPS on thyroid gland histology. Specifically, no effects were reported in F0 and F1 male SD rats in the EOGRT study (BASF 2019a), in adult male SD rats treated with up to 600 mg/kg-day BPS for 28 days (BASF 2020), and in adult male Wistar rats when treated with up to 1,000/[600] mg/kg-day for 90 days (BASF 2014).

Gonadosomatic index

BPS effects on gonadosomatic index (GSI) were assessed in four rat studies where exposures began at or soon after weaning (Darghouthi et al. 2022; Ullah et al. 2018b; Ullah et al. 2021) or during puberty (Jeminiwa et al. 2021) and four zebrafish studies (Ji et al. 2013; Naderi et al. 2014; Park et al. 2022). Decreases in GSI were reported in two studies in male SD rats treated for 48 weeks starting on PND 23 with 50 µg/L BPS (Ullah et al. 2018b; Ullah et al. 2021), while no effects on GSI was reported in male Wistar rats treated with 50 µg/L BPS, starting on PND 21, for 10 weeks (Darghouthi et al. 2022). There were also no effects on GSI observed in a study that treated prepubertal (PND 21) or pubertal (PND 35) male Long-Evans rats with 5 µg/L BPS via drinking water for 14 days (Jeminiwa et al. 2021).

In three zebrafish studies, decreased GSI was observed in male zebrafish exposed to 10 and 100 µg/L BPS from 2-75 days post fertilization (dpf) (Naderi et al. 2014), in adult males treated with 50 µg/L BPS for 21 days (Ji et al. 2013), and in the male grandoffspring (F2) of F0 zebrafish exposed to 1 and 100 µg/L BPS from 3 hpf to 120 dpf, while no effects on GSI were observed in their offspring (F1 males) (Hao et al. 2022). No effects were observed in adult male zebrafish exposed up to 200 µg/mL BPS for 21 days (Park et al. 2022).

4.2.3 Effects on Sperm

Several studies reported adverse effects of BPS treatment on sperm, including effects on germ cell development and commonly measured sperm parameters (e.g., count, motility).

Spermatogenesis

The effects of BPS exposure on spermatogenesis were assessed in six mouse studies, five rat studies, one hamster study, and one zebrafish study. All studies except for one mouse study reported effects.

Mouse

Two studies in mice exposed to BPS during gestation observed effects on germ cell development. In one study, pregnant CD-1 mice were treated from GD 11 until birth. In PND 12 male progeny, there was no effect on germ cell numbers per tubule, however, at all doses (0.5, 20, 50 µg/kg-day), there were increases in TUNEL-positive cells and TUNEL-positive germ cells per tubule, indicating germ cell apoptosis (see Section 4.3) (Shi et al. 2018). In PND 12 male mice, germ cells located closer to the lumen of seminiferous tubules are generally primary spermatocytes (Saitou and Yamaji 2012), therefore, the TUNEL-positive germ cells in BPS-treated mice on PND 12 are most likely spermatocytes. This effect was not observed on PND 60. However, on PND 60 there was a decreased percentage of tubules in stage VII and increased percentage of tubules in stage VIII at 50 µg/kg-day (Shi et al. 2018). In another study, pregnant CD-1 mice (F0) were treated with BPS from GD 7 until birth, and their great-grandoffspring (F3 males) were evaluated. On PND 60, F3 males had an increased proportion of seminiferous tubules in stages I–VI and a decreased percentage at stage IX at 0.5 µg/kg-day (Shi et al. 2019).

One study in male 129S1/SvImJ mice orally administered 20 µg/kg-day BPS from PND 1 to 8 reported decreased meiotic recombination levels indicated by a decrease in the mean number of MLH1 foci in pachytene spermatocytes, and a non-significant increase in the frequency of pachytene spermatocytes with at least one synaptonemal complex lacking an MLH1 focus (Horan et al. 2018). One study in male CD-1 mice administered a subcutaneous injection of BPS every 3 days from PND 0 to 60 found an increased proportion of tubules in stage VII and a non-significant decrease of tubules in stage VIII at 10,000 µg/kg on PND 60 (Shi et al. 2017). In adult male C57BL/6 mice treated with BPS for 28 days, there was a decreased percentage of seminiferous tubules in stages VII-VIII at all BPS doses, with a shift toward stages I-VI at 200,000 µg/kg-day and stages IX-XII at 2,000 and 20,000 µg/kg-day (Dai et al. 2021). One study in adult ICR

mice reported no significant effects on spermatogenesis exposed up to 100 µg/kg-day BPS for eight weeks (Řimnáčová et al. 2020).

Rat

In rats, one study in the male progeny of pregnant SD females treated from GD 1 to 21, reported decreased numbers of spermatogonia, spermatocytes, and spermatids in the seminiferous tubules on PND 80 at 50 µg/L (Ullah et al. 2019b).

Three studies evaluated the effects on germ cell development in male juvenile rats after BPS exposure for 10- or 48-weeks reporting effects at several stages of spermatogenesis. Two of these studies treated male SD rats with BPS from PND 23 for 48 weeks. This treatment resulted in cellular arrest at the spermatogonial stage and round spermatids at 25 and 50 µg/L (Ullah et al. 2018b), and at 5 and 50 µg/L (Ullah et al. 2021). There were fewer spermatogonia, spermatocytes, and spermatids per cross-section of seminiferous tubules at 50 µg/L (Ullah et al. 2018b), and a decreased number of spermatocytes and spermatids at 5 and 50 µg/L (Ullah et al. 2021). In the third study, juvenile male Wistar rats treated with 50 µg/L BPS from PND 21 for 10 weeks had hypospermatogenesis, characterized by decreased germ cell and spermatozoa numbers in the seminiferous tubule walls and decreased numbers of spermatogonia, spermatocytes I & II, and spermatids. There were also decreased numbers and cytoplasmic vacuolization of Sertoli cells (Darghouthi et al. 2022).

One study in adult rats reported effects of BPS on germ cell development. Treatment with 1 to 50 µg/kg-day BPS for 28 days in adult male SD rats resulted in a relatively dispersed population of secondary spermatocytes at all BPS doses, and there were a few tubules containing few to no elongated spermatids in the seminiferous tubule lumen, at 25 and 50 µg/kg-day (Ullah et al. 2016).

Hamster

In adult male golden (Syrian) hamsters treated with BPS for 28 days, there was a decrease in germ cell number. These effects did not occur in a group co-treated with a 10 mg/kg-alternate day melatonin injection (Kumar et al. 2020).

Zebrafish

In the offspring (F1 males; 120 dpf) of F0 zebrafish exposed to 1 and 100 µg/L BPS from 3 hpf to 120 dpf, there was an increased number of spermatogonia and spermatocytes with a decreased number of spermatozoa in the lumen. In the grandoffspring (F2 males; 120 dpf), sperm pyknosis and increased number of spermatogonia and spermatocytes were observed at 1 µg/L, decreased number of spermatozoa was observed at 1 and 100 µg/L, and spermatid leakage, interstitial cell

loss, and increased proportion of spermatogonia and spermatids was observed at 100 µg/L (Hao et al. 2022).

Sperm parameters

Seven studies in mice, eight studies in rats, and one study each in hamsters and zebrafish assessed the effects of BPS on sperm count, daily sperm production, and/or morphology.

Sperm count

Five studies in mice, five studies in rats, and the hamster and zebrafish studies reported decreases in sperm count with BPS exposure.

Mouse

Decreased sperm count in mice was observed at different times of exposure: gestational (Shi et al. 2018; Shi et al. 2019), juvenile (Shi et al. 2017), and adults (Dai et al. 2021; Sahu and Verma 2023), and no effects on sperm count were reported in one lactational exposure study (Fenclová et al. 2022b) and one study in adult mice (Řimnáčová et al. 2020).

In two studies in CD-1 mice by Shi et al., maternal (F0) BPS exposure decreased sperm count in F1 male progeny at 0.5 and 20 µg/kg-day (Shi et al. 2018), and in F3 great-grandoffspring at 0.5 and 50 µg/kg-day (Shi et al. 2019). Juvenile CD-1 mice treated with a subcutaneous injection of BPS every three days from PND 0 to 60 had decreased sperm count at 0.05 and 10 mg/kg on PND 60, and at 0.05 mg/kg on PND 90 (Shi et al. 2017). There were no effects on sperm count in adult mice exposed during lactation with up to 20 µg/kg-day BPS (Fenclová et al. 2022b).

Two studies in adult mice reported decreased sperm count after exposure to BPS for 28 days. The first study in adult Parkes mice treated with 150 mg/kg-day BPS reported decreased sperm count and viability (Sahu and Verma 2023). The second study in adult C57BL/6 mice observed decreases in the caput/corpus epididymis sperm count at 200 mg/kg-day, and cauda epididymis sperm count at 20 and 200 mg/kg-day (Dai et al. 2021). There were no effects on sperm count in adult ICR mice treated with up to 100 µg/kg-day BPS for 8 weeks (Řimnáčová et al. 2020).

Rat

Five rat studies reported decreased sperm count with exposure occurring at different life stages.

BPS exposure during gestation in SD rats from GD 1-21 resulted in decreased caput/corpus epididymis sperm number at 25 and 50 µg/L in the male progeny on PND 80 (Ullah et al. 2019b). Two studies in juvenile SD rats reported decreases in caput epididymis sperm number at 5, 25 and 50 µg/L, and decreased cauda epididymis sperm number at 50 µg/L for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021). One study in juvenile Wistar rats reported decreases in sperm count and viability at 50 µg/L for ten weeks (Darghouthi et al. 2022). One study in adult SD rats reported decreased sperm count and cauda epididymis sperm number in rats treated with 50 and 100 mg/kg-day BPS for 30 days (Wu et al. 2021).

There were no effects on sperm head counts in the testis and cauda epididymis in F0 and F1 males in the EOGRT study (BASF 2019a)

Hamster

One study in adult golden (Syrian) hamsters reported decreases in sperm count and viability with 75 mg/kg-day BPS. These effects were not seen in the group co-treated with 10 mg/kg-alternate day melatonin for 28 days (Kumar et al. 2020).

Zebrafish

One study in zebrafish treated with BPS from 2-75 dpf reported decreased sperm counts at 10 and 100 µg/L (Naderi et al. 2014).

Daily sperm production

Rat

Five rat studies reported decreased daily sperm production with exposure occurring at different life stages.

BPS exposure during gestation in SD rats from GD 1-21 resulted in decreased daily sperm production at 50 µg/L in the male progeny on PND 80 (Ullah et al. 2019b). Two studies in juvenile SD rats reported decreases in daily sperm production at 50 µg/L (Ullah et al. 2018b; Ullah et al. 2021). Two studies in adult SD rats exposed for 28 days reported a decrease in daily sperm production at 50 µg/kg-day BPS (Ullah et al. 2017), and at 50 mg/kg-day BPS (Ullah et al. 2019a).

Sperm morphology

Mouse

Two studies in adult mice reported morphological abnormalities in sperm. In adult Parkes mice treated with 150 mg/kg-day BPS for 28 days, there was a decrease in the percentage of normal sperm (Sahu and Verma 2023). In the second study, there was an increased percentage of morphological abnormalities in sperm characterized by the presence of banana-shaped, chubby head, double-head, and no-hook morphologies in adult C57BL/6 mice after exposure to 200 mg/kg-day BPS (Dai et al. 2021).

Rat

One study in juvenile Wistar rats reported a decreased percentage of cells with normal morphology at 50 µg/L (Darghouthi et al. 2022), and in adult SD rats, there was an increased percentage of sperm with abnormalities in rats treated with 50 and 100 mg/kg-day BPS for 30 days (Wu et al. 2021). There were no effects on sperm morphology in F0 and F1 males in the EOGRT study (BASF 2019a)

Sperm motility

Sperm motility was assessed in seven studies each in mice and rats; of which six mouse and five rat studies reported decreased sperm motility.

Mouse

Two studies in CD-1 mice reported decreased sperm motility in males after BPS exposure to 0.5 µg/kg-day during gestation. In the first study, decreased sperm motility was observed in PND 60 male progeny of F0 dams treated from GD 11 until birth (Shi et al. 2018). In the second study, a transgenerational effect of decreased sperm motility was observed in the male great-grandoffspring (F3) of F0 dams treated with BPS from GD 7 until birth (Shi et al. 2019). There were no effects on sperm motility in 14-week-old male ICR mice exposed during lactation (PND 0 to 15) with up to 20 µg/kg-day BPS (Fenclová et al. 2022b). One study in adult CD-1 mice reported reduced sperm motility in PND 60 male CD-1 mice subcutaneously administered 0.05 and 10 mg/kg BPS every three days from PND 0 to 60, with no effects on sperm motility observed on PND 90 (Shi et al. 2017).

Three studies in adult mice reported decreased sperm motility: in ICR mice exposed to BPS at 0.001 µg/kg-day for 8 weeks (Řimnáčová et al. 2020); in Parkes mice treated with 150 mg/kg-day BPS for 28 days (Sahu and Verma 2023); and in C57BL/6 mice after exposure to 20 and 200 mg/kg-day BPS for 28 days (Dai et al. 2021).

Rat

Five studies in rats reported decreased sperm motility: in SD rats exposed to 25 and 50 µg/L BPS during gestation from GD 1-21 (Ullah et al. 2019b); in two studies in juvenile rats treated from PND 23 for 48 weeks at 25 and 50 µg/L (Ullah et al. 2018b), and 50 µg/L (Ullah et al. 2021), and one study in juvenile Wistar rats treated with 50 µg/L from PND 21 for 10 weeks (Darghouthi et al. 2022). In the EOGRT study, parental SD rats (F0) had a reduction in the percentage of motile sperm at all doses (20, 60, and 180 mg/kg-day) with no effects reported in their male progeny (BASF 2019a). No effects on sperm motility were reported in two studies in adult male SD rats (PND 70-80) treated with 0.001 mg/kg-day to 50 mg/kg-day BPS for 28 days (Ullah et al. 2019a; Ullah et al. 2017).

4.2.4 Endocrine Effects

To evaluate the effects of BPS on the endocrine system as it relates to male reproductive function, several studies assessed hormone levels and gene or protein expression of endocrine-related factors. Other studies assessed the effect of BPS exposure on biomarkers of endocrine disruption such as anogenital distance.

Hormone levels and gene/protein expression of endocrine-related factors

Effects of BPS on hormone levels were reported in various body fluids and tissues in studies conducted in mice, rats, hamsters, zebrafish and included altered levels of gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH); and steroid hormones: androgens (testosterone), estrogens (estradiol, E2); and thyroid hormones. Effects of BPS on mRNA and/or protein expression of endocrine-related factors including steroidogenic enzymes and hormone receptors for gonadotropins (LHR, FSHR), androgen (AR), estrogen (ER), and thyrotropin or thyroid-stimulating hormone receptor (TSHR) were also reported in studies conducted in mice, rats, hamsters, gerbils, and zebrafish. These findings are presented in more detail in section 4.3 Mechanistic considerations and other data relevant to male reproductive toxicity, in subsection 4.3.3 Effects on the endocrine system.

Anogenital distance

Anogenital distance (AGD) is a classic marker of developmental endocrine disruption. It may be influenced by both gestational and early postnatal androgen or antiandrogen effects (Thankamony et al. 2009). AGD plasticity in adulthood has also been observed (Mitchell et al. 2015). AGD has been found to be associated with male fertility in humans (Foresta et al. 2018).

Two studies in mice and five studies in rats assessed the effects of BPS on AGD. One mouse study and two rat studies reported effects, and one mouse study and three rat studies reported no effects on AGD.

Mouse

In the male progeny of lactating ICR mice administered 0, 0.375 or 37.5 ng/mL (equivalent to 0, 0.2, or 20 µg/kg-day) BPS in drinking water from PND 0-15, there was a decrease in AGD at the low dose and a non-significant increase at the high dose. The authors did not report when AGD was measured (presumed to be PND 21 from other data in the report) (Fenclová et al. 2022b). There were no observed effects on anogenital index (AGD divided by body weight) in the male offspring (PND 31) of female CD-1 mice treated with 2, 200, or 2,000 µg/kg-day from GD 9 to PND 20 (Kolla and Vandenberg 2019)

Rat

A decrease in AGD was reported in two studies in rats exposed during gestation and/or lactation. In the male progeny of Wistar rats treated with BPS at 50 µg/kg-day or 20,000 µg/kg-day from GD 6 to PND 21, there was a decrease in relative AGD at both doses assessed on PND 21 (Morimoto et al. 2022). A second study in Wistar rats, reported no effects on AGD in PND 90 male offspring of pregnant rats treated up to 40 µg/kg-day BPS from GD 4 to 21 (Molangiri et al. 2022). Decreased AGD was reported in PND 1 male offspring of pregnant SD rats orally administered 5 µg/kg-day BPS from GD 6-21 (Kaimal et al. 2021). No effects on AGD were observed in the EOGRT study on PND 1 in F1 male progeny after gestational exposure with up to 180 mg/kg-day BPS (BASF 2019a), or in the male progeny (PND 1) of pregnant SD rats administered 5 to 50 µg/L (estimated to be 0.9 to 9.0 µg/kg-day) BPS from GD 1 to 21 (Ullah et al. 2019b).

4.2.5 Mammary Gland Development

The effects of BPS exposure on male mammary gland histopathology were assessed in one mouse study and four rat studies, and all studies reported effects.

Mouse

In male offspring of female CD-1 mice exposed from GD 9 up through PND 20, there were mammary gland effects at 200 µg/kg-day including increased space between cells consistent with the earliest stages of lumen formation on GD 16, and a decrease in ductal tree size in the left mammary gland on PND 24 at 200 µg/kg-day with no effect in the right mammary gland, indicating suppressed growth of the ductal tree prior to puberty. At 9 weeks of age (post puberty), there were increases in right epithelial trees at 2 and 200 µg/kg-day and left epithelial trees at 200 µg/kg-day. It has previously been shown that the right mammary epithelial tree of male mice is typically larger than the left

mammary epithelial tree, and the right glands are more sensitive to either perinatal or peripubertal exposures to estrogens (Kolla et al. 2019).

Rat

Three studies in adult male rats reported atrophy (diffuse or multifocal) of the mammary gland after BPS exposure. Diffuse atrophy was observed in SD rats exposed to BPS for up to 12 weeks at 300 mg/kg-day in Anonymous Study 14, as described in ECHA (2019), and after 28 days treatment at 600 mg/kg-day (BASF 2020). Multifocal atrophy was reported in the mammary glands of adult male Wistar rats exposed at 300 and 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).

In the EOGRT study in SD rats, there was an increased incidence of multifocal atrophy of the mammary gland in F1 males (13 weeks old; Cohort 1A) at 180 mg/kg-day along with multifocal atrophy of the mammary gland fat pad at 180 mg/kg-day. There were no effects in the other cohort of F1 males (19-25 weeks of age; Cohort 1B) (BASF 2019a).

Two rat studies reported no effects of BPS on nipple/areola retention in male SD rats. The first assessed PND 14 males exposed to 5-25 µg/L BPS throughout gestation (Ullah et al. 2019b), and the second study assessed F1 and F2 males (progeny of Cohort 1B) in the EOGRT study (BASF 2019a).

4.2.6 Reproductive Performance

Two studies in mice evaluated the effects of BPS treatment on male reproductive performance. In male CD-1 mice exposed to 0.05 or 10 mg/kg BPS every 3 days from PND 0 to 60 and then mated to untreated female mice, there was a non-significant increase in the number of days to successful mating at both doses, and there were no effects on pregnancy rate or litter size (Shi et al. 2017). The second study treated female ICR mice to 0.2 or 20 µg/kg-day BPS during lactation (PND 0-15). The BPS-exposed males were mated to untreated female mice, and the zygotes were flushed and cultured in the blastocyst stage. There was a decrease in zygote cleavage rate at 20 µg/kg-day and a decrease in the number of blastomeres per blastocyst (an indicator of embryo development) at both doses, with no effect on pregnancy rate, fertilization rate, or blastocyst rate (Fenclová et al. 2022b).

Additional studies evaluating the effects of BPS on reproductive performance treated both males and females, therefore it is not possible to determine whether any effects observed (i.e., implantation loss, sex ratio) were due to impaired female or male reproductive function, paternal genetic damage, or epigenetic modifications, and/or a combined effect from both parents. Brief summaries of these studies, three in rats and two in zebrafish, are included for completeness:

- In male and female SD rats treated with BPS for six weeks of pre-mating through four weeks post-mating there was a decrease in the number of implantation sites, an increase in post-implantation loss, and a decrease in the mean number of pups delivered at 300 mg/kg-day (Anonymous Study 14, as described in ECHA 2019).
- In male and female SD rats treated for a total of 40-46 days (from premating), there were no effects on F0 copulation index, but there was a decrease in fertility index at 300 mg/kg-day (Anonymous Study 12, as described in ECHA 2019).
- In the EOGRT study, F0 male and female SD rats were treated from premating, to mating and post-mating, and increased post-implantation loss was reported at both 60 and 180 mg/kg-day. The total number of stillborn F1 pups was increased and the total number of liveborn F1 pups was decreased at 180 mg/kg-day. There was a decrease in the number of F2 pups delivered to F1 parents exposed to 180 mg/kg-day BPS during gestation, lactation, and 15-16 weeks post-weaning (BASF 2019a).
- In zebrafish exposed to BPS from 2-75 dpf, there was an increase in the female/male sex ratio at 10 and 100 µg/L with a decreased survival rate at 100 µg/L. After spawning of the F0 generation, there were decreases in egg count and hatching rate, and an increased time to hatching at 10 and 100 µg/L (Naderi et al. 2014).
- In zebrafish exposed from 3 hpf to 120 dpf, there was an increase in the female/male sex ratio in the F0 generation at 100 µg/L, and a decreased total number of eggs produced in the F1 generation at 100 µg/L. In the F2 generation, there was an increase in the gamete fertilization rate at 1 µg/L. There was no effect on gamete fertilization rate in the F3 generation (Hao et al. 2022).

Table 4.2.1 BPS: Evidence on the male reproductive toxicity in studies in mammals

Study Design	Outcomes assessed	Major Findings
BASF 2014 Male Wistar rats, 42 days old at the start of treatment,10 rats per group. Treatment: Bisphenol S (BPS) (99.4% purity) in 1% carboxymethylcellulose (CMC) at 0, 100, 300 or 1000 milligrams per kilogram per day (mg/kg-day) by gavage for 90 days. Animals were sacrificed after 16 hours of fasting. Due to severe reductions in body weight in animals treated with 1000 mg/kg-day on day 63 compared to control (-20%), animals in this test group were treated at 600 mg/kg-day from day 70 onwards.	Organ weight. Histopathology. Cholesterol levels (matrix not reported) . General toxicity: body weight.	Organ weight: Decreased absolute brain weight at 100 (-5%), 300 (-6%), and 1000/[600] (-6%) mg/kg-day. Increased absolute (+40%) and relative adrenal gland weight (+77%) at 1000/[600] mg/kg-day. Decreased absolute weight (-11%) and increased relative weight (+12%) of epididymides at 1000/[600] mg/kg-day. Decreased absolute testes weight (-7 to -8%) at ≥300 mg/kg-day and increased relative testes weight (+16%) at 1000/[600] mg/kg-day. Increased relative thyroid gland weight (+16%) at 1000/[600] mg/kg-day. Histopathology: Hypertrophy and hyperplasia present in the adrenal cortex in 8/10 males at 1000/[600] mg/kg-day vs. 0/10 male controls. Multifocal atrophy present in the mammary gland of 7/10 males at 300 mg/kg-day and 10/10 males at 1000/[600] mg/kg-day vs. 0/10 male controls. Decreased cholesterol levels at 300 (-34%) and 1000/[600] (-44%) mg/kg-day. General toxicity: Lower mean body weight at 300 mg/kg-day
BASF 2019a Adult male and female Sprague-Dawley (SD) Crl:CD rats, 24 rats/sex/dose. Treatment: BPS (99.9% purity) in 0.5% CMC suspension at doses of 0, 20, 60 or 180 mg/kg-day by gavage for 10 weeks premating, up to 2 weeks mating, and a maximum of 6 weeks post-mating. F0 males were mated with treated females from the same dose group after 10 weeks of treatment. F0 males and females were sacrificed before and after weaning of the F1 pups, respectively. F1 rats (n = 74 animals/sex/group) were randomly placed into cohorts after weaning: Cohort 1A: 20 F1 male rats per dose group treated by gavage for 10 weeks post-weaning and sacrificed at 13 weeks old. Cohort 1B: 24 F1 male rats per dose group were selected to produce F2 pups and treated by gavage for 10 weeks post-weaning/premating, up to 2 weeks mating, and a maximum of 4 weeks post-mating. Other F1 cohorts selected for neurotoxicity and immunotoxicity assays (not reported here).	Sperm parameters. Organ weight. Histopathology. Mating performance (mating index and fertility index). Reproductive performance. Sexual maturation (F1): preputial separation from PND 38 Anogenital distance for F1 progeny on PND 1. General toxicity: body weight.	F0 males: Sperm parameters: Decreases in percentage of motile sperm at all doses 20 (-5%), 60 (-3%), and 180 (-2%) mg/kg-day. Organ weight: Increased absolute and relative adrenal gland weight at 60 (+9% each) and 180 (+12-16%) mg/kg-day. Histopathology: No relevant effects. Mating performance: No effects. Reproductive performance: Increased post-implantation loss at 20 (non-significant [NS]; +90%), 60 (+203%) and 180 (+239%) mg/kg-day. Increased stillborn F1 pups at 20 (NS; +183%), 60 (NS; +50%), and 180 (+350%) mg/kg-day. Decreased total number of liveborn F1 pups at 180 mg/kg-day (-16%). Sexual maturation in F1 males: No effect on timing of preputial separation General toxicity: No relevant effects. F1 pup data: No relevant effects on any evaluated parameters. **F1 Cohort 1A males (13 weeks old):** No effect on sperm parameters. Organ weight: Decreased absolute prostate weight at 60 (-9%) and 180 (-10%) mg/kg-day. Increased relative adrenal gland weight at 180 mg/kg-day (+13%).

Study Design	Outcomes assessed	Major Findings
		Histopathology: Increased multifocal atrophy of mammary glands in 7/20 males vs.in 1/20 male controls and of mammary gland fat pad in 7/10 males vs. in 1/10 male controls, at 180 mg/kg-day. General toxicity: No relevant effects. **F1 Cohort 1B males (19-25 weeks old):** Reproductive performance: Increased % post-implantation loss at 180 mg/kg-day (+284%) and complete litter loss in two females at 180 mg/kg-day. Decreased mean pups delivered at 180 mg/kg-day (-20%). F2 pup data: No relevant effects. Organ weight: Increased absolute and relative adrenal gland weights at 60 (+13% and +11%, respectively) and 180 (+8% [NS] and +13%, respectively) mg/kg-day. Histopathology: No relevant effects. General toxicity: No relevant effects.
BASF 2020 Adult male SD rats, 5 rats per group. Treatment: BPS (99.9% purity) in 0.5% CMC by gavage at 0, 100, 300 or 600 mg/kg-day BPS for a total of 28 days. Animals were sacrificed on Day 29; clinical evaluations were performed prior to sacrifice.	Organ weight. Histopathology. Cholesterol levels (matrix not reported) General toxicity: body weight.	Organ weight: Decreased absolute weights of epididymides (-15%), prostate (-25%), and seminal vesicles (-25%) at 600 mg/kg-day. No effect on testis or thyroid weight. Decreased terminal body weight at 600 mg/kg-day (-12%). Increased (NS) relative adrenal weight at 300 (+18%) and 600 (+39%) mg/kg-day. Histopathology: Hypertrophy/hyperplasia observed in adrenal cortex in 3/5 males at 600 mg/kg-day vs. in 0/5 male controls. Diffuse atrophy observed in mammary glands in 3/5 males at 300 and 4/5 males at 600 mg/kg-day vs. 0/5 male controls. Decreased cholesterol at 300 (-47%) and 600 (-36%) mg/kg-day. General toxicity: Lower body weight on days 7, 14, 21 at 600 mg/kg-day (-8-12%) but were comparable to control by Day 28. Reduced body weight from Days 0 to 7 (-96%) and the overall treatment period (Days 0 to 28; -52%) at 600 mg/kg-day. Reduced body weight from day 7 to 14 at ≥ 300 mg/kg-day (-47-52%).
Dai et al. 2021 Male C57BL/6 mice, 8 weeks old; 10 mice per group. Treatment: BPS (purity ≥99%) in sesame oil, daily oral gavage at doses of 0, 2, 20, or 200 mg/kg-day for 28 consecutive days. All animals were sacrificed on day 29 for analyses.	Testis weight (with epididymides; absolute). Sperm parameters (count, motility, and deformity rate). Testis histopathology. Oxidative stress in testicular tissue (superoxide dismutase [SOD] and glutathione [GSH] peroxidase (GPX) activities, GSH and lipid peroxidation (LPO) by	No effect on absolute testis weight (with epididymis). Sperm parameters: Decreased cauda sperm count at 20 and 200 mg/kg-day (-19% and -40%, respectively), Decreased sperm motility at 20 and 200 mg/kg-day (-24% and -38%, respectively), Decreased caput/corpus sperm count at 200 mg/kg/day (-34%), and

Study Design	Outcomes assessed	Major Findings
	malondialdehyde [MDA, indicator of LPO activity] levels. Spermatogenic cell apoptosis (Terminal deoxynucleotidyl transferase dUTP Nick-End Labeling [TUNEL] Assay). Expression of apoptosis signaling pathway proteins in testis: BCL2, BAX, cleaved caspase-8 (CASP8), cleaved caspase-9 (CASP9), cleaved caspase-3 (CASP3), FAS, and FASL. General toxicity: body weight.	Increased sperm with abnormal morphology at 200 mg/kg-day (+108%) indicated by the presence of banana-shaped, chubby head, double head, and no-hook morphologies. Testicular histopathology: Irregular spermatogenic cell arrangements at ≥2 mg/kg-day, Spermatocytes were more scattered ≥2 mg/kg-day, Decreased epithelial height (-11%, -21%, and -24%, respectively) with few elongated spermatids present in the lumen of tubules at ≥2 mg/kg-day, Decreased percentage of seminiferous tubules in stages VII-VIII at ≥2 mg/kg-day (-22%, -30%, and -48%, respectively) with a shift toward stages I-VI at 200 mg/kg-day (48% treated vs. 20% control) and stages IX-XII at 2 and 20 mg/kg-day (25% each vs. 14% control), Swollen and vacuolated mitochondria (cristae disappeared) at ≥2 mg/kg-day, Autophagic vacuoles at ≥20 mg/kg-day, and Condensed and marginated chromatin at ≥20 mg/kg-day. Oxidative stress in testicular tissue: Decrease in SOD activity, GPX activity, and GSH level at ≥2 mg/kg-day Increase in MDA (LPO indicator) level at ≥20 mg/kg-day. Apoptosis: Increase in spermatogenic apoptotic index at 200 mg/kg-day Mitochondria-mediated apoptotic protein changes: Decreased BCL2 and BCL2/BAX at ≥20 mg/kg-day Increased: BAX at 200 mg/kg-day, cleaved CASP8 at ≥2 mg/kg-day, cleaved CASP9 and cleaved CASP3 at ≥20 mg/kg-day, and FAS and FASL (mediated apoptotic protein) at ≥2 mg/kg-day. General toxicity: No effect on body weight.
Darghouthi et al. 2022 Male Wistar rats, 21 days old, 6 rats per group. Treatment: BPS (98% pure) in dimethyl sulfoxide (DMSO) (% not provided), in drinking water at 0 or 50 micrograms per liter (µg/L) for 10 weeks. Animals were sacrificed at the end of the 10-week dosing period.	Reproductive organ weight (testis, epididymis, whole prostate, and seminal vesicle). Gonadosomatic index (GSI; relative gonadal weight x 100). Testes histopathology. Caudal epididymal sperm parameters (count, viability, motility, and morphology). Oxidative stress in testes: MDA (marker of LPO) levels; GPX, SOD, and catalase activity; MTT (mitochondrial function assay) levels. Computational docking studies used to investigate the potential interaction pattern between BPS and steroidogenic acute regulatory protein (STAR). General toxicity: body weight.	Reproductive organ weights: Decreased absolute (-29%) and relative (-31%) prostate weights with treatment. No effects on testis, epididymis, and seminal vesicle weights. No effect on GSI. Testes histopathology: Based on the histological images, testicular tissues appear to be poorly fixed. Decreased germ cells and spermatozoa numbers in the seminiferous tubule walls. Clearer lumen of the tubules. Decreased number of spermatogonia, spermatocytes I, spermatocytes II, and spermatids. Decreased number and altered (cytoplasmic vacuolization) Sertoli cells (while not explicitly stated in the study, context indicates reduced Sertoli cell numbers). Sperm parameters: Decreased count (-60%), viability (-20%), mobility (-41%), and percentage of sperm with normal morphology (-43%).

Study Design	Outcomes assessed	Major Findings
		Oxidative stress: Increased MDA levels and GPX activity Decreased MTT levels (-79%). Computational docking: BPS molecule docked into the crystal structure of S*t*AR protein under X-ray crystal structural analysis. General toxicity: Increased body weight (+8%).
ECHA 2019 - Anonymous Study 12, 2000 OECD TG 421 protocol. Adult male SD rats, 12 rats per group. Treatment: BPS (purity ≥ 99.7%) in 0.5% aqueous sodium CMC solution with 0.1% Tween 80 by gavage at 0, 10, 60, or 300 mg/kg-day for 45 days, including 14 days of premating through mating to the day before necropsy. Rats were mated with females exposed for 40–46 days (from premating, mating, gestation until lactation day 3).	Organ weight. Reproductive performance (mating index, fertility index). General toxicity: body weight.	Organ weight: Increase in relative pituitary weight and decrease in absolute seminal vesicle weight at 300 mg/kg-day. No effect on absolute weights of testis, epididymis, or prostate. Reproductive performance (unclear if mating and fertility indices are reported in males or females): No effect on copulation index. Decreased fertility index at 300 mg/kg-day (-36%). General toxicity: Reduced body weight on treatment days 3 and 14 at 300 mg/kg-day. No effect on body weight gain 0–42 days.
ECHA 2019 - Anonymous Study 14, 2017 Performed as dose range finding study. Adult male and female SD rats, 10 rats/sex/dose. Treatment: BPS (purity ≥ 99.7%) in CMC by gavage at 0, 30, 100 or 300 mg/kg-day for 12 weeks (6 weeks premating, 2 weeks mating, and 4 weeks post mating). Rats were mated with females that were treated from premating (6 weeks), through gestation and lactation until postnatal day (PND) 21.	Organ weight. Histopathology. Reproductive parameters (implantation sites, post-implantation loss). General toxicity: body weight.	Organ weight: No effect on the absolute weights of testis, prostate, seminal vesicles, or adrenal. Histopathology: Diffuse atrophy observed in the mammary gland of 10/10 males at 300 mg/kg-day vs. in 0/10 male controls. Reproductive parameters: Decreased implantation sites at 300 mg/kg-day (-34%). Increased % post-implantation loss at 300 mg/kg-day (+861%). Decreased mean number of pups delivered at 300 mg/kg-day (-29%). General toxicity: Reduced body weight (-7%) at 300 mg/kg-day.
ECHA 2019 - Anonymous Study 16, 1999 Adult male SD rat, 6 rats per group (28-day main study), 6 rats per group (2-week recovery study). Treatment: BPS (purity ≥ 99.7%) in 0.5% methylcellulose by gavage at 0, 40, 200, or 1000 mg/kg-day BPS for 28 days. Recovery group animals (0, 200, or 1000 mg/kg/day groups only) were untreated for a 2-week observation period before sacrifice.	Organ weight. Histopathology. General toxicity: body weight.	Decreased total cholesterol at 1000 mg/kg-day with no effect after recovery period. Organ weight: Increased absolute and relative adrenal weight at 1000 mg/kg-day. Increased relative testes weight at 1000 mg/kg-day. Histopathology: Adrenal hypertrophy observed in 4/5 males at 1000 mg/kg-day vs. in 0/6 male controls. General toxicity: Lower body weight on days 14 and 28 at 1000 mg/kg-day; no effect on body weight after recovery period. Lower body weight gain on days 1–28 at 1000 mg/kg-day.

Study Design	Outcomes assessed	Major Findings
Fenclová et al. 2022a Lactational exposure experiment: Lactating female mice (ICR), 6 per group. Litters reduced to 10 pups at birth, with an equal ratio of male and female offspring. Treatment: BPS (purity not reported) in 0.1% ethanol, in drinking water at 0, 0.375, and 37.5 ng/mL), resulting in doses of 0, 0.216, or 21.6 µg/kg-day based on recorded water intake or DES at 0.375 ng/mL as a positive control from PND 0-15. Male offspring were sacrificed on PND 15 or PND 90 for analysis (n≥3 male pups per litter).	Testis histopathology (PND 15 and 90) and histomorphometry including seminiferous tubule height and diameter (PND 90). Spermatogenesis staging (PND 90). DNA integrity of seminiferous tubules in middle-stage spermatogenesis on PND 90 (testis TUNEL and γH2AX levels). Seminiferous tubule integrity in middle-stage spermatogenesis on PND 15 and 90 (CX43, OCL, and ZO protein levels). Oxidative stress protein levels and markers on PND 90 (HSP90, PRDX6, and 8-OHdG).	PND 15 Testis histopathology: No effect. Seminiferous tubule integrity: Increased relative density of CX43 in positive control only. PND 90: Testis histopathology: No effect. Testis histomorphometry: Decreased height of seminiferous epithelia in middle-stage tubules at 21.6 µg/kg-day and in positive control. Spermatogenesis staging: No effect. DNA integrity: No effect. Seminiferous tubule integrity: Increased CX43 % area at 21.6 µg/kg-day, Decreased relative density levels of OCL at 0.216 µg/kg-day. Increased relative density and % area of ZO at 0.216 and 21.6 µg/kg-day. Oxidative stress: Increased 8-OHdG at 21.6 µg/kg-day. No effects on HSP90 and PRDX6 levels.
Fenclová et al. 2022b Lactational exposure experiment: Lactating female mice (ICR), ≥6 per group. Treatment: BPS (purity not reported) in 0.1% ethanol, in drinking water at 0, 0.375, and 37.5 ng/mL, resulting in doses of 0, 0.2, or 20 ng/g-day (µg/kg-day) based on assumed water intake or (DES) as a positive control at 0.2 ng/g-day, from PND 0–15. The male offspring were weaned on PND 21 and sacrificed at 14 weeks of age (n≥3 male pups per litter). Embryo flushing and in vitro culture experiment: Male mice (ICR), age not reported, sample size not reported. Zygotes from untreated female mice mated to BPS-treated male mice were flushed and cultured in vitro until the blastocyst stage for analysis.	Lactational exposure experiment: Anogenital distance (AGD), age at assessment unclear, presumed to be PND 21. Assessments at 14 weeks of age: Sperm parameters (count and motility). Sperm chromatin structure assay (sperm chromatin immaturity indicated by DNA fragmentation index and high DNA stainability. Markers of DNA damage (double-strand breaks [DSB]) in testicular tissues and sperm (H3K4me2 and γH2AX protein levels). Embryo flushing and in vitro culture experiment (females mated to BPS-treated males): Pregnancy rate. Fertilization rate. Blastocyst rate. Cleavage rate. Blastomeres per blastocyst. Apoptotic index (TUNEL Assay) on blastocysts.	Lactational exposure experiment: AGD: Decreased at 0.2 µg/kg-day, NS increase at 20 µg/kg-day. No effect in positive control. Sperm parameters: No effect, positive control data not shown. Sperm chromatin structure: No effect, positive control data not shown. Markers of DNA damage (DSB) in testicular germ cells: Increased H3K4me2 protein in the epithelia of stage VII-VIII seminiferous tubules at 20 µg/kg-day. No effect on γH2AX protein level at either dose, positive control data not shown. Markers of DNA damage (DSB) in epididymal spermatozoa: No effects. Embryo flushing and in vitro culture experiment: No effects on pregnancy, fertilization, or blastocyst rate Cleavage rate: Decreased at 20 µg/kg-day (-29%), no effect with positive control. Decreased number of blastomeres per blastocyst at ≥0.2 µg/kg-day, no effect with positive control. Apoptotic index: No effect with BPS treatment, increased with positive control. Increased γH2AX in blastomeres at 20 µg/kg-day.

Study Design	Outcomes assessed	Major Findings
	Markers of DNA damage (DSB) in fixed zygotes (H3K4me2 and γH2AX protein levels).	Markers of DNA damage (DSB) in fixed zygotes: Increase in the number of γH2AX loci in paternal pronuclei ≥0.2 µg/kg-day and positive control, Decrease in the number of H3K4me2 loci in paternal pronuclei in positive control. Correlation analysis: Negative correlations between H3K4me2 levels in testicular germ cells and cleavage rate of early embryos (-0.65), and between H3K4me2 and double-strand breaks in blastocysts (-0.733).
Horan et al. 2018 Male 129S1/SvImJ mice, n = 11 treated males and 14 control males. Treatment: BPS (purity not reported) in 1% (v/v) ethanol solution in tocopherol-stripped corn oil, orally via pipette at 20 µg/kg-day from PND 1 to PND 8. Control or placebo: equal volume ethanol and corn oil Males were sacrificed and assessed at 6 weeks of age.	Meiotic recombination levels measured by: The mean number of MLH1 foci in pachytene spermatocytes. Frequency of pachytene spermatocytes with at least one synaptonemal complex lacking an MLH1 focus (expressed as mean % MLH1 null).	Decreased meiotic recombination levels as indicated by: Decreased mean MLH1 counts. Increased (NS) mean % MLH1 null.
Hu et al. 2023 Male C57BL/6 mice, 6-8 weeks old, 10 mice per group. Treatment: BPS (purity not reported) in 0.1% DMSO, daily oral dose at 0 (corn oil), 0.002, 0.2, 2, or 20 mg/kg-day for five weeks. Animals were sacrificed after treatment.	Thyroid morphology: thyroid follicular epithelial height Thyroid histopathological analysis Serum Thyroid Hormone levels: triiodothyronine (T3), thyroxine (T4), and thyroid-stimulating hormone (TSH). Protein expression by immunoblotting analysis: Thyrotropin receptor (thyroid-stimulating hormone receptor; TSHR), thyroperoxidase, thyroglobulin, sodium iodide symporter.	Thyroid follicular epithelium: Increased height at 20 mg/kg-day Serum levels: Increased TSH at 20 mg/kg-day NS increase at 20 mg/kg-day in T3. NS increase at ≥ 2 mg/kg-day in T4 levels. Protein expression levels: Decreased TSHR at 0.02 mg/kg-day. Decreased sodium iodide symporter at 0.02 and 20 mg/kg-day Decreased thyroglobulin and thyroperoxidase at 20 mg/kg-day.
Jeminiwa et al. 2021 Male Long-Evans rats, prepubertal (PND 21) and pubertal (PND 35), 6 or 9 rats per group. Experiment 1: Treatment: BPS (purity not reported) in DMSO (0.001%), in drinking water at doses of 0 (vehicle) and 5 µg/L for 14 days, 6 rats per group. Experiment 2: Treatment: BPS (purity not reported) in DMSO (0.001%), in drinking water at doses of 0 and 5 µg/L, from PND 21-35, prepubertal rats only; 9 animals per group.	Testes weights, GSI. Serum T and E2 concentrations. Testicular T and E2 concentrations produced by testicular explants in the absence (basal) or presence of ovine luteinizing hormone (LH; 100 ng/mL). Leydig cell (isolated from testes) T and E2 concentrations (basal and LH-stimulated). Steroidogenic enzyme expression in testes of pubertal rats. Testicular protein expression in testes of pubertal rats: AMH and Desert hedgehog protein (DHH) involved in Sertoli and sperm maturation respectively. General toxicity: body weight, mortality	Organ weights: No effects. Prepubertal rats: No effects on serum hormone concentrations. Decreased testicular T (basal only) Decreased Leydig cell T levels (basal and LH-stimulated) Increased testicular E2 (basal and LH-stimulated) No effect on Leydig cell E2 levels (basal or LH-stimulated) Pubertal rats: No effects on serum hormone concentrations. No effect on testicular T or E2 (basal or LH-stimulated) Decreased CYP11A1 and HSD17B in pubertal testes Decreased AMH, and increased DHH in pubertal testes. General toxicity: No effect on body weight or mortality.

Study Design	Outcomes assessed	Major Findings
For both experiments, animals were sacrificed at the end of the treatment period and samples were collected. For experiment 2, Leydig cells were isolated and cultured for analysis.		
John et al. 2019 Male SD rats,6 rats per group. Treatment: BPS (purity not reported) in olive oil, daily subcutaneous injection at doses of 0, 2, or 200 µg/kg-day from PND 1–27. Animals were sacrificed on PND 27.	Organ weights, assumed to be absolute (testes, seminal vesicle, prostate). Testes histopathology. Hypothalamus immunocytochemistry. General toxicity: body weight	Organ weights: NS decreases in absolute testes (-64% and -68%), prostate (-50% each) and seminal vesicle (-74% and -94%) weights at both doses. Testes histopathology: Tissue appears to be poorly processed for morphometric analysis and seminiferous tubules were not appropriately staged for comparison. Altered seminiferous tubule appearance at 2 µg/kg-day No effects on seminiferous tubule diameter . Decreased tubular lumen diameter and epithelial height at both doses. Hypothalamus immunocytochemistry: Increased TH-immunoreactive cell bodies at 2 (+29%) and 200 µg/kg-day (+90%). General toxicity: Increased body weight at both doses on PND 16 (+60% and +39%, respectively) and at the high dose on PND 24 (+54%).
Kaimal et al. 2021 Pregnant female SD rats, approximately 3 months old, 9 (control) or 13 rats per group (animals were pooled from 2 experiments). Treatment: BPS (≥98% purity) in DMSO and diluted in phosphate buffered saline (PBS, 10 µL), orally via micropipette at 0 (PBS), or 5 µg/kg-day from GD 6-21. Dams were moved to individual cages on GD 22 and remained with offspring until weaning; offspring were sacrificed at 16–24 weeks of age (blood, organs, and tissues were collected).	AGD (PND 1). Organ weight (pituitary, thymus, heart, lungs, liver, spleen, adrenals, kidneys, abdominal adipose tissue, epididymal adipose tissue, perirenal adipose tissue, paired testes, prostate, seminal vesicles). Oxidative damage to DNA in testes (immunohistochemistry for 8-OHdG). Serum T levels.	Decreased AGD (0.26 centimeter in BPS vs. 0.32 centimeter in controls) Organ weights: No effect. Oxidative damage to DNA in testes: Increased 8-OHdG; likely localized in primary spermatocytes in the germinal epithelium. Serum T: No effect.
Kolla et al. 2019 Experiment 1: Pregnant female mice (CD-1), 6–7 mice per group. Treatment: BPS (purity not reported) in 70% ethanol (dried before feeding), daily wafer treated with 0, 2, or 200 µg/kg-day) from GD 9 to GD 16 or LD 20. Dams were sacrificed on GD 16 or LD 20, litters culled to 10 pups/litter on LD1, 1 male/litter was necropsied at PND 24, 1 male/litter was necropsied at 9 weeks of age.	Experiment 1: Fetuses were sexed by genotyping on GD16. Immunohistochemical analysis of mammary tissues on GD16 estrogen receptor α (Erα) and androgen receptor (AR). Histology of mammary gland on E16, PND 24, and 9 weeks old. Experiment 2 (PND 31): AGI. Seminal vesicle weight.	Experiment 1: Male offspring mammary gland histology on GD 16: Increased (NS) size of epithelial anlagen with highest value at 200 µg/kg-day, NS Increased number of epithelial cells with the highest value at 200 µg/kg-day, Increased space between cells consistent with the earliest stages of lumen formation at 200 µg/kg-day, No staining for AR in epithelial cells Dose-dependent decrease in AR expression in the mesenchyme Inconsistent results for ERα in epithelial cells with no difference between treatment groups.

Study Design	Outcomes assessed	Major Findings
Experiment 2: Pregnant female mice (CD-1), 10–12 mice per group. Treatment: BPS (purity not reported) in tocopherol-stripped corn oil, daily oral dose at 0, 2, 200, or 2000 µg/kg-day from GD 9 to LD 2, two males/litter were randomly selected on PND 21 and orally dosed with vehicle or 1 µg/kg-day of 17α-ethinyl estradiol (EE2) for 10 days. Male offspring were euthanized on PND 31 for analysis.	Histology of mammary gland.	Male offspring mammary gland histology on PND24: No effect on ductal area or number of branching points in the right mammary gland. Decreased ductal tree size in the left mammary gland at 200 µg/kg-day. Male offspring mammary gland histology at 9 weeks old: Increased right epithelial trees at ≥2 µg/kg-day and left epithelial trees at 200 µg/kg-day. Experiment 2: AGI: No effects. Seminal vesicle weight: No effects. Male offspring left mammary gland histology: NS increased areas and branching points at ≥200 µg/kg-day with EE2 challenge, Increased total TEB area at ≥200 µg/kg-day with EE2 challenge. NS increased number of TEBs at ≥200 µg/kg-day with EE2 challenge, and Male offspring right mammary gland histology: Increased growth parameters at ≥200 µg/kg-day with EE2 challenge.
Kumar et al. 2020 Male golden (Syrian) hamsters, 90-100 days old, 6 hamsters per group. Treatment: BPS (purity not reported) in corn oil, daily oral administration (specific method not reported) at doses of 0 (vehicle control) or 75 mg/kg-day BPS for 28 days. BPS treatment occurred in the presence or absence of melatonin at 10 mg/kg-day in 0.9% normal saline administered via intraperitoneal injection every other day for 28 days. Animals were sacrificed 24 hours after the final dose.	Relative testis weight. Sperm parameters (count and viability). Testes histopathology. Serum melatonin and T concentrations. Testicular melatonin receptor 1 (MT1) and AR protein expression. Testicular oxidative stress (superoxide dismutase [SOD] and catalase [CAT] activities, MDA levels). Testicular protein expression (nuclear factor [erythroid-derived 2]-like 2 [NRF2], heme oxygenase-1 [HO1], silent information regulator-1 [SIRT1], fork head Box O-1 [FOXO1], nuclear factor-kappa B [NFKB], cyclooxygenase-2 [COX2], connexin-43 [CX43], caspase-3 [CASP3], and B cell lymphoma-2 [BCL2]. Testicular proliferating cell nuclear antigen (PCNA) immunohistochemistry. General toxicity: body weight	Note: Findings from BPS-only group. Decreased relative testes weight. Decreased sperm count and viability. Testes histopathology: Marked testicular degeneration characterized by the presence of vacuoles, giant cells, atrophied Leydig cells, reduced germ cell number, and lumen devoid of sperm, and decreased seminiferous tubule diameter and area, and germinal epithelium height (attenuation of morphometric parameters were observed in the BPS-melatonin group). Serum melatonin and T concentrations: Decreased melatonin and T concentrations. Testicular MT1 and AR protein expression: Decreased MT1 and AR protein expression. Testicular oxidative stress: Decreased SOD & CAT activities. Increased MDA levels. Testicular protein expression: Increased NFKB, COX2, and CASP3., Decreased NRF2, HO1, SIRT1, FOXO1, CX43, and BCL2. Testicular PCNA immunohistochemistry. Decreased PCNA-positive cells and PCNA expression. Melatonin co-treatment ameliorated BPS-induced effects. General toxicity: Decreased body weight.

Study Design	Outcomes assessed	Major Findings
Molangiri et al. 2022 Pregnant female Wistar rats, 3 months old, 5-6 dams per group. Treatment: BPS (purity not reported) in olive oil, gavage at doses of 0, 0.4, 4 or 40 µg/kg-day from GD 4 to GD 21. Male offspring were sacrificed on PND 90 for all evaluations, 5-20 pups per group. Note: other than body and organ weight, findings in the 40 µg/kg-day exposure were not reported	Testis weight (assumed absolute). AGD (at PND 90). Plasma T concentration. AR and estrogen-related receptor gamma (ESRRG) protein expression in testes. Testicular gene expression (*Ar*, estrogen receptor 1 [*Esr1*], progesterone receptor [*Pgr*], FSH receptor [*Fshr*], luteinizing hormone/choriogonadotropin hormone receptor [*Lhcgr*], and prolactin receptor [*Prlr*]). Testes histopathology. Pro-inflammatory (IL6 and COX2), -adhesion (VCAM1 and MMP9), and -proliferation (TIMP1) protein expression in testes. Lipid peroxidation (LPO) in testes (thiobarbituric acid-reactive substances,[TBARS (MDA)], a measure of LPO). Corticoid stress-related gene expression in testes (*Hsd11b2, Hsd17b7, Hsd3b1, Hsd11b1,* and *Hsd17b1*). Apoptosis protein marker expression in testes (CASP8, CASP3, and cleaved CASP3). Sperm DNA damage (DNA fragmentation index, acridine orange assay). Global DNA methylation of sperm. Epigenetic-related protein expression (DNMT3A, DNMT3B, and pAKT/AKT protein levels). Spermatogenesis-related and testes-expressed genes (Tex101, Spo11, Akap4, Catsper1, Catsper2, Gpr56, Ddx4, Tssk1, Tex11, Tex12, Tex14, and Tex15). Spermatogenesis-related testes-expressed protein (TEX11).	Dose-dependent increases at all doses in testes weight. AGD: No effect. Increased plasma T concentration at 0.4 µg/kg-day. Receptor protein expression in testes: Decreased AR at 0.4 and 4.0 µg/kg-day. Decreased ESRRG at 0.4 µg/kg-day. Receptor gene expression in testes: Decreased *Ar* at 0.4 and 4.0 µg/kg-day. Decreased *Esr1* at 4.0 µg/kg-day. Testes histopathology: Widely spaced seminiferous tubules surrounded by fewer Leydig cells and congested interstitial arteries at 0.4 µg/kg-day. Seminiferous tubules divided by huge interstitial gaps and partly damaged interstitial Leydig cells at 4.0 µg/kg-day. Decreased seminiferous tubule diameter at 0.4 and 4.0 µg/kg-day. Pro-inflammatory, -adhesion, and -proliferation protein expression in testes: Increased IL6 at 4.0 µg/kg-day. Increased COX2 and TIMP1 at 0.4 and 4.0 µg/kg-day. Decreased VCAM1 at 0.4 and 4.0 µg/kg-day. LPO in testes (TBARS): Increased MDA levels at 0.4 and 4.0 µg/kg-day. Corticoid stress in testes: Decreased *Hsd11b2* and *Hsd17b7* expression at 0.4 and 4.0 µg/kg-day. Apoptosis protein markers in testes: Increased CASP8 and CASP3 at 4.0 µg/kg-day Increased cleaved CASP3 at 0.4 and 4.0 µg/kg-day. Increased DNA fragmentation index in sperm at 0.4 and 4.0 µg/kg-day. Global DNA methylation of sperm: No effect. Epigenetic-related protein expression: Increased DNMT3B at 4.0 µg/kg-day. Increased DNMT3A at 0.4 and 4.0 µg/kg-day. Decreased pAKT/AKT ratio at 0.4 and 4.0 µg/kg-day. Spermatogenesis-related and testes-expressed genes: Decreased *Tex101* and *Spo11* at 0.4 µg/kg-day. Spermatogenesis-related and testes-expressed protein: Increased TEX11 at 0.4 and 4.0 µg/kg-day.

Study Design	Outcomes assessed	Major Findings
Morimoto et al. 2022 Pregnant Wistar rats, 7 rats per group. Treatment: BPS (purity not reported) in 0.1% ethanol, administered via drinking water at 0, 0.05, or 20 mg/kg-day from GD 6 to PND 21. Male offspring evaluated on PND 21, 36, and 120.	AGD/(body weight)$^{1/3}$ (PND 21). Serum T level (PND 36 and 120).	Relative AGD decreased at ≥0.05 mg/kg-day. Serum T levels: No effect.
Nguyen JL et al. 2022 Male C57BL/6 mice, 6–8 weeks old, 6 mice per group. Treatment: BPS (purity not reported) in a slow-releasing subcutaneous implant, 0 or 25 mg with or without co-implant of 25 mg testosterone, for 2 months. BPS in serum was determined at the end of the 2-months = 0.02 ng/ml in BPS-only group, and 0.64 ng/ml in T+BPS group	Serum BPS concentration. Urinary bladder weight. Prostate weight. Prostate histology. Urinary bladder function. General toxicity: body weight.	Urinary bladder weight: No effect in BPS-only group. Increased absolute bladder weight (+35%) in T+BPS group. Effects on prostate: No effect on prostate weight in BPS-only group, In T+BPS group: Increased absolute hemiprostate weight (+44%), Increased ventral prostate weight (+134%) Increased dorsolateral prostate weight (+56%) Increased number of prostatic ducts. Urinary bladder function: Non-voiding bladder contractions in T+BPS group, and Decreased threshold pressure (-39%) in T+BPS group. General toxicity: No effect on body weight.
Pollock et al. 2019 Male CF-1 mice, 3-4 months old, 7–10 animals per group. Treatment: BPS (≥ 98% purity) in peanut oil (0.1 mL final volume), single subcutaneous injection at doses of 0, 1, 3, or 9 mg/animal (final doses specific for each experiment were provided by author and they are below) Exp. 1: BPS plus BPA (not presented here) Exp. 2: BPS at doses of 0, 25.4, 76.8, or 218.9 mg/kg. 30 min after BPS injection, animals were fed 14.5 ng of 5 µCi ^{3}H-E2. One hour after oral administration (in food) of radiolabeled compound blood was collected by cardiac puncture, tissues were collected for further analysis of the radiolabel. Exp. 3: BPS at doses of 0, 22.5, or 65.0 mg/kg (only the two lowest BPS doses were tested in this experiment)	Exp 2: Distribution of ^{3}H-E2 in various organs (heart, lung, muscle, adipose, testes, epididymides, vesicular coagulating (VC) glands, preputial glands, and serum) Experiment 3: Urinary concentrations of endogenous E2	Experiment 2: ^{3}H-E2 concentration: Dose-dependent decrease (NS) of serum E2 levels Experiment 3: Urinary concentrations of endogenous E2: At 65.0 mg/kg, creatinine-adjusted and unadjusted levels were increased at 4 hours post-injection, and decreased at 22.5 and 65.0 mg/kg at 10 hours post-injection

Study Design	Outcomes assessed	Major Findings
Urine was collected at 2, 4, 6-, 8-, 10-, and 12-hours post-injection for hormone measurement.		
Řimnáčová et al. 2020 Male ICR mice, 8 weeks old, 5-9 mice per group. Treatment: BPS (purity not reported) in 0.1% ethanol, in drinking water at 0, 0.001, 1, or 100 µg/kg-day for 8 weeks.	Relative testis weight. Sperm parameters (count and motility). Spermatogenesis staging. Testis histopathology. Testis proteome profiling. Serum hormone analysis (adrenocorticotropic hormone, LH, FSH, growth hormone, TSH, T3, T4, cortisol, progesterone, testosterone). DNA damage in testes (phosphorylated γH2AX protein). Post-translational protein modifications in sperm (acetylation and phosphorylation).	Relative testis weight: No effect. Sperm parameters: Decreased percentage of motile sperm at 0.001 ug/kg-day (-50%). Spermatogenesis staging: No effect. Testicular histopathology: Increased incidence of total abnormalities at 100 µg/kg-day as indicated by: Increased atypical residual bodies at 100 µg/kg-day, Increased (NS) vacuolization in germ cell layer at 100 µg/kg-day, Increased (NS) incidence of enlarged multi-nuclear germ cells at 100 µg/kg-day. Decreased mature spermatozoa in germ cell layer at 1 µg/kg-day. Testes proteome profiling: No effect. Serum hormone concentrations: No effect. DNA damage (double-stranded breaks): Increased γH2AX signal at 100 µg/kg-day (~+300%). Protein modifications: Altered acetylation of proteins with molecular weights of approximately 37, 40, and 50 kDa at all doses (significant at low dose only), Altered phosphorylation of proteins with molecular weights of approximately 37, 40, 85, and 100 kDa at all doses (significant at low dose only), Analysis indicated the involvement of housekeeping proteins (ATP synthase subunit, hexokinase-1) and enzymes (DNA repair protein, E3 ubiquitin-protein ligase).
Sahu and Verma 2023 Adult male Parkes mice, n = 5 animals per treatment group. Treatment: BPS (purity not reported) in corn oil (vehicle control) at a concentration of 0 or 150 mg/kg-day via oral gavage for 28 days. Sacrificed 24 hours after last treatment and samples collected for evaluation.	Relative testicular weight. Thyroid gland structure. Testicular structure. Sperm quality: Motility, count, viability, and % normal sperm. Serum hormone concentrations: T, thyroid hormones (T3 and T4), and insulin. Testicular protein expression: Thyroid hormone-related (THRA and DIO2), redox-related (MT1 and SIRT1), and survival-related (pJAK2, and pSTAT3) Testicular mRNA expression: Steroidogenic (Star, Cyp11a1) and redox (Mt1, Sirt1, Pgc1α (Ppargc1a), Foxo1, Nrf2, and Ho1.) Testicular metabolic markers: glucose, IR, pAKT, and GLUT1.	Decreased relative testicular weight (-40%). Thyroid gland structure: Disordered histoarchitecture with epithelial cell thinning and presence of vacuoles in BPS group. Decreased follicular epithelial cell height (-55%). Testicular structure: Seminiferous tubules were not appropriately staged for comparison, limiting interpretation of the histologic data. Observed presence of vacuoles, reduced germ cell count, germ cell loosening, and lumen without sperm in BPS-treated testes. Decreased germinal epithelium height (-50%), seminiferous tubule diameter (-48%), and seminiferous tubule area (-36%). Sperm quality: Decreased motility (-75%), count (-37%), viability (-55%), and % normal sperm (-25%). Serum hormone concentrations: Decreased T (-73%), T3 (-53%), and T4 (-67%), and increased insulin (+98%).

Study Design	Outcomes assessed	Major Findings
	Testicular oxidative stress: SOD activity, CAT activity, MDA (LPO), nitrite-nitrate levels, and intracellular ROS content. Testicular cell proliferation and apoptotic markers: PCNA positive cells/tubule, CASP3, TUNEL positive cells. General toxicity: body weight	Testicular protein expression: Thyroid hormone-related: Decreased THRA (-22%) and DIO2 (-14%). Redox-related: Decreased MT1 (-13%) and SIRT1 (-8%). Survival-related: Decreased pJAK2 (-15%) and pSTAT3 (-18%). Testicular mRNA expression: Steroidogenic: Decreased Star (-76%) and Cyp11a1 (-55%). Redox: Decreased Mt1 (-38%), Sirt1 (-40%), Pgc1α (-50%), Foxo1 (-25%), Nrf2 (-48%), and Ho1 (-45%). Testicular metabolic markers: Decreased glucose (-79%), IR (-18%), pAKT (-31%), and GLUT1 (-28%). Testicular oxidative stress: Decreased SOD (-67%) and CAT (-31%) activity. Increased MDA (+44%), nitrite-nitrate levels (+270%), and intracellular ROS content (+43%). Testicular cell proliferation and apoptotic markers: Decreased PCNA positive cells/tubule (-62%). Increased CASP3 (+50%) and TUNEL positive cells (+405%). General toxicity: Decreased body weight (-25%)
Shi et al. 2017 Male CD-1 mice pups, ≥5 pups per litter, 5-6 litters per group. Treatment: BPS (purity >98%) in tocopherol-stripped corn oil containing less than 1.0% of ethanol, subcutaneous administration at 0, 50 µg/kg, or 10 mg/kg (20-50 µl) every 3 days from PND 0-60. Some male offspring (15-18/dose) were sacrificed on PND 60 and the remaining (12/dose) were mated to untreated females on PND 60 and sacrificed on PND 90.	Testis weight. Gross pathology (liver, kidneys, lungs, heart, intestines, and muscle). Sperm parameters (count and motility). Spermatogenesis staging on PND 60. Serum levels of E2 and T on PND 60. Expression of steroidogenesis-related genes in testes on PND 60 (Cyp11a1, Cyp17a1, Cyp19a1, Hsd3b1, Hsd17b3, and Star). Expression of androgen-dependent (Rhox5) and androgen-independent (Rhox8) Sertoli cell markers on PND 60. Reproductive performance (pregnancy rate, gestational days, litter size, sex ratio, and pup weights). General toxicity: mortality and body weight.	PND 60 males: Absolute and relative testis weights: No effects. Gross pathology: No effect. Sperm parameters: Decreased sperm counts at 50 µg/kg (-61%) and 10 mg/kg (-41%), Decreased sperm motility at 50 µg/kg (-13%) and 10 mg/kg (-18%). Spermatogenesis staging: Increased proportion of tubules in stage VII at 10 mg/kg, Decrease (NS) in proportion of tubules in stage VIII at 10 mg/kg. Serum E2 and T levels: Increased serum E2 at 50 µg/kg and 10 mg/kg Increased T at 10 mg/kg. Gene expression in testes: No effect on steroidogenesis-related genes. Increased expression of Rhox5 (androgen-dependent Sertoli cell marker) at 10 mg/kg. Reproductive performance: No effect on pregnancy rate, Increased (NS) number of days to successful mating at both doses (2 low-dose and 4 high-dose males took 10–17 days for a successful mating). PND 90 males: No effect on absolute and relative testis weight. Decreased sperm count at 50 µg/kg (-34%). General toxicity: No effects on mortality or body weight.

Study Design	Outcomes assessed	Major Findings
Shi et al. 2018 Pregnant female CD-1 mice, 7–8-week-old, 5 mice per group. Treatment: BPS (purity not reported) in corn oil, daily oral administration at 0, 0.5, 20, or 50 µg/kg-day from GD 11 until birth. Male pups were sacrificed on PND 12 or 60 for analysis, 2–3 male offspring per dose per dosing period were evaluated.	PND 12 assessment: Testis weights (absolute and relative). Apoptosis of germ cells (TUNEL). Serum E2 and T levels on PND 60. Expression of germ and Sertoli cell marker genes in testes (Stra8, Sox9, Rhox5, and Rhox8). Expression of apoptotic genes in testes (Apaf1, Bad, Bak1, Bax, Bcl2, Bcl2l1, Cycs, and Fasl). Expression of autophagy-related genes in testes (Atg3, Atg5, Atg7, Atg12, and Atg16l1). Expression of oxidative stress-related genes in testes (Cat, Gpx4, Gsr, Sod1, and Sod2). Expression of epigenetic-related genes in testes (Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l, Setd1a, Setd1b, Kmt2b, Kmt2c, Kmt2d, Kmt2e, Ezh2, Eed, Suz12, and Dot1l). PND 60 assessment: Testis weight (absolute and relative). Sperm parameters (sperm count and motility). Spermatogenesis staging. Apoptosis of germ cells (TUNEL). Serum E2 and T levels. Expression of steroidogenic-related genes in testes (Star, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b3, and Cyp19a1).	PND 12 assessment: Testes weights: No effects. Apoptosis of germ cells (TUNEL): Increased proportion of TUNEL-positive at all doses on PND 12 only. Increased number of TUNEL-positive cells per tubule at all doses, with no effect on germ cell number per tubule. Expression of germ and Sertoli cell marker genes in testes: No effects Expression of apoptotic genes in testes: Decreased Bad and Bax at all doses, Increased Cycs at 0.5 µg/kg/day. Expression of autophagy-related genes in testes: Decreased Atg3 at ≥20 µg/kg/day, Decreased Atg5 and Atg7 at all doses, Decreased Atg16l1 at 20 µg/kg/day. Expression of oxidative stress-related genes in testes: Decreased Cat at 0.5 and 20 µg/kg/day, Decreased Gpx4 at 0.5 µg/kg/day, Decreased Sod2 at all doses, Increased Sod1 at 20 µg/kg/day. Expression of epigenetic-related genes in testes: Increased Dnmt1 at 20 µg/kg/day, Increased Dnmt3b at 50 µg/kg/day, Increased Dot1l at ≥20 µg/kg/day, Decreased Setd1a at 0.5 µg/kg/day, Decreased Setd1b, Kmt2e, and Suz12 at all doses. PND 60 Assessment: Testis weights: No effect. Sperm parameters: Decreased sperm count at 0.5 (-34%), 20 (-45%), and 50 (NS) µg/kg-day, and Decreased sperm motility at 0.5 µg/kg-day (-40%). Spermatogenesis staging: Decreased percentage of tubules in stage VII at 50 µg/kg-day, Increased percentage of tubules in stage VIII at 50 µg/kg-day. Apoptosis of germ cells (TUNEL): No effect. Serum hormone levels: Increased E2 at 50 µg/kg-day. Expression of steroidogenic-related genes in testes: Increased Star at 0.5 and 20 µg/kg-day,

Study Design	Outcomes assessed	Major Findings
		Increased Cyp11a1 at 0.5 and 50 µg/kg-day, Increased Cyp19a1 at 50 µg/kg/day.
Shi et al. 2019 CD-1 mice, F0 females (6 mice per group) generated the F1 generation; 6–7-week-old F1 offspring from the same treatment group (but not littermates) were bred to produce the F2 generation; F2 offspring were similarly mated to produce the F3 generation. Treatment: BPS (purity not reported) in tocopherol-stripped corn oil, F0 pregnant dams were administered a daily oral dose at 0, 0.5, or 50 ug/kg-day from GD 7 until birth. One F3 male offspring per litter was assessed on PND 6 and/or 60.	All assessments are of F3 males. PND 6: Testes apoptosis on (TUNEL and phosphorylated γH2AX). Expression of epigenetic-related genes in testes (Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l, Setd1a, Setd1b, Kmt2a, Kmt2b, Kmt2c, Kmt2d, Kmt2e, Ezh2, Eed, Suz12, and Dot1l). Immunolocalization of phosphorylated forms of DNMT1, DNMT3A, DNMT3B, H3K4me3, H3K9me2, H3K9me3, H3K27ac, DDX4, and GATA4 PND 60: Body weight. Combined left and right testes weight. Sperm parameters (count and motility). Spermatogenesis staging Serum E2 and T levels on Expression of steroidogenesis-related genes in testes (Star, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b3, and Cyp19a1). Immunolocalization of phosphorylated forms of DNMT1, DNMT3A, DNMT3B, H3K4me3, H3K9me2, H3K9me3, H3K27ac, DDX4, and GATA4.	PND 6: Testes apoptosis: No effect. Expression of epigenetic-related genes in testes: Increased Dnmt1, Dnmt3a, Dnmt3b, Dnmt3l, Kmt2b, Kmt2e, Dot1l, Eed, and Suz12 at 50 µg/kg-day, and Increased Set1b, Kmt2a, Kmt2c, and Kmt2d at ≥0.5 µg/kg-day. Immunolocalization of epigenetic-related proteins in testes: Upregulation of phosphorylated DNMT3A in Sertoli cells at ≥0.5 µg/kg-day, Aberrant DNMT3B in germ cells at ≥0.5 µg/kg-day, Larger proportion of phosphorylated DNMT3B-positive tubules and positive cells per tubule at ≥0.5 µg/kg-day, and Decreased phosphorylated H3K4me3 and H3K27me3 in germ cells at ≥0.5 µg/kg-day. PND 60: Decreased body weight at ≥0.5 µg/kg-day. Increased relative testis weight at 0.5 µg/kg-day. Sperm parameters: Decreased sperm count at 0.5 (-40%) and 50 (-48%) µg/kg-day, Decreased sperm motility at 0.5 µg/kg-day (~-30%). Spermatogenesis staging: Increased proportion of tubules in stages I–VI at 0.5 µg/kg-day, Decreased percentage in stage IX at 0.5 µg/kg-day. Serum hormone levels: Decreased T at 50 µg/kg-day Expression of steroidogenesis-related genes in testes: Increased Star and Cyp19a1 at ≥0.5 µg/kg-day. Immunolocalization of epigenetic-related proteins in testes: Moderate phosphorylated DNMT3B in preleptotene and Sertoli cells at 50 µg/kg-day, Increased phosphorylated DNMT3B-positive cells (spermatogonia and Sertoli cells) at stages IX-XII at ≥0.5 µg/kg-day.
Silva et al. 2019 Male gerbils (Meriones unguiculatus), 90 days old, 10 gerbils per group. Treatment: BPS (purity not reported) in mineral oil, oral administration at doses of 0 or 40 µg/kg-day for 28 days.	Organ weight (prostatic complex and testes). Serum hormone concentrations (E2 and T; n=5). Morphology, stereology, and histopathology of the prostatic complex (urethral segment and ventral, dorsolateral and dorsal prostate lobes) and testes (n=5); ultrastructural analysis of ventral prostate (n=2).	Organ weights: No effect. Serum hormone concentrations: No effects. Morphology, stereology, and histopathology: Ventral prostate: Increased tissue relative frequency of epithelium, muscular stroma, and non-muscular stroma. Decreased tissue relative frequency of the luminal compartment. Increased hyperplasia (41.7% in BPS-treated vs. 3.5% in controls).

Study Design	Outcomes assessed	Major Findings
Animals were sacrificed 24 hours after the last treatment.	Secretory activity in ventral prostate (periodic acid-Schiff [PAS] test and transmission electron microscopy; n=8). Immunohistochemistry of ventral prostate (AR, ERα, and PCNA; n=5). Quantification of AR-, ERα-, and PCNA-positive prostatic tissue (n=5).	Secretory activity of ventral prostate: Decreased PAS-positive secretion area present at the secretory epithelial cell apex and in the prostatic lumen. Decreased number of secretory vesicles in the apical cytoplasm. Immunohistochemistry of ventral prostate: Increased PCNA-positive cell frequency in the epithelium and stroma of the ventral prostate, Increased AR-positive epithelial and stromal cells.
Ullah et al. 2016 Male SD rats SD, 70-80 days old, 6 rats per group. Treatment: BPS (99% purity) in 0.1–0.5% ethanol, oral administration at doses of 0, 1, 5, 25, or 50 µg/kg-day for 28 consecutive days. Animals were sacrificed on Day 29.	Absolute organ weights (testes and epididymis). Oxidative stress in testes (CAT, peroxidases, and SOD activities; LPO [TBARS]; and total ROS). Testes protein content. Plasma and testicular T concentrations. Histopathology (testes and epididymis). Morphology of the testis (areas of seminiferous tubule and interstitium, seminiferous tubule diameter, and epithelial height). Number of cell types in seminiferous tubules (spermatids, spermatogonia, and spermatocytes). Morphology of the caput and cauda epididymis (tubular diameter, lumen diameter, epithelial height, epithelium, and lumen).	Organ weights: No effects. Oxidative stress in testes Decreased SOD (-56–63%), peroxidases (-39–53%), and CAT (-49–54%) activities at ≥5 µg/kg-day, Increased LPO at 50 µg/kg-day (+23%), Increased total ROS at 1 µg/kg (+42%), 5 µg/kg (NS; +33%) and 25 µg/kg (+55%), and 50 µg/kg (NS; +34%). Testes protein content: Decreased total protein content at 5 µg/kg (-36%) and 25 µg/kg (-30%). T concentrations: Decreased in plasma at 1 and 50 µg/kg-day (-25% and -18%, respectively), Decreased in intratesticular tissue at 50 µg/kg-day (-35%). Histopathology and morphology: Testis: Authors did not note whether seminiferous tubules of the same stage were compared among the groups. Thin epithelium of seminiferous tubules and population of secondary spermatocytes relatively disperse at all doses, Fewer to no elongated spermatids in the lumen of a few seminiferous tubules observed at ≥25 µg/kg-day, Decreased epithelial height at 25 (-15%) and 50 µg/kg-day (-16%). Number of cell types in seminiferous tubules: No effects. Epididymis: Some empty lumen in epididymal section at ≥25 µg/kg-day.
Ullah et al. 2017 Male SD rats, 70–80 days old, 6 rats per group. Treatment: BPS (purity 99%) in 0.1% ethanol, daily gavage at doses of 0,1, 5, 25, or 50 µg/kg-day for 28 consecutive days. Animals were sacrificed on Day 29.	Sperm motility (cauda epididymis sperm). Daily sperm production (DSP). DNA damage (modified Comet assay, cauda epididymis sperm).	No effect on sperm motility. Decreased DSP at 50 µg/kg-day (-19%). DNA damage in sperm: Increased % tail DNA at ≥25 µg/kg-day (+31% and +44%).
Ullah et al. 2018a Male SD rats, 70–90 days old (study reported both 70–80 and 80–90 days old), 7 rats per group.	Organ weights (testes, assumed with epididymis). Oxidative stress (CAT, SOD, and peroxidases activities; LPO [TBARS]; and total ROS).	Organ weights: No effects. Oxidative Stress: Decreased peroxidases (-9-16%; NS at ≥25 mg/kg-day) and CAT (-8-15%; NS at 25 mg/kg-day)

Study Design	Outcomes assessed	Major Findings
Treatment: BPS (99% purity) in 0.1–0.5% ethanol, daily gavage at doses of 0, 5, 25, or 50 mg/kg-day for 28 consecutive days. Animals were sacrificed on Day 29. Note: The methods report dosing of 0, 5, 50, or 500 mg/kg-day, however, a dosing regimen of 0, 5, 25, or 50 mg/kg-day is reported in all other parts of the study, including data presentation.	Plasma and testicular T concentrations. Histopathology (testes and epididymis). Morphology of the testis (areas of seminiferous tubule and interstitium, seminiferous tubule diameter, and epithelial height). Number of cell types in seminiferous tubules (spermatids, spermatogonia, and spermatocytes). Morphology of the caput and cauda epididymis (tubular and lumen diameter, epithelial height, and percentage of epithelium and lumen). General toxicity: body weight gain	activities at all doses, Increased LPO (+14%) and total ROS (+59%) at 50 mg/kg-day, with decreased total protein content at all doses (-15-18%). T concentrations: Decreased plasma (-25-33%; NS at 5 and 25 mg/kg-day) and intra-testicular (-12-18%; NS at 25 mg/kg-day) T concentrations at all doses. Histopathology and morphology: Testis: Tissue appears to be poorly processed for morphometric analysis and seminiferous tubules were not appropriately staged for comparison. Decreased epithelial height at 50 mg/kg-day (-18%) and absence of sperm in tubule lumen at 50 mg/kg-day. Number of cell types in seminiferous tubules: Not reported. Epididymis: No effects. General toxicity: No effect on body weight gain.
Ullah et al. 2018b Male SD rats, 23 days old (prepubertal), 7 rats per group. Treatment: BPS (purity not reported) in 0.1% ethanol, drinking water at concentrations of 0, 5, 25, or 50 µg/L for 48 weeks. Animals were sacrificed after the exposure period.	Organ weights (testes, epididymis, seminal vesicle, and prostate; absolute, relative [to body weight]; and GSI). Oxidative stress in testes (CAT, SOD, and peroxidases activities; LPO [TBARS], and total ROS). Plasma hormone concentrations (T, estrogen, LH, and FSH). Sperm parameters (DSP, number [caput and cauda epididymis], motility, and viability). Histopathology (testes and epididymis). Morphology of the testis (areas of seminiferous tubule and interstitium, seminiferous tubule diameter, and epithelial height). Number of cell types in seminiferous tubules (spermatids, spermatogonia, and spermatocytes). Morphology of the caput and cauda epididymis (tubular and lumen diameter, epithelial height, and percentage of epithelium and lumen). General toxicity: Body weight and body weight gain.	Organ Weights: Decreased absolute seminal vesicle weight at ≥25 µg/L (-4–6%), Decreased relative epididymis and seminal vesicle weights at 50 µg/L (-3% and -6%, respectively), Decreased GSI at 50 µg/L (-6%). Oxidative Stress: Decreased CAT -15% at 50 µg/L, and peroxidases (-10% each) activities at 25 and 50 µg/L. Decreased SOD activity at 50 µg/L (-5%), Increased LPO (+11%) and total ROS (+23%) at 50 µg/L. Plasma hormone concentrations: Decreased T at 25 µg/L (-14%) and at 50 µL (-21%), Increased E2 at 50 µg/L (+56%), Decreased LH (-17%) and FSH (-27%) at 50 µg/L Sperm Parameters: Decreased motility (-5–7%) at ≥25 µg/L. Decreased caput epididymal sperm number (-3–4%) at ≥25 µg/L. Decreased DSP (-10%) and cauda epididymal sperm number (-2%) at 50 µg/L. Histopathology and morphometry: Testis: Seminiferous tubules were not appropriately staged for comparison, limiting interpretation of the histologic data. Seminiferous tubules were relatively small with larger interstitial spaces and emptier lumen with cellular arrest at spermatogonial stage and round spermatids at ≥25 µg/L, Decreased epithelial height (-13%) at 50 µg/L.

Study Design	Outcomes assessed	Major Findings
		Number of cell types in seminiferous tubules: Decreased numbers of spermatogonia (-6%), spermatocytes (-6%), and spermatids (-5%) per cross-section of seminiferous tubules at 50 µg/L. Epididymis: Decreased number of sperm in the caput and cauda regions at 50 µg/L. General toxicity: Increased final body weight at 50 µg/L (+1%).
Ullah et al. 2019a Adult male SD rats, 70–80 days old, 7 animals per group. Treatment: BPS (99% purity) in 0.1% ethanol, daily gavage at doses of 0 (0.1% ethanol), 5, 25, or 50 mg/kg-day for 28 consecutive days. Animals were sacrificed on Day 29.	Sperm parameters (motility and DSP). DNA damage in sperm (Comet assay).	Sperm parameters: No effects on sperm motility. Decreased DSP at 50 mg/kg-day (quantitative data is not provided, but based on statements in the study, OEHHA assumes that this effect was observed at this dose). Increased DNA damage at 50 mg/kg-day as indicated by: Increased number of comets per 100 cells (+29%), tail moment (+31%), and % sperm tail DNA (+37%) at 50 mg/kg-day.
Ullah et al. 2019b Pregnant SD rats, 80-90 days old, 8 dams per group. Treatment: BPS (purity not reported) in 0.1–0.5% ethanol diluted in water, orally in drinking water at concentrations of 5, 25, or 50 µg/L from GD 1–21. Male offspring were sacrificed on PND 16 (2 males/litter) and PND 80 (8 males/group).	Early development: AGD (PND 1). Nipple retention (PND 14). Organ and tissue weights on PND 16 (absolute; testes, prostate, epididymis, seminal vesicle, bulbourethral gland, adrenals, bulbocavernosus muscles, fat pad, and liver). Late development: Puberty onset determined by preputial skin separation (checked daily from PND 35). Organ and tissue weights on PND 80 (testes, epididymides, seminal vesicle, prostate, fat pad, kidney, liver, and adrenals). Histopathology on PND 80 (testes and epididymides). Testis morphology: Number of spermatogonia, spermatocytes, and spermatids in seminiferous tubules. Epididymis (caput and cauda regions) morphology. Sperm parameters on PND 80 (DSP, count in different regions of epididymis, and transit time). Oxidative stress in testes on PND 80 (LPO [TBARS] and total ROS levels, and CAT, peroxidases, and SOD activities).	No effects on AGD No effect on nipple retention. PND 16: No effect on organ and tissue weight PND 35: No effect on puberty onset. PND 80: Organ and tissue weights: Decreased absolute seminal vesicle weight at 50 µg/L. Histopathology and morphology Testis: Seminiferous tubules were not appropriately staged for comparison, limiting interpretation of the histologic data. 25 µg/L: Decreased area % (-4%) of seminiferous tubule. 50 µg/L: Increased seminiferous tubule epithelial height (+4%) Decreased area % of seminiferous tubule (-5%), interstitial space (-14%), lumen (-4%), and seminiferous tubule diameter (-2%). Number of spermatogonia, spermatocytes, and spermatids in seminiferous tubules: Decreased numbers of spermatogonia (-6%), spermatocytes (-7%), and spermatids (-5%) at 50 µg/L. Caput epididymis: No effects. Cauda epididymis: No effects. Sperm parameters: Decreased DSP (-17%) at 50 µg/L Decreased number of sperm in caput/corpus epididymis at ≥25 µg/L (-3% each). Decreased % (-5%) motile sperm at 25 µg/L. Decreased % (-7%) motile sperm at 50 µg/L.

Study Design	Outcomes assessed	Major Findings
	Plasma hormone concentrations on PND 80 (T, E2, LH, and FSH).	Oxidative stress in testes: Increased LPO (+20%) and total ROS (+24%) at 50 µg/L, Decreased CAT (-21%) and SOD (-5%) activities at 50 µg/L, Decreased peroxidases activity at ≥25 µg/L (-14%). Plasma hormone concentrations: Increased E2 (+198%) at 50 µg/L, Decreased T (-28%), LH (-28%), and FSH (-62%) at 50 µg/L.
Ullah et al. 2021 Male SD rats, 23 days old (prepubertal), 8 animals per group (n=10 initially). Treatment: BPS (purity not reported) in 0.1% ethanol, drinking water at concentrations of 0, 0.5, 5, or 50 µg/L for 48 weeks. Animals were sacrificed at the end of the exposure period.	Organ weights (absolute and relative): testes, epididymis, seminal vesicle, and prostate; GSI. Oxidative stress in testes (CAT, SOD, and peroxidases activities; LPO; and total ROS). Plasma hormone concentrations (T, E2, FSH, and LH). Sperm parameters (DSP, number [caput/carpus and cauda epididymis], motility, and viability). Histopathology (testes and epididymis). Morphology of the testis (areas of seminiferous tubule and interstitium, seminiferous tubule diameter, and epithelial height). Morphology of the caput and cauda epididymis (tubular and lumen diameter, epithelial height, and percentage of epithelium and lumen). Number of cell types in seminiferous tubules (spermatids, spermatogonia, and spermatocytes). General toxicity: body weight.	Organ weights: Decreased absolute seminal vesicle weight at ≥5 µg/L (-4–5%), Decreased relative seminal vesicle (-7%) and epididymis (-5%) weights at 50 µg/L, Decreased GSI at 50 µg/L (-6%). Oxidative stress in testes: Decreased CAT (-10% and -18%, respectively) and peroxidases (-5% and -13%, respectively) activities at ≥5 µg/L Decreased SOD activity at 50 µg/L (-11%), Increased LPO (+16%) and total ROS (+23%) at 50 µg/L. Plasma hormone concentrations: Decreased T at 5 µg/L (-15%) and 50 µg/L (-21%), Dose-dependent increase (NS at 0.5 µg/L) in E2 at all doses (+30–52%), Decreased FSH (-21%) and LH (-39%) at 50 µg/L. Epididymis sperm parameters: Decreased motility (-6%) and DSP (-9%) at 50 µg/L, Decreased cauda epididymal sperm number at 50 µg/L (-8%), Decreased caput/carpus epididymal sperm number at ≥5 µg/L (-7% each). Histology and morphology: Testis: Tubules were relatively alternated with larger interstitial spaces and less filled lumen with cellular arrest at the spermatogonial stage and round spermatids (stronger effect at high dose) at ≥5 µg/L, Decreased area of seminiferous tubules (-5%) and seminiferous tubular diameter (-8%) at 50 µg/L, Increased area of interstitium at 50 µg/L (+28%), Decreased epithelial height (-7%) at ≥5 µg/L. Number of cell types in seminiferous tubules: Decreased spermatocytes (-7–9%) and spermatids (-3–5%) in the seminiferous tubules at ≥5 µg/L. Epididymis: Decreased tubular diameter in the caput region at 50 µg/L (-2%). General toxicity: Body weight increased (+1%) at 50 µg/L after 48 weeks exposure.

Study Design	Outcomes assessed	Major Findings
Wang et al. 2022 Male C57BL/6 mice, 8 weeks old, 10 mice per group. Treatment: BPS (≥99% purity, sesame oil vehicle) at 0 (control), 2, 20, and 200 mg/kg-day (0.1 mL/10 g) via oral gavage for 28 consecutive days. Animals were sacrificed on Day 29 and samples were collected for evaluation.	Serum and testis T concentration. Steroidogenesis-related gene expression in testes: *Star, Cyp11a1, Hsd3b1, Cyp17a1, Hsd17b3*. Steroidogenesis-related protein expression in testes: STAR, CYP11A1, HSD3B1, CYP17A1, HSD17B3. Oxidative stress-related gene expression in testes: *Keap1, Nrf2, Ho1, Gpx4, Cat, Sod1*. Oxidative stress-related protein expression in testes: KEAP1, NRF2, HO1, GPX4, CAT, SOD1.	Dose dependent decrease in testicular T concentrations at 2 (-18%), 20 (-30%), and 200 (-49%) mg/kg-day. Decreased serum T concentrations at 20 (-21%) and 200 (-33%) mg/kg-day. Steroidogenesis-related gene expression: Decreased *Star, Cyp17a1, Hsd17b3* at 200 mg/kg-day Decreased *Cyp11a1* at ≥2 mg/kg-day. Increased *Hsd17b3* at 20 mg/kg-day. Steroidogenesis-related protein expression: Decreased STAR at ≥20 mg/kg-day, Decreased CYP11A1 and HSD17B3 at 200 mg/kg-day, and Decreased HSD3B1 and CYP17A1 at ≥2 mg/kg-day. Oxidative stress-related gene expression: Increased *Keap1* at ≥20 mg/kg-day. Decreased *Nrf2* and *Cat* at 200 mg/kg-day, *HO-1* at ≥2 mg/kg-day, Decreased *Gpx4* and *Sod1* at ≥20 mg/kg-day. Oxidative stress-related protein expression: Increased KEAP1 at ≥2 mg/kg-day. Decreased NRF2 and CAT at ≥2 mg/kg-day, and Decreased HO1, GPX4, and SOD1 at ≥20 mg/kg-day.
Wu et al. 2021 Adult male SD rats, 80–90 days old, 8 rats per group. Treatment: BPS (>98% purity) in ethanol (0.5%) and corn oil, daily gavage at doses of 0 (corn oil), 50, or 100 mg/kg-day for 30 consecutive days. Animals were sacrificed on Day 31.	Organ weights (testes and epididymides (relative [to body weight]). Sperm parameters (count and morphology). Morphology and histopathology of the testis and epididymis. Immunohistochemistry (testis and epididymis). Blood-testis barrier (BTB) integrity. BTB integrity assay, protein expression, and immunolocalization: ZO1, Claudin 11 (CLDN11), β-catenin, N-cadherin, and Connexin-43 (CX43). Apical ectoplasmic specialization function (immunofluorescence) of nectin-3 and β1-integrin in Sertoli cells). Immunohistochemistry of seminiferous epithelium (F-actin and α-tubulin). Actin-binding protein expression and localization in testes (EPS8 and ARP3). BTB- and apical ectoplasmic specialization -associated genes (mTORC1 and mTORC2 signaling pathway genes [*Rps6, Rictor, Akt*, and *Mmp9*]).	Organ weights: No effects. Sperm parameters: (all effects at both doses) Decreased sperm count. Increased sperm with abnormalities. Decreased number of sperm in cauda epididymis. Testes and epididymides morphology and histopathology: At both doses: Altered spermatogenesis in stage VIII-IX seminiferous tubules: disrupted transport of spermatids and phagosomes across the seminiferous epithelium (likely due to F-actin and microtubule disorganization) with histopathological observations below: Spermatids: found deeply embedded in the epithelium instead of lining the tubular lumen to be released at stage VIII, with loss of polarity where heads were shifted ~90°-180° away from the basement membrane. Phagosomes: found in the adluminal compartment near the tubular lumen instead of being transported to the basal epithelium for lysosomal degradation at Stage IX. BTB integrity assay: Fluorescent biotin widely distributed throughout the adluminal compartment. Control limited to basal epithelium and interstitial space. BTB integrity-related junctional protein expression: Dose-dependent decrease (NS) in ZO1. Dose-dependent decrease in CLDN11 (NS at 50 mg/kg-day).

Study Design	Outcomes assessed	Major Findings
	BTB- and apical ectoplasmic specialization -associated proteins (mTORC1 and mTORC2 signaling pathway proteins [RPS6, p-RPS6, RICTOR, AKT1/2, p-AKT, and MMP9]).	Increased β-catenin and N-cadherin at 100 mg/kg-day, Decreased CX43 at both doses. BTB integrity: Immunolocalization: No effects on immunolocalization of BTB integrity-related junctional proteins. Apical ectoplasmic specialization: Location of β1-integrin was no longer restricted to the convex (dorsal) side of spermatid heads at stage VII, Decreased expression of β1-integrin and nectin-3 at both doses (indications of impaired ectoplasmic specialization). Seminiferous epithelium immunohistochemistry: Grossly disrupted organization of F-actin and α-tubulin in the seminiferous epithelium, and MTs were no longer observed as track-like structures at both doses. Actin-binding proteins: Decreased EPS8 at both doses (NS at 50 mg/kg-day), Decreased expression of ARP3 and EPS8 on the concave side of the spermatid heads at both doses. BTB- and apical ectoplasmic specialization-associated gene expression: Decreased *Rictor* at 100 mg/kg-day, Increased *Mmp9* at both doses. BTB- and apical ectoplasmic specialization-associated proteins: Increased p-RPS6/RPS6 ratio and MMP9 at both doses, Decreased RICTOR at 100 mg/kg-day Decreased p-AKT/AKT ratio at both doses.

Table 4.2.2 BPS: Evidence on the male reproductive toxicity in studies in zebrafish

Study Design	Outcomes assessed	Major Findings
Hao et al. 2022 Zebrafish (wild-type, AB). Treatment: BPS (purity ≥ 98%) in 0.002% DMSO, concentrations of 0 (blank control), 0 (vehicle control), 1, or 100 µg/L from 3 hours postfertilization (hpf) to 120 dpf in F0 generation only. F0 females and males mated on 110-120 dpf to produce the F1 generation (with successive matings to produce the F2 and F3 generations) and sacrificed on 120 dpf for sample collection; F1, F2, and F3 embryos were untreated and maintained in water. F1-2 samples were collected on 120 dpf; and F3 samples were collected on 5 dpf (120 hpf).	F0 generation: Organ weights (gonads, gonad-somatic index [GSI]). Sex ratio. Global DNA methylation levels in gonads (pooled from 5 fish per replicate, n=6). F1 generation: Number of total eggs produced. Organ weights (GSI and hepatosomatic index [HSI]). Gonad histopathology (n=5). Whole blood analysis (pooled from 10 fish) of sex hormones: testosterone (T) and estradiol (E2). Global DNA methylation levels in gonads (pooled from 5 fish per replicate, n=6). DNA methylation- and demethylation-related gene expression in gonads (pooled from 5 fish per replicate, n=6; *dnmt1, dnmt3-dnmt8, tet3*, and *tet1*). Steroidogenesis-related gene expression in testes (pooled from 5 fish per replicate, n=6; *cyp11a, cyp17, cyp19a, hsd3b, hsd17b*, and *star*; and in testis only: *spata17* and *spata4*). Steroidogenesis-related gene expression in brain (pooled from 5 fish per replicate, n=6; *ar, cyp19b, erα (esr1)*, *erβ* (*esr2b)*, *fshb*, *gnrh2*, *gnrh3, gnrhr1, gnrhr2, gnrh4*, and *lhb*). F2 generation: Organ weights (GSI and HSI). Testes histopathology (n=5). Whole blood analysis (pooled from 10 fish) of sex hormones (T and E2). Global DNA methylation levels in gonads (pooled from 5 fish per replicate, n=6).	F0 effects: Organ weights: Not reported. Sex ratio: Increased female/male sex ratio at 100 µg/L. Global DNA methylation levels (gonads): Decreased in testes at 100 µg/L. F1 effects: Number of total eggs produced: Decreased at 100 µg/L. Organ weights: No effects on GSI or HSI. Testis histopathology: Increased number of spermatogonia and spermatocytes, and decreased number of spermatozoa ≥ 1 µg/L. Plasma sex steroid hormones: Increased E2 and E2/T ratio, and decreased T in 100 µg/L males. Global DNA methylation levels in testes: Increased at 100 µg/L. DNA methylation- and demethylation-related gene expression in testes: 1 µg/L: Increased *tet1*; Decreased *dnmt1, dnmt3, dnmt4, dnmt5, dnmt7*, and *dnmt8*. 100 µg/L: Increased *tet1* and *tet3*; Decreased *dnmt1, dnmt3-dnmt8*. Steroidogenesis-related gene expression in testes: 1 µg/L and 100 µg/L: Increased *hsd17b* and *star*. Decreased *cyp11a, cyp17*, and *hsd3b*. Steroidogenesis-related gene expression in brain: 1 µg/L: Increased *ar, gnrh2, and gnrh3*; Decreased *lhb*. 100 µg/L: Increased *gnrh3* and *gnrhr2*; Decreased *erα* and *lhb*. F2 effects: Gamete fertilization rate: Increased slightly at 1 µg/L. Organ weights: decreased GSI at both concentrations. Testis histopathology: Sperm pyknosis in the lumen of seminiferous tubules, gap between seminiferous tubules and spermatozoa.

Study Design	Outcomes assessed	Major Findings
	DNA methylation- and demethylation-related gene expression in gonads: (pooled from 5 fish per replicate, n=6; *dnmt1, dnmt3-dnmt8, tet3,* and *tet1*). Steroidogenesis-related gene expression in gonads (pooled from 5 fish per replicate, n=6; *cyp11a, cyp17, cyp19a, hsd3b, hsd17b*, and *star*; and in testis only: *spata17* and *spata4*). Steroidogenesis-related gene expression in brain (pooled from 5 fish per replicate, n=6; *ar, cyp19b, erα (esr1), erβ (esr2b), fshb, gnrh2, gnrh3, gnrhr1, gnrhr2, gnrh4,* and *lhb*). F3 generation: Hatchability (24, 48, 60, and 72 hpf). Gamete fertilization rate.	Increased number of spermatogonia and spermatocytes in seminiferous tubules at 1 µg/L. Spermatid leakage and interstitial cell loss and increased proportion of spermatogonia and spermatids at 100 µg/L. Decreased number of spermatozoa ≥ 1 µg/L. Plasma sex steroid hormones: Increased E2 and E2/T ratio in 100 µg/L. DNA methylation- and demethylation-related gene expression in testes: 1 µg/L: Increased *dnmt8*; Decreased *dnmt4, dnmt5, dnmt6*, and *tet3*. 100 µg/L: Decreased *dnmt3-dnmt8*. Steroidogenesis-related gene expression in testes: 1 µg/L: Increased *hsd17b*. Decreased *cyp11a, cyp17*, and *hsd3b*. 100 µg/L: Decreased *cyp17, cyp19a, hsd3b*, *spata4, spata17*, and *star*. Steroidogenesis-related gene expression in brain: 1 µg/L: Increased *erα* and *gnrhr4*; Decreased *fshb*. 100 µg/L: Increased *gnrhr2* and *lhb*; Decreased *fshb*. F3 effects: Gamete fertilization rate: No effect.
Ji et al. 2013 Zebrafish (AB-type), 3–4 months old, 4 males and 6 females per group (duplicate tanks per group). Only data on males reported here. Treatment: BPS (purity not reported) in methanol (0.1%) at doses of 0 (water only), 0 (vehicle), 0.5, 5, or 50 µg/L for 21 days. F0 adults were sacrificed at the end of the exposure period for analyses. It should be noted that statistics were analyzed between treatment and water-only control groups (no differences were observed between the two control groups).	Snout-to-vent length. Body weight indices (Condition Factor [K] (body weight/body length), Brain Somatic Index [BSI], HSI, and GSI). Plasma hormone measurement (E2 and T concentration, E2/T ratio). Hypothalamic-pituitary-gonadal (HPG) axis gene expression (21 genes representing key signaling pathways and functional processes).	Body weight indices: Decreased GSI at 50 µg/L (-27%). Plasma hormone: Increased E2 at ≥0.5 µg/L, Decreased T at 50 µg/L, and Dose-related increase in E2/T ratio at ≥0.5 µg/L. HPG axis gene expression: Brain: Increased *cyp19b* (+165%), *gnrh3* (+122%), *gnrhr1* (+80%), *gnrhr2* (+119%), *fshb* (+109%), and *lhb* (+132%) at the 50 µg/L. Testes: Increased *cyp19a* (+181%), *fshr* (+165%), *lhr* (+218%), *hmgra* (+129%), *hmgrb* (+96%), *cyp11a* (+137%), and *3βhsd (hsd3b*; +107%) at 50 µg/L, and Decreased *cyp17* (-53%) and *17βhsd (hsd17b*; -61%) at 50 µg/L.

Study Design	Outcomes assessed	Major Findings
Naderi et al. 2014 Zebrafish (*Danio rerio*) embryos collected 2 hpf, normal embryos that had reached the blastula stage were selected and placed in untreated Petri dishes until 2 dpf. Approximately 700 of these 2 dpf embryos were divided into 51 tanks (5 treatment groups in triplicate). Treatment: BPS (purity >98%) in 0.01% acetone, concentrations of 0 (vehicle), 0.1, 1, 10, and 100 µg/L from 2–75 dpf. Spawned eggs and dead embryos/larvae were removed, 50% exposure solution was renewed daily. Zebrafish males were assessed at 75 dpf.	Sex ratio. Males: Organ weight indices (GSI and HSI). Sperm count. Plasma E2, T, Vtg, T4, and T3 concentrations (4-6 fish per sex per group pooled, 3 replicates). General toxicity: body weight and total length	Skew in the sex ratio towards females. Males: Organ weight indices (GSI and HSI): Decreased GSI at ≥10 µg/L (-24% and -31%, respectively), and Increased HSI at 100 µg/L (+28%). Plasma hormone and VTG concentrations: Increased E2 at ≥1 µg/L Increased Vtg at 100 µg/L Decreased T, T4, and T3 at ≥10 µg/L. Decreased sperm count at ≥10 µg/L. General toxicity: Decreased body weight at 100 µg/L (-26%). Decreased total length at 100 µg/L (-20%).
Park et al. 2022 Zebrafish (AB-strain), 3–4 months old, 5 males per group. Treatment: BPS (purity not reported) in 0.1% DMSO, concentrations of 0 (vehicle), 8, 40, or 200 µg/mL for 21 days, performed in triplicate. Outcomes assessed after exposure period. There was an increased mean bioaccumulation of BPS in males in a concentration-dependent manner	Organ weight indices (GSI and HSI). Testes morphology (testicular developmental stage). Gene expression in liver: *vtg1*, *erα* (*esr1*), and *erβ* (*esr2b*/*esr2a*). Whole body hormone concentrations: Sex hormones (11-ketotestosterone [11-KT], T, E2, aromatase [measured by the metabolic ratio of T:E2], progesterone). Thyroid hormones (T3 and T4). Cortisol. Whole body BPS concentration. General toxicity: survival.	Organ weight indices: Increased HSI at 200 µg/mL (+79%). Testes morphology: No effect. Gene expression in liver: Increased *vtg1* at 200 µg/mL. Decreased *erβ* at ≥40 µg/mL. No effect on *erα*. Whole body hormone concentrations: Decreased T at all concentrations. Decreased 11-KT at ≥40 µg/mL. Dose-dependent increase at all concentrations in E2. Increase in aromatase (T:E2) at all concentrations Dose-dependent increases at all concentrations in T3 (+108-192%) and T4 (+32-98%). No effect on cortisol concentration. General toxicity: No effect on survival rate

4.3 Mechanistic Considerations and Other Data Relevant to Male Reproductive Toxicity

This section presents *in vivo* and *in vitro* mechanistic evidence relevant to the effects of BPS on the male reproductive system. Study findings are generally summarized as concise bullet points organized first by the type and direction of effect, followed by species (ordered from mouse, rat, other mammalian, non-mammalian species, and *in vitro* studies), and lastly exposure period (ordered from gestation through adulthood). Summary tables are included throughout this section and in Appendix C to provide a consolidated overview of effects of BPS on specific enzymes, genes, and other proteins across all studies. All effects are significant ($p \leq 0.05$) unless otherwise noted (e.g., not significant [NS]). Magnitude of effects can be found (when available) along with further study details in Table 4.3.10.

When discussing data related to BPS-related effects on expression of genes and proteins, only those studies reporting changes are summarized in the text of this section. More detailed information on each study's findings (i.e., increases, decreases, or no changes) on gene or protein expression is included, when available, in Tables 4.2.1, 4.2.2, and 4.3.10.

The mechanistic evidence is discussed, where appropriate, in the context of the key characteristics (KCs) of male reproductive toxicants (Arzuaga et al. 2019). As discussed by Arzuaga et al. (2019), one or more of these KCs (listed in Table 4.3.1) are frequently exhibited by exogenous agents that cause male reproductive toxicity. A male reproductive toxicant need not exhibit, nor is it expected to exhibit, each of these KCs.

Table 4.3.1 Key characteristics of male reproductive toxicants (from Arzuaga et al., 2019)

Key characteristic	Example of relevant evidence
1. Alters germ cell development, function, or death	Increased germ cell apoptosis; alterations in sperm acrosome reaction and motility
2. Alters somatic cell development, functions, or death	Increased Sertoli cell apoptosis; alterations in Sertoli cell functions, cytoskeleton, and interactions with germ cells; alterations in Leydig cell development
3. Alters production and levels of reproductive hormones	Decreased Leydig cell steroidogenic functions; increased hepatic metabolism and excretion of sex hormones
4. Alters hormone receptor levels/functions	Androgen receptor antagonism, estrogen receptor activation, decreased LH receptor expression
5. Is genotoxic	DNA damage, chromosome fragmentation, altered sperm cell chromosome numbers
6. Induces epigenetic alterations	Altered sperm noncoding RNA (ncRNAs), germ cell DNA methylation patterns, and histone retention sites
7 Induces oxidative stress	Reduced tissue antioxidant levels
8. Induces inflammation	Increased testicular expression of pro-inflammatory markers and prostaglandin levels

4.3.1 Oxidative Stress, Genetic Damage, and Apoptosis

Oxidative stress, DNA damage, and apoptosis are complex cellular and molecular processes implicated in male reproductive toxicity. Indeed, “induces oxidative stress” (KC7), “is genotoxic” (KC5), and “alters germ [or somatic] cell development, function, or death (KC1, KC2)” are among the KCs of male reproductive toxicants (Table 4.3.1). There is abundant evidence that BPS induces these effects in experimental models, as summarized below.

Oxidative Stress

Oxidative stress results from an imbalance of reactive oxygen species (ROS) and antioxidants. ROS can be generated in the male reproductive tract by cells of the immune system, within sperm cells by reactions occurring in the mitochondria and plasma membrane, and within other cells and tissues important to male reproduction. Physiological levels of ROS play an important role in normal male reproductive function, including sperm capacitation and oocyte fertilization (Agarwal et al. 2014; Chen et al. 2013; Lavranos et al. 2012; Sabeti et al. 2016). However, when increases in ROS levels overwhelm cellular and tissue antioxidant capacity, this can potentially lead to damage or cell death, with pathological implications on male fertility (Agarwal et al. 2014; Sabeti et al. 2016). For example, oxidative stress can contribute to adverse effects on sperm, including tail defects, acrosome abnormalities, increased DNA damage, and decreased motility and viability (Chen et al. 2013; Lavranos et al. 2012; Sabeti et al. 2016). Spermatozoa are highly susceptible to oxidative damage due to their lack of DNA repair systems, low levels of antioxidant enzymes, and a plasma membrane rich in polyunsaturated fatty acids that are particularly susceptible to lipid peroxidation (Agarwal et al. 2014; Chen et al. 2013; Sabeti et al. 2016; Saleh and Agarwal 2002).

There is substantial *in vivo* and *in vitro* evidence demonstrating that BPS induces oxidative stress in testicular tissue, sperm cells, Leydig cells, and prostate cells. Evidence of oxidative stress was observed in animal models with gross and microscopic pathological findings in reproductive organs and altered spermatogenesis and sperm parameters. The following studies reported increased ROS production in BPS-treated rat testicular tissue, rat sperm cells, and a mouse Leydig cell line:

- Peroxide species (including generic peroxides, alkoxyl radicals, and peroxyl radicals), measured using the derivatives of reactive oxygen metabolites (D-ROM) assay (Hayashi et al. 2007) unless otherwise noted, were increased in:
    - The testes of adult male offspring of pregnant rats administered 50 µg/L BPS in drinking water from GD 1-21 (Ullah et al. 2019b).
    - The testes of PND 23 male rats administered 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021).
    - The testes of adult male rats orally administered (specific method not reported) 1, 5 (non-significant), 25, or 50 (non-significant) µg/kg-day BPS for 28 days (Ullah et al. 2016).
    - The testes of adult male rats gavaged with 50 mg/kg-day BPS for 28 days (Ullah et al. 2018a).
    - Adult rat testicular explants incubated with 100 ng/mL BPS for two hours (Ullah et al. 2018a; Ullah et al. 2016).

    - Adult rat sperm incubated with 100 µg/L BPS for two hours (Ullah et al. 2019a; Ullah et al. 2017).
    - Mouse TM3 Leydig cells incubated with 100, 200, or 400 µM BPS for 48 hours (dose-dependent), as measured by the dichlorodihydrofluorescein diacetate (DCFH-DA) assay which is reliant on the fluorescence emitted from the free radical oxidation of a DCFH-DA metabolite (Zhang W et al. 2022).
- Superoxide anion radical production, measured by blue formazan deposits formed by the reduction of nitroblue tetrazolium, was increased in mouse TM3 Leydig cell cultures incubated with 5 or 10 µg/mL BPS for 48 hours (Jambor et al. 2023).

One study reported a decrease in ROS levels in bovine adult spermatozoa from one bull with known fertility treated with 0.05 mg/mL BPS for four hours (Nguyen M et al. 2022).

Some studies found no effect on ROS and/or superoxide anion levels in prostate cell cultures and/or human spermatozoa (Caglayan and Ozden 2024; Castellini et al. 2021; Hyun et al. 2021).

The following studies reported increased lipid peroxidation in BPS-treated mouse, rat and hamster testicular tissue, rat sperm cells, and a mouse Leydig cell line:

- The testes of adult male mice gavaged with 20 or 200 mg/kg-day BPS for 28 days (Dai et al. 2021).
- The testes of adult male offspring of pregnant rats gavaged with 0.4 or 4 µg/kg-day BPS from GD 4-21 (Molangiri et al. 2022).
- The testes of adult male offspring of pregnant rats administered 50 µg/L BPS in drinking water from GD 1-21 (Ullah et al. 2019b).
- The testes of PND 21 male rats administered 50 µg/L BPS in drinking water for 10 weeks (Darghouthi et al. 2022).
- The testes of PND 21 and 23 male rats administered 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021).
- The testes of adult male rats orally administered (specific method not reported) 50 µg/kg-day BPS for 28 days (Ullah et al. 2016).
- The testes of adult male rats gavaged with 50 mg/kg-day BPS for 28 days (Ullah et al. 2018a).

- Adult rat testicular explants incubated with 1 (non-significant), 10 (non-significant), or 100 ng/mL BPS for two hours (dose-dependent) (Ullah et al. 2018a).
- Adult rat testicular explants incubated with 0.5, 1, 10, or 100 ng/mL BPS for two hours (Ullah et al. 2016).
- The testes of adult male hamsters orally administered (specific method not reported) 75 mg/kg-day BPS for 28 days (Kumar et al. 2020).
    - When BPS administration was accompanied by a 10 mg/kg/alternate day intraperitoneal injection of melatonin, no increase in lipid peroxidation was observed in the testes (Kumar et al. 2020). The study authors suggested that melatonin may have antioxidant effects in the testes by increasing levels of the antioxidant proteins NRF2/HO1 and SIRT1/FOXO1, and decreasing levels of the inflammatory proteins NFκB/COX2 (Kumar et al. 2020). Melatonin has been shown to reverse adverse effects on spermatogenesis caused by endocrine disruptors and other toxicants (Bhattacharya et al. 2019; Espino et al. 2011).
- Sperm from adult rats incubated with 100 µg/L BPS for two hours (Ullah et al. 2019a; Ullah et al. 2017).
- Mouse TM3 Leydig cells incubated with 100, 200, or 400 µM BPS for 48 hours (Zhang W et al. 2022).

BPS altered key components of the antioxidant system in mouse, rat and hamster testicular tissue, rat sperm cells, a mouse Leydig cell line, and a human prostate cell line. A summary of the observed effects is provided in Appendix C Table C1.

- Glutathione (GSH) levels were increased after 24 hours BPS treatment, likely as an initial response to an oxidative challenge, whereas a longer treatment caused a decrease, potentially demonstrating an overwhelmed antioxidant system.
    - Increased in RWPE-1 human prostate epithelial cells incubated with 108 µM BPS for 24 hours (Kose et al. 2020).
    - Decreased in the testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Dai et al. 2021).
- Glutathione reductase activity was increased in RWPE-1 human prostate epithelial cells incubated with 108 µM BPS for 24 hours (Kose et al. 2020).

- Superoxide dismutase (SOD) activity was altered with BPS treatment and demonstrated a directionality that was consistent with an overwhelmed antioxidant system.
    - Increased in adult rat testicular explants incubated with 100 µg/L BPS for two hours (Ullah et al. 2016).
    - Increased in adult rat sperm incubated with 100 µg/L BPS for two hours (Ullah et al. 2019a; Ullah et al. 2017).
    - Decreased in the testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Dai et al. 2021).
    - Decreased in the testes of adult male offspring of treated pregnant rats administered 50 µg/L BPS in drinking water from GD 1-21 (Ullah et al. 2019b).
    - Decreased in the testes of PND 21 and 23 male rats administered 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021).
    - Decreased in the testes of adult male rats orally administered (specific method not reported) 5, 25, or 50 µg/kg-day BPS for 28 days (Ullah et al. 2016).
    - Decreased in the testes of adult male hamsters orally administered (specific method not reported) 75 mg/kg-day BPS for 28 days (Kumar et al. 2020).
        - When BPS administration was accompanied by a 10 mg/kg/alternate day intraperitoneal injection of melatonin, no decrease in SOD activity was observed in the testes. It is suggested that melatonin may have antioxidant effects in the testes (see lipid peroxidation) (Kumar et al. 2020).
    - Decreased in mouse TM3 Leydig cells incubated with 200 or 400 µM BPS for 48 hours (Zhang W et al. 2022).
- One study found no effect on SOD level in prostate cells (Kose et al. 2020).
- Glutathione peroxidase (GPX) (and general peroxidase) activities were altered with BPS treatment and demonstrated a directionality that was consistent with an overwhelmed antioxidant system.

- Increased (non-significant) peroxidase activity in testicular explants of adult rats incubated with 0.5 or 100 ng/mL BPS for two hours (Ullah et al. 2016).
- Decreased GPX in testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Dai et al. 2021).
- Decreased peroxidase activity in testes of adult male offspring of pregnant rats administered 25 or 50 µg/L BPS in drinking water from GD 1-21 (Ullah et al. 2019b).
- Decreased GPX in testes of PND 21 male rats administered 50 µg/L BPS in drinking water for 10 weeks (Darghouthi et al. 2022).
- Decreased peroxidase activity in testes of PND 23 male rats administered 25 or 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b).
- Decreased peroxidase activity in testes of PND 22 male rats administered 5 or 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2021).
- Decreased peroxidase activity in testes of adult male rats orally administered (specific method not reported) 5, 25, or 50 µg/kg-day BPS for 28 days (Ullah et al. 2016).
- Decreased peroxidase activity in testes of adult male rats gavaged with 5, 25 (non-significant), or 50 (non-significant) mg/kg-day BPS for 28 days (Ullah et al. 2018a).
- Decreased GPX1 in RWPE-1 human prostate cell cultures incubated with 108 µM BPS for 24 hours (Kose et al. 2020).

- Catalase (CAT) activity was altered with BPS treatment and demonstrated a directionality that was consistent with an overwhelmed antioxidant system.
  - Increased (non-significant) in adult rat testicular explants incubated with 1, 10, or 100 ng/mL BPS for two hours (Ullah et al. 2016).
  - Decreased in the testes of adult male offspring of pregnant rats administered 50 µg/L BPS in drinking water from GD 1-21 (Ullah et al. 2019b).
  - Decreased in the testes of PND 22 male rats administered 5 or 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2021).
  - Decreased in the testes of PND 23 male rats administered 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b).

    - Decreased in the testes of adult male rats orally administered (specific method not reported) 5, 25, or 50 µg/kg-day BPS for 28 days (Ullah et al. 2016).
    - Decreased in the testes of adult male rats gavaged with 5, 25 (non-significant), or 50 mg/kg-day BPS for 28 days (Ullah et al. 2018a).
    - Decreased in the testes of adult male hamsters orally administered (specific method not reported) 75 mg/kg-day BPS for 28 days (Kumar et al. 2020).
        - When BPS administration was accompanied by a 10 mg/kg/alternate day intraperitoneal injection of melatonin, no effect on CAT activity was observed in the testes. It is suggested that melatonin may have antioxidant effects in the testes (see lipid peroxidation) (Kumar et al. 2020).
    - Decreased in mouse TM3 Leydig cell cultures incubated with 200 or 400 µM BPS for 48 hours (Zhang W et al. 2022).
- One study found no effect on the total antioxidant capacity level in prostate cells (Kose et al. 2020).

BPS altered expression of oxidative stress-related genes in mouse testicular tissue and bovine spermatozoa (See Table 4.3.2):

- The testes of PND 12 male offspring of pregnant mice administered 0.5, 20, or 50 µg/kg-day BPS by oral pipetting from GD 11 until birth (Shi et al. 2018).
- The testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Wang et al. 2022).
- Bovine adult spermatozoa from one bull with known fertility treated with 0.05 mg/mL BPS for four hours (Nguyen M et al. 2022).

BPS altered expression of oxidative stress-related proteins in mouse and hamster testicular tissue (See Table 4.3.3):

- The testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Wang et al. 2022).
- The testes of hamsters orally administered (specific method not reported) 75 mg/kg-day BPS for 28 days (Kumar et al., 2020). However, there were no effects on oxidative stress-related protein expression in the testes when BPS

administration was accompanied by a 10 mg/kg/alternate day intraperitoneal injection of melatonin. It is suggested that melatonin may have antioxidant effects in the testes (see lipid peroxidation) (Kumar et al. 2020).

One study found no effects on oxidative stress-related protein levels in bovine spermatozoa (Nguyen M et al. 2022).

Table 4.3.2 BPS effects on oxidative stress-related gene expression in testicular tissue, unless otherwise noted.

Gene	System	Exposure method	Dose/Duration	Direction	Reference
Cat	Mice, PND12 (*in utero* exposure)	Oral pipetting	0.5, 20 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Cat	Mice, adult	Gavage	200 mg/kg-day, 28 days	Downregulated	Wang et al. 2022
Gpx4	Mice, PND 12 (*in utero* exposure)	Oral pipetting	0.5 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Gpx4	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Downregulated	Wang et al. 2022
Gpx4	Bovine, adult spermatozoa	*In vitro* incubation	0.05 mg/mL of BPS for 4 hours	Downregulated	Nguyen M et al. 2022
Sod1	Mice, PND 12 (*in utero* exposure)	Oral pipetting	20 µg/kg-day, GD 11-birth	Upregulated	Shi et al. 2018
Sod1	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Downregulated	Wang et al. 2022
Sod1	Bovine, adult spermatozoa	*In vitro* incubation	0.05 mg/mL of BPS for 4 hours	Upregulated	Nguyen M et al. 2022
Sod2	Mice, PND 12 (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Keap1	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Upregulated	Wang et al. 2022
Nrf2, Nfe2l2	Mice, adult	Gavage	200 mg/kg-day, 28 days	Downregulated	Wang et al. 2022
Ho1, Hmox1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Downregulated	Wang et al. 2022

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).

Table 4.3.3 BPS effects on oxidative stress-related protein levels in testicular tissue.

Protein	System	Exposure method	Dose/Duration	Direction	Reference
KEAP1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Increased	Wang et al. 2022
NRF2, NFE2L2	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Decreased	Wang et al. 2022
NRF2, NFE2L2	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Decreased	Kumar et al. 2020
CAT	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Decreased	Wang et al. 2022
GPX4	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Decreased	Wang et al. 2022
SOD1	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Decreased	Wang et al. 2022
HO1, HMOX1	Mice, adult	Gavage	20, 200 mg/kg-day, 28 days	Decreased	Wang et al. 2022
HO1, HMOX1	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Decreased	Kumar et al. 2020
SIRT1	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Decreased	Kumar et al. 2020
FOXO1	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Decreased	Kumar et al. 2020

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).

Genetic damage

Toxicants can damage sperm DNA directly (e.g. DNA binding) or indirectly through processes such as oxidative stress (Delbes et al. 2009). Sustained cellular oxidative stress causes surges in mitochondrial ROS production that can lead to DNA strand breaks and base oxidation (Agarwal and Said 2005). Mature sperm have no DNA repair capabilities, leaving DNA damage unrepaired unless the spermatozoa successfully fertilize an egg, at which point the oocyte drives the remainder of the repair process. Without successful fertilization, sperm cells often undergo apoptosis (Aitken and Baker 2013; González-Marín et al. 2012). Additionally, there are natural characteristics of sperm cells that make them susceptible to DNA damage, such as protamine deficiency and a high level of endogenous ROS (González-Marín et al. 2012; Zini and Sigman 2009). Clinically, DNA damage in sperm is associated with decreased fertility rates, diminished embryo quality, and pregnancy loss (Ioannou et al. 2016; Zini and Sigman 2009).

There is *in vivo* and *in vitro* evidence that BPS induces genetic damage in testicular tissue, sperm cells, and prostate cells. Evidence of genetic damage was reported in animal studies with gross and microscopic pathological findings of reproductive organs, altered spermatogenesis and sperm parameters, and diminished reproductive performance.

The following studies reported increased genetic damage in BPS-treated mouse and rat testicular tissue, rat sperm, mouse paternal pronuclei in zygotes, a mouse spermatogonial cell line, and a human prostate cell line:

- 8-hydroxy-2′-deoxyguanosine (8-OHdG), a marker of oxidative DNA damage, was increased in the testes of:
    - Adult male offspring of lactating mice exposed to 21.6 µg/kg-day BPS via drinking water from PND 0-15, with no effects on HSP90 or PRDX6 levels at 0.216 or 21.6 µg/kg-day (Fenclová et al. 2022a).
    - Adult male offspring of pregnant rats administered 5 µg/kg-day BPS by oral pipetting from GD 6-21 with localization in primary spermatocytes (Kaimal et al. 2021).
- The DNA fragmentation index (also a marker of apoptosis) in sperm was increased in adult male offspring of pregnant rats gavaged with 0.4 or 4 µg/kg-day BPS from GD 4-21 (Molangiri et al. 2022).

- DNA damage, as measured using the Comet assay under conditions that may have included damage related to apoptosis, was increased in:
    - The sperm of adult rats gavaged with 25 and 50 µg/kg-day BPS for 28 days, with increased % tail DNA, number of comets per 100 cells, and/or tail moment (Ullah et al. 2019a; Ullah et al. 2017).
    - Adult rat sperm incubated with 1 µg/L (increased tail moment) and 100 µg/L BPS (increased tail moment and percent tail DNA) for two hours (Ullah et al. 2017).
    - Adult rat sperm incubated with 100 ng/mL BPS for two hours with increased % tail DNA, number of comets per 100 cells, and tail moment (Ullah et al. 2019a).
    - RWPE-1 human prostate epithelial cells incubated with 108 µM BPS for 24 hours with increased % tail intensity [with and without the oxidative lesion-specific enzyme, formamidopyrimidine-DNA glycosylase (FPG)] (Kose et al. 2020).
- Phosphorylated γH2AX, a marker of double-strand breaks and genomic instability, was increased in:
    - The testes of male mice administered 100 µg/kg-day BPS in drinking water for eight weeks (Řimnáčová et al. 2020).
    - Cultured C18-4 mouse spermatogonial cells incubated with 50 µM BPS for 1-3 days (increases in γHA2X-positive cells) with a decrease in DNA synthesis (Liang et al. 2017).
    - Increased number of γH2AX loci in paternal pronuclei of zygotes produced by mice males exposed to 0.2 or 20 ng/g-day BPS during lactation (by treating the dams) from PND 0-15 and untreated females. γH2AX was also increased in blastomeres at 20 ng/g-day. There was no effect on the apoptotic index at 0.2 or 20 ng/g-day (Fenclová et al., 2022a).
- Expression of the DNA damage repair gene, *MUTYH*, was decreased in RWPE-1 human prostate epithelial cells incubated with 108 µM BPS for 24 hours (Kose et al. 2020).

Some assays found no effects of genetic damage, including double-strand breaks or DNA integrity, in testicular germ cells, epididymal spermatozoa, a testicular cell co-culture model, and/or seminal vesicles (Fenclová et al. 2022a; Fenclová et al. 2022b; Yin et al. 2020).

Apoptosis

Apoptosis, or programed cell death, may occur as part of the normal life cycle of a cell, or as a "self-destruct" process when a cell's damage response and repair mechanisms are overcome by injury. It is initiated by a variety of triggers and comprised of multiple pathways and has both beneficial and detrimental consequences within the male reproductive system. Apoptosis has been described as a "quality control" mechanism in the testes, removing irreparably damaged germ cells from the seminiferous tubules, and is necessary for controlling spermatogenesis, including germ cell formation and maintenance of the germ to Sertoli cell ratio (Aitken and Baker 2013; Asadi et al. 2021; Latchoumycandane et al. 2020). Alterations in apoptosis in germ and somatic cells of the male reproductive system can adversely affect sperm production, motility, and viability. Studies have shown that infertile men have a higher level of germ cell apoptosis than fertile men (Aitken and Baker 2013; Asadi et al. 2021; Koppers et al. 2011; Makker et al. 2009).

There is *in vivo* and *in vitro* evidence that BPS induces apoptosis in testicular tissue, germ (and sperm) cells, Leydig cells, and prostate cells. Evidence of apoptosis was observed in animals with gross and microscopic pathological findings of the testes, altered spermatogenesis and sperm parameters.

The following studies reported increased apoptosis in BPS-treated mouse testicular tissue (germ cells), a mouse Leydig cell line, and a human prostate adenocarcinoma cell line:

- Shi et al. (2018) used the TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) assay to detect DNA fragmentation during apoptosis. There was an increased proportion of TUNEL-positive germ cells and TUNEL-positive germ cells per seminiferous tubule in PND 12 male offspring of pregnant mice administered 0.5, 20, or 50 µg/kg-day by oral pipetting from GD 11 until birth. There was no effect on germ cell number per tubule. The TUNEL assay was negative for PND 60 animals.
- Dai et al. (2021) reported that the spermatogenic apoptotic index, calculated as a ratio of apoptotic cells to non-apoptotic cells, was increased in adult male mice gavaged with 200 mg/kg-day BPS for 28 days.
- Li et al. (2023) observed that apoptosis rate was dose-dependently increased in mouse TM3 Leydig cells incubated with 20, 40, or 80 µM BPS for 72 hours.

- There was an increase in late apoptosis (identified by Annexin -V/-FITC/PI flow cytometry) in human PC-3 prostate cells incubated with 1 or 10 µM BPS for 48 hours, with no effect on PTN1A prostate cells (Caglayan and Ozden 2024).

One study found no effects on apoptosis in the testes (Shi et al. 2019).

Alterations in apoptosis-related gene expression were observed in the testes of PND 12 male offspring of pregnant mice administered 0.5, 20, or 50 µg/kg-day BPS by oral pipetting from GD 11 until birth. There was a downregulation of the *Bad* and *Bax* genes, and an upregulation of the *Cycs* genes (Shi et al. 2018). There were no effects on apoptosis-related gene expression in PC-3 or PTN1A prostate cells (Caglayan and Ozden 2024).

BPS altered apoptosis-related protein expression in mouse, rat, and hamster testicular tissue, a human prostate cell line, and a mouse Leydig cell line (See Table 4.3.4):

- Testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Dai et al. 2021).
- Testes of adult male offspring of pregnant rats gavaged with 0.4 or 4 µg/kg-day BPS from GD 4-21 (Molangiri et al. 2022).
- Testes of adult male hamsters orally administered 75 mg/kg-day BPS for 28 days (Kumar et al. 2020).
    - When BPS administration was accompanied by a 10 mg/kg/alternate day intraperitoneal injection of melatonin, no effect on apoptosis-related protein expression was observed in the testes. It is suggested that melatonin may have antioxidant effects in the testes (see lipid peroxidation) (Kumar et al. 2020).
- Human PNT1A prostate cells incubated with 10 µM BPS for 48 hours, with no effect in PC-3 prostate cells (Caglayan and Ozden 2024). Further, in a molecular mechanism investigation of BPS-induced prostate injury, core targets of BPS included cell cycle-, apoptosis-, and proliferation-related proteins (Huang 2024).
- Mouse TM3 Leydig cells incubated with 100, 200 or 400 µM BPS for 48 hours (Zhang W et al. 2022).
- Mouse TM3 Leydig cells incubated with 20, 40, or 80 µM BPS for 72 hours (Li et al. 2023).

Table 4.3.4 BPS effects on apoptosis-related proteins

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
BCL2	Mouse, adult	Gavage	20, 200 mg/kg-day, 28 days	Testes	Decreased	Dai et al. 2021
BCL2	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Decreased	Kumar et al. 2020
BCL2	Mouse TM3 cell culture	-	20, 40, 80 μM, 72 hours	Leydig cells	Decreased dose-dependently	Li et al. 2023
BCL2	Mouse TM3 cell culture	-	200, 400 μM, 48 hours	Leydig cells	Decreased	Zhang W et al. 2022
BAX	Mouse, adult	Gavage	200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
BAX	Mouse TM3 cell culture	-	100, 200, 400 μM, 48 hours	Leydig cells	Increased	Zhang W et al. 2022
BAX	Mouse TM3 cell culture	-	20, 40, 80 μM, 72 hours	Leydig cells	Increased	Li et al. 2023
BCL2/BAX ratio	Mouse, adult	Gavage	20, 200 mg/kg-day, 28 days	Testes	Decreased	Dai et al. 2021
BCL2/BAX ratio	Mouse TM3 cell culture	-	100, 200, 400 μM, 48 hours	Leydig cells	Decreased	Zhang W et al. 2022

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
Cleaved CASP3	Mouse, adult	Gavage	20, 200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
Cleaved CASP3	Rats, adult (*in utero* exposure)	Gavage	0.4, 4 µg/kg-day, GD 4-21	Testes	Increased	Molangiri et al. 2022
Cleaved CASP8	Mouse, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
Cleaved CASP9	Mouse	Gavage	20, 200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
FAS	Mouse, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
FASL	Mouse, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Increased	Dai et al. 2021
CHOP, DDIT3	Human PNT1A cell culture	-	10 µM, 48 hours	Prostate	Increased	Caglayan and Ozden 2024
CASP3	Rats, adult (*in utero* exposure)	Gavage	4 µg/kg-day, GD 4-21	Testes	Increased	Molangiri et al. 2022
CASP3	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Increased	Kumar et al. 2020
CASP3	Mouse TM3 cell culture	-	20, 40, 80 µM, 72 hours	Leydig cells	Increased	Li et al. 2023

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
CASP8	Rats, adult (*in utero* exposure)	Gavage	4 µg/kg-day, GD 4-21	Testes	Increased	Molangiri et al. 2022
CASP9	Mouse TM3 cell culture	-	40, 80 µM, 72 hours	Leydig cells	Increased	Li et al. 2023

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).

4.3.2 Epigenetic Alterations

Mechanistic evidence suggests that BPS induces epigenetic alterations in the male reproductive system. “Induces epigenetic alterations” is KC6 of male reproductive toxicants (Table 4.3.1) (Arzuaga et al. 2019).

Epigenetics refers to the regulation of gene expression through heritable and stable modifications that do not involve changes to the DNA sequence. Epigenetic processes, including DNA/RNA methylation, post-translational histone modification, chromatin packaging, and regulation of gene expression by non-coding RNAs (e.g., microRNAs), affect the activity and availability of DNA for expression (Arzuaga et al. 2019; Donkin and Barrès 2018; Rotondo et al. 2021). Throughout spermatogenesis, epigenetic modifications drive the regulation of the genes necessary to produce functionally mature sperm (Jenkins and Carrell 2011; Rotondo et al. 2021). During sperm maturation, protamines replace histones to create tightly packaged DNA protected from damage. However, histones that are retained play an important role in offspring development and transgenerational inheritance and, with other components of the sperm epigenome, have the potential to be altered by environmental toxicants (Belleau et al. 2018; Ben Maamar et al. 2018; Carrell 2012; Siklenka et al. 2015). Epigenetic modifications in sperm may be passed on to offspring with potential for inheritance across multiple generations (Youngson and Whitelaw 2008). For example, sperm DNA methylation is important for genomic imprinting, the determination of allelic expression in offspring via methylation of specific genes in parental gametes, and altering sperm DNA methylation patterns may have adverse effects on male fertility, embryonic development, and offspring disease susceptibility (Aston et al. 2015; Jenkins et al. 2014; Jenkins et al. 2016; Kitamura et al. 2015; Urdinguio et al. 2015).

There is some *in vivo* and *in vitro* evidence that BPS induces epigenetic alterations in global DNA methylation, adenosine methylation, and changes in the expression of genes and proteins involved in epigenetic processes in testicular tissue, sperm cells, Sertoli cells, and Leydig cells. Evidence of epigenetic changes was observed in animals with gross and microscopic pathological findings of the testes, altered spermatogenesis and sperm parameters, altered anogenital distance, and diminished reproductive performance (see section 4.2 for details).

The following studies reported evidence of epigenetic alterations in global DNA methylation in BPS-treated zebrafish testicular tissue and a mouse spermatocyte cell line and increases in adenosine methylation in a mouse Leydig cell line.

- Increases in global DNA methylation:
    - Testes of 120 day post-fertilization (dpf) F1 offspring of F0 zebrafish exposed to 100 µg/L BPS from 3 hours post-fertilization (hpf) to 120 dpf (Hao et al. 2022).
    - Mouse spermatocyte GC-2 cells incubated with 10^{-8} M BPS for 1–3 days at 72 hours (Sidorkiewicz et al. 2018).
    - Decreases in global DNA methylation in the testes of 120 dpf zebrafish exposed to 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).
    - Decreased expression of N6 methyladenosine (m6A) in mouse TM3 Leydig cell cultures incubated with 20, 40, or 80 µM BPS for 72 hours (Li et al. 2023). N6-methyladenosine (m6A) is a reversible mRNA modification that is essential in the male reproductive system and is controlled by a methyltransferase complex, demethylases including FTO, and regulatory binding proteins such as YT521-B homology (YTH) proteins (Lan et al. 2021; Li et al. 2023; Lin et al. 2022; Qi et al. 2022; Zhang W et al. 2022).

One study found no effect on global DNA methylation in rat sperm (Molangiri et al. 2022).

BPS altered the expression of several genes involved in DNA and histone methylation and demethylation and other epigenetic processes in mouse, rat, and zebrafish testicular tissue (See Table 4.3.5):

- Testes of PND 12 male offspring of pregnant mice administered 0.5, 20, or 50 µg/kg-day BPS by oral pipetting from GD 11 until birth (Shi et al. 2018).
- Testes of PND 6 F3 male offspring of pregnant mice administered 0.5 or 50 µg/kg-day BPS by oral pipetting from GD 7 until birth (Shi et al. 2019).
- Testes of adult male rats administered a subcutaneous dose of 10 mg/kg BPS every three days from PND 0–60 (Shi et al. 2017).
- Testes of 120 dpf F1 and F2 offspring of F0 zebrafish exposed to 1 or 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).

BPS altered epigenetic-related proteins (post-translational changes, and changes in protein levels) in mouse germ and Sertoli cells, and rat sperm cells (Table 4.3.6):

- Increased H3K4me2 in testicular germ cells of adult male offspring of nursing mice administered 37.5 ng/mL BPS via drinking water from PND 0–15 (Fenclová et al. 2022b). H3K4me2 (dimethylation of lysine [K4] on histone H3) is involved in chromatin opening during the final stages of spermatogenesis, with altered levels being associated with decreased sperm quality (Štiavnická et al. 2020).
- Altered phosphorylation of DNMT3A & B, H3K4me3, and H3K27me3 in Sertoli and germ cells in adult F3 male offspring of F0 pregnant mice administered 0.5 or 50 µg/kg-day BPS by oral pipetting from GD 7 until birth (Shi et al. 2019).
- Increased DNMT protein expression in the sperm of adult offspring of pregnant rats gavaged with 0.4 or 4.0 µg/kg-day BPS from GD 4-21 (Molangiri et al. 2022).

Table 4.3.5 BPS effects on epigenetic-related genes in testes

Gene	System	Exposure method	Dose/Duration	Direction	Reference
tet1	Zebrafish, whole body, 120 dpf F1 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Upregulated	Hao et al. 2022
tet3	Zebrafish, whole body, 120 dpf F1 and F2 offspring of treated F0	-	1 (F2), 100 (F1) µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
Dnmt1	Mice, PND 12 offspring (*in utero* exposure)	Oral pipetting	20 µg/kg-day, GD 11-birth	Upregulated	Shi et al. 2018
Dnmt1	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
dnmt1	Zebrafish, whole body, 120 dpf F1 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
Dnmt3a	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Dnmt3b	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	50 µg/kg-day, GD 11-birth	Upregulated	Shi et al. 2018
Dnmt3b	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Dnmt3l	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
dnmt3, dnmt3bb.2	Zebrafish, whole body, 120 dpf F1 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022

Gene	System	Exposure method	Dose/Duration	Direction	Reference
dnmt4, dnmt3bb.1	Zebrafish, whole body, 120 dpf F1 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
dnmt5, dnmt3bb.3	Zebrafish, whole body, 120 dpf F1 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
dnmt6, dnmt3ab	Zebrafish, whole body, 120 dpf F2 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
dnmt7, dnmt3ba	Zebrafish, whole body, 120 dpf F1 and F2 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated	Hao et al. 2022
dnmt8, dnmt3aa	Zebrafish, whole body, 120 dpf F1 and F2 offspring of treated F0	-	1, 100 µg/L, 3 hpf to 120 dpf	Downregulated (upregulated in F2 low dose)	Hao et al. 2022
Dot1l	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	20, 50 µg/kg-day, GD 11-birth	Upregulated	Shi et al. 2018
Dot1l	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Setd1a	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	0.5 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Setd1b	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Setd1b	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019

Gene	System	Exposure method	Dose/Duration	Direction	Reference
Suz12	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Suz12	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Downregulated	Shi et al. 2018
Kmt2a	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Kmt2b	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Kmt2c	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Kmt2d	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Kmt2e	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Kmt2e	Mice, PND 12 males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Upregulated	Shi et al. 2018
Eed	Mice, PND 6 F3 males (F0 exposure)	Oral pipetting	50 µg/kg-day, GD 7-birth	Upregulated	Shi et al. 2019
Rhox5	Mice, adult	sc injection	10 mg/kg every 3 days, PND 0–60	Upregulated	Shi et al. 2017

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).
dpf = days post-fertilization, GD = gestation day, hpf = hours post-fertilization, PND = post-natal day, sc = subcutaneous

Table 4.3.6 BPS effects on epigenetic-related proteins

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
H3K4me2	Mice, adult (nursing exposure)	Drinking water	37.5 ng/mL, PND 0-12.	Testes	Increased	Fenclová et al. 2022b
p-DNMT3A	Mice, PND 6 F3 offspring (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Sertoli cells	Increased	Shi et al. 2019
p-DNMT3B	Mice, PND 6 F3 offspring (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Sertoli cells	Increased	Shi et al. 2019
p-H3K4me3	Mice, PND 6 F3 offspring (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Germ cells	Decreased	Shi et al. 2019
p-H3K27me3	Mice, PND 6 F3 offspring (F0 exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Germ cells	Decreased	Shi et al. 2019
DNMT3B	Rats, adult offspring (*in utero* exposure)	Gavage	4 µg/kg-day, GD 4-21	Sperm	Increased	Molangiri et al. 2022
DNMT3A	Rats, adult offspring (*in utero* exposure)	Gavage	0.4, 4 µg/kg-day, GD 4-21	Sperm	Increased	Molangiri et al. 2022
YTHDF1	Mouse TM3 cell line	-	20, 40, 80 µM, 72 hours	Leydig cells	Decreased	Li et al. 2023
FTO	Mouse TM3 cell line	-	20, 40, 80 µM, 72 hours	Leydig cells	Increased	Li et al. 2023

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).
GD = gestation day, PND = post-natal day

4.3.3 Effects on the Endocrine System

Mechanistic evidence suggests that BPS alters the production and levels of reproductive hormones and hormone receptor levels. “Alters production and levels of reproductive hormones” and “alters hormone receptor levels/functions” are two of the KCs of male reproductive toxicants (KCs 3 & 4) (See Table 4.3.1). In addition, a related set of key characteristics of endocrine-disrupting chemicals (EDCs) has been described by La Merrill et al. (2020) and is presented in Table 4.3.7. Relevant findings from studies of BPS can be considered in the context of both the KCs of male reproductive toxicants and the KCs of EDCs.

Table 4.3.7 Key characteristics of endocrine-disrupting chemicals (EDC)

EDC KC 1 Interacts with or activates hormone receptors
EDC KC 2 Antagonizes hormone receptors
EDC KC 3 Alters hormone receptor expression
EDC KC4 Alters signal transduction in hormone-responsive cells
EDC KC5 Induces epigenetic modifications in hormone-producing or hormone-responsive cells
EDC KC6 Alters hormone synthesis
EDC KC7 Alters hormone transport across cell membranes
EDC KC8 Alters hormone distribution or circulating hormone levels
EDC KC9 Alters hormone metabolism or clearance
EDC KC10 Alters fate of hormone-producing or hormone-responsive cells

The endocrine system controlling male reproduction is comprised of organs along the hypothalamic-pituitary-gonadal (HPG) axis, secreting glands, circulating hormones, and regulatory molecules, and is responsible for maintaining various aspects of male reproduction, including sexual behavior, testicular function, and semen quality.

Several chemicals are known to cause adverse effects on male reproductive function by interfering with one or more components of the endocrine system. Agonism/antagonism of the androgen receptor (AR) and/or estrogen receptors (ERα, ERβ, and G protein-

coupled ER) has been shown to cause adverse effects on sexual development, the male phenotype, and the maintenance of reproductive functions, among other adverse male reproductive effects (Arzuaga et al. 2019; Dent et al. 2015; Gill et al. 1979; Henley and Korach 2006; Hotchkiss et al. 2008; Matsumoto et al. 2008; Ramesh et al. 2022; Scott et al. 2009; Semet et al. 2017; Wilson et al. 2008). Changes in the concentration of testosterone, the primary circulating androgen in males, are associated with impaired spermatogenesis, sperm maturation, and sperm release, resulting in decreased semen quality (Dutta et al. 2019). The gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are essential in androgen production and spermatogenesis and are key regulators of testicular function (De Ronde et al. 2005; Dutta et al. 2019; Sengupta and Dutta 2019). Disruption of LH and FSH have direct impacts on androgen production and sperm parameters as well as sexual behavior (Dutta et al. 2019; Sengupta and Dutta 2019). Estradiol (E2), the primary form of estrogen, plays an important role in brain masculinization during development and is necessary for regulating male libido, erectile function, spermatogenesis, and sperm quality. It plays several supportive roles in Leydig and Sertoli cell function, and in germ cells during spermatogenesis. Low testosterone and high E2 levels are associated with erectile dysfunction, decreased semen parameters, and infertility in men (Dutta et al. 2019; Mancini et al. 2005; Schulster et al. 2016).

There is considerable *in vivo* and *in vitro* evidence that BPS induces endocrine changes, as observed in testicular tissue, Leydig cells, spermatocytes, prostate, brain, liver, mammary tissue, blood, and urine. Evidence of endocrine effects was observed in animals with gross and microscopic pathological findings in the testes and epididymides, altered spermatogenesis and sperm parameters, altered mammary gland development, and diminished reproductive performance.

The following summarizes studies reporting evidence of endocrine effects in BPS-treated human, mouse, rat, hamster, and zebrafish testicular tissue; isolated rat Leydig cells and mouse Leydig cell lines; a mouse spermatocyte cell line; gerbil prostate; zebrafish brain; zebrafish and guppy liver; mouse mammary tissue; mouse thyroid; mouse, rat, hamster, and zebrafish blood; and mouse urine.

Gonadotropins

- Decreased plasma LH and FSH levels:
    - Adult male offspring of pregnant rats treated with 50 µg/L BPS in drinking water from GD 1–21 (Ullah et al. 2019b).
    - PND 23 male rats exposed to 50 µg/L BPS in drinking water for 48 weeks (Ullah et al. 2018b; Ullah et al. 2021).

One study found no effect on gonadotropin levels in serum (Řimnáčová et al. 2020).

Cholesterol

- Decreased cholesterol levels (matrix not reported):
    - Adult male rats gavaged with 300 or 1,000/[600] mg/kg-day BPS for 90 days (BASF 2014).
    - Adult male rats gavaged with 300 or 600 mg/kg-day BPS for 28 days (BASF 2020).

Androgens

- Altered testosterone levels:
    - Increased in the serum of adult male mice treated subcutaneously with 10 mg/kg BPS every three days from PND 0–60 (Shi et al. 2017).
    - Increased in the plasma of adult male offspring of pregnant rats gavaged with 0.4 µg/kg-day BPS from GD 4–21 (Molangiri et al. 2022).
    - Decreased in the serum of F3 adult male offspring of F0 pregnant mice administered 50 µg/kg-day BPS by oral pipetting from GD 7 until birth (Shi et al. 2019).
    - Decreased in the serum of adult male mice gavaged with 20 or 200 mg/kg-day BPS for 28 days (Wang et al. 2022).
    - Decreased dose-dependently in the testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS for 28 days (Wang et al. 2022).
    - Decreased in human and mouse testicular explant cultures incubated with 1,000 or 10,000 nM BPS and 100, 1,000, or 10,000 nM BPS, respectively, for 3–4 days in basal conditions on Days 1, 2, and 3 (Eladak et al. 2015).

- Decreased in the plasma of adult male offspring of pregnant rats administered 50 µg/L BPS in drinking water from GD 1–21 (Ullah et al. 2019b).
- Decreased in the plasma of PND 22-23 male rats administered in drinking water for 48 weeks with 25 or 50 µg/L BPS (Ullah et al. 2018b), or 5 or 50 µg/L BPS (Ullah et al. 2021).
- Decreased in testicular explants (basal) and isolated Leydig cells (basal and LH stimulated) of PND 21 male rats administered 5 µg/L BPS in drinking water for 14 days, with no effects on serum levels on PND 21 or 35 or testicular levels on PND 35 (Jeminiwa et al. 2021).
- Decreased in the serum of adult male rats orally administered for 28 days with 1 or 50 µg/kg-day BPS (Ullah et al. 2016), or with 5 (non-significant), 25 (non-significant), or 50 mg/kg-day BPS (Ullah et al. 2018a).
- Decreased in the testes of adult male rats orally administered for 28 days with 50 µg/kg-day BPS (Ullah et al. 2016) or with 5, 25 (non-significant), or 50 mg/kg-day BPS (Ullah et al. 2018a).
- Decreased in the serum of adult male hamsters orally administered 75 mg/kg-day BPS for 28 days (Kumar et al. 2020).
- Decreased in the plasma of male zebrafish exposed to 10 or 100 µg/L BPS from 2–75 dpf (Naderi et al. 2014).
- Decreased in the whole blood of 120 dpf F1 male offspring of F0 zebrafish exposed to 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).
- Decreased in the plasma of adult male zebrafish exposed to 50 µg/L BPS for 21 days (Ji et al. 2013).
- Decreased in the whole body of adult male zebrafish exposed to 8, 40, or 200 µg/L BPS for 21 days (Park et al. 2022).
- Decreased (non-significant) in mouse TM3 Leydig cells incubated with 50 µg/mL BPS for 24 hours (it should be noted that there was no adjustment for significant decreases in cell viability at these doses) (Jambor et al. 2019).
- Decreased in mouse TM3 Leydig cells incubated with 10 (non-significant), 25, or 50 µg/mL BPS for 48 hours (it should be noted that there was no adjustment for significant decreases in cell viability at these doses) (Jambor et al. 2023).

- Decreased levels of 11-ketotestosterone, a testosterone derivative, in the testes of adult male zebrafish exposed to 40 or 200 µg/L BPS for 21 days (Park et al. 2022).

Some studies found no effects on testosterone levels in serum, testicular tissue, or Leydig cells (Kaimal et al. 2021; Morimoto et al. 2022; Řimnáčová et al. 2020; Roelofs et al. 2015; Silva et al. 2019; Ullah et al. 2018a; Ullah et al. 2016).

Estradiol

- E2 levels were increased in the following biological matrices:
    - Serum of adult male offspring of pregnant mice administered 50 µg/kg-day BPS by oral pipetting from GD 11 until birth (Shi et al. 2018).
    - Serum of adult male mice administered a 50 µg/kg or 10 mg/kg subcutaneous dose of BPS every three days from PND 0–60 (Shi et al. 2017).
    - Urine (creatinine adjusted and unadjusted) of adult male mice administered a single subcutaneous dose of 3 mg BPS (~65 mg/kg) at 4 hours post-injection and decreased at 1 (~22.5 mg/kg) or 3 mg BPS at 10 hours post-injection (Pollock et al. 2019).
    - Plasma of adult male offspring of pregnant rats administered 50 µg/L BPS in drinking water from GD 1–21 (Ullah et al. 2019b).
    - Plasma of PND 21 and 23 male rats orally administered in drinking water for 48 weeks with 50 µg/L BPS (Ullah et al. 2018b) or with 0.5 (non-significant), 5, or 50 µg/L BPS (dose-dependent) (Ullah et al. 2021).
    - Testes of PND 21 male rats treated with 5 µg/L BPS in drinking water for 14 days, with no effects on levels in serum on PND 21 or 35 or testicular or Leydig cell on PND 35 (Jeminiwa et al. 2021).
    - Plasma of male zebrafish exposed to 0.5, 5, or 50 µg/L BPS for 21 days (Ji et al. 2013), or 1, 10, or 100 µg/L BPS from 2–75 dpf (Naderi et al. 2014).
    - Whole blood of F1 and F2 male offspring of F0 zebrafish exposed to 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).
    - Testes of adult male zebrafish exposed to 8, 40, or 200 µg/L BPS for 21 days (dose-dependent) (Park et al. 2022).

- Serum E2 was decreased (NS; dose-dependently) in adult male mice one hour after administration of a single subcutaneous dose of 1 (~25.4 mg/kg), 3 (~76.8 mg/kg), or 9 mg (~218.9 mg/kg) followed by a 14.5 ng dietary dose of 5 µCi ^{3}H-E2 30 minutes post-injection (Pollock et al. 2019).

One study found no effect on E2 level in serum (Silva et al. 2019).

- Increased E2/testosterone ratio in:
    - Plasma of zebrafish (3–4 months old) exposed to 0.5, 5, or 50 µg/L BPS for 21 days (Ji et al. 2013).
    - Whole blood of F1 and F2 offspring of F0 zebrafish exposed to 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).

Increased aromatase activity, an enzyme responsible for converting androgens into estrogens, was observed in the testes of adult male zebrafish exposed to 8, 40, or 200 µg/L BPS for 21 days (Park et al. 2022).

HPG axis signaling

Estrogen stimulates cellular proliferation via signaling cascades such as the epidermal growth factor receptor/extracellular signal-regulated kinases (EGFR/ERK) pathway (Albanito et al. 2007).

- In mouse spermatocyte GC-2 cells incubated with 10^{-14} – 10^{-4} M BPS for 24-72 hours, cell viability increased at 10^{-9} – 10^{-6} M at 24 hours, decreased in the presence of estrogen receptor 1 and 2 agonists and an EGFR inhibitor, and increased with combined GPR30 (a membrane estrogen receptor) and estrogen receptor inhibitors and combined EGFR and mitogen-activated ERK-activating protein kinase (MEK1/2) inhibitors (Sidorkiewicz et al. 2018).
- There were increases in tyrosine hydroxylase immunoreactive cell bodies in the hypothalamus of male rats administered a daily subcutaneous injection of 2 or 200 µg/kg-day BPS from PND 1–27 (John et al. 2019). The anteroventral periventricular nucleus (AVPV) of the hypothalamus contains sexually dimorphic tyrosine hydroxylase-positive cells which are generally fewer in number in male brains. Tyrosine hydroxylase neurons are rate-limiting in dopamine synthesis and dopaminergic innervations of the AVPV regulate gonadotropin-releasing hormone. Thus, tyrosine hydroxylase-positive neurons in the male hypothalamus are a sensitive region for endocrine disruption and may be indicative of demasculinization within the HPG axis (John et al. 2019; Simerly et al. 1985).

Thyroid hormones and receptors

The thyroid hormones triiodothyronine (T3) and thyroxine (T4) are important for regulating male reproductive function and testicular development. Alterations in thyroid hormones are associated with decreased testosterone, increased E2, increased testicular oxidative damage, deteriorated semen quality, and male reproductive disorders (Abalovich et al. 1999; Alahmar et al. 2019; Carani et al. 2005; Hernández et al. 1990; Sahoo et al. 2008; Wortsman et al. 1987).

- Thyroid hormones and receptors were altered in mice and zebrafish:
    - Decreased thyroid stimulating hormone receptor (TSHR) in the thyroid gland at 0.02 mg/kg-day, increased serum thyroid-stimulating hormone (TSH) and T3 (non-significant) levels at 20 mg/kg-day, and increased serum T4 levels (non-significant) at 2 or 20 mg/kg-day BPS in adult male mice orally administered BPS for five weeks (Hu et al. 2023).
    - Decreased in serum T3 and T4 levels in adult male mice gavaged with 150 mg/kg-day BPS for 28 days (Sahu and Verma 2023).
    - Decreased plasma T3 and T4 levels in male zebrafish exposed to 10 or 100 µg/L BPS from 2–75 dpf (Naderi et al. 2014).
    - Increased whole body T3 and T4 in adult (3–4 month old) male zebrafish exposed to 8, 40, or 200 µg/L BPS for 21 days (dose-dependent) (Park et al. 2022).
- One study found no effect on thyroid hormone levels in serum (Řimnáčová et al. 2020).

Melatonin and Progestins

- Serum melatonin was decreased in hamsters orally administered 75 mg/kg-day BPS (specific method not reported) for 28 days (Kumar et al. 2020). Melatonin regulates male reproduction by influencing gonadotropin release, gonadal steroids, and testicular function. It has antioxidant and anti-apoptotic properties, helping to protect the testes from oxidative damage (Bhattacharya et al. 2019; Shiu et al. 2003). Melatonin has been shown to attenuate adverse effects on spermatogenesis caused by endocrine disruptors and other toxicants (Bhattacharya et al. 2019; Espino et al. 2011).
- Pregnenolone and progesterone levels were increased in mouse MA-10 Leydig cells incubated with 10 µM BPS for 48 hours (Roelofs et al. 2015).

One study found no effect on serum progesterone level (Řimnáčová et al. 2020).

Some studies found no effects on other hormone levels, including growth hormone, cortisol, or adrenocorticotropic hormone, in serum or whole-body zebrafish (Park et al. 2022; Řimnáčová et al. 2020).

Endocrine-related genes

BPS altered the expression of several endocrine-related genes (See Table 4.3.8 for details):

- Increased steroidogenic-related gene expression in:
    - Testes of adult male offspring of pregnant mice administered 0.5, 20, or 50 µg/kg-day BPS by oral pipetting from GD 11 until birth (Shi et al. 2018).
    - Testes of adult F3 male offspring of F0 pregnant mice administered 0.5 or 50 µg/kg-day BPS from GD 7 until birth (Shi et al. 2019).
    - Testes of adult male mice gavaged daily with 2, 20, or 200 mg/kg-day BPS for 28 days (Wang et al. 2022).
    - Brain and testes of 120 dpf F1 and F2 adult male offspring of F0 zebrafish exposed to 1 or 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).
    - Brain and testes of adult zebrafish exposed to 50 µg/L BPS for 21 days (Ji et al. 2013).
    - Mouse spermatocyte GC-2 cells incubated with 10^{-10} or 10^{-8} M BPS for 24-72 hours (Sidorkiewicz et al. 2018).
    - Mouse MA-10 Leydig cells incubated with 10 µM BPS for 48 hours (Roelofs et al. 2015).
- Steroid receptor gene expression was altered in:
    - Brain and testes of 120 dpf F1 and F2 adult male offspring of F0 zebrafish exposed to 1 or 100 µg/L BPS from 3 hpf to 120 dpf (Hao et al. 2022).
    - Liver of adult male zebrafish exposed to 40 or 200 µg/L BPS for 21 days (*erβ* but not *erα*) (Park et al. 2022).
    - Mouse spermatocyte GC-2 cells incubated with 10^{-10} or 10^{-8} M BPS for 24-72 hours (Sidorkiewicz et al. 2018).
- Increased *vtg1* in the liver of adult male zebrafish exposed to 200 µg/mL BPS for 21 days (Park et al. 2022).

- There was a decrease (non-significant) in the expression of the Leydig cell-specific gene, *Insl3* (insulin-like 3 peptide) and *Star* (steroidogenic acute regulatory protein), which encodes a hormone essential in the development of the gubernaculum for the initial descent of the testes, in mouse testicular explants incubated with 10,000 nM BPS for 3 days (basal and LH-stimulated) (Eladak et al. 2015).

One study found no effect on steroidogenesis-related gene expression in the testes (Shi et al. 2017).

Endocrine-related proteins

BPS altered the expression of several endocrine-related proteins (See Table 4.3.9 for details):

- Steroidogenic proteins were altered in:
    - Testes of adult male mice gavaged with 2, 20, or 200 mg/kg-day BPS (Wang et al. 2022).
    - Testes of PND 35 male rats administered 5 µg/L BPS in drinking water for 14 days (Jeminiwa et al. 2021).
- Steroid receptor proteins were altered in:
    - Mammary mesenchyme of GD 16 male offspring of pregnant mice administered 2 or 200 µg/kg-day BPS in drinking water from GD 9–16 with no AR staining in epithelial cells (Kolla et al. 2019).
    - Testes of adult male offspring of pregnant rats gavaged with 0.4 or 4 µg/kg-day BPS from GD 4–21 (Molangiri et al. 2022).
    - Testes of adult hamsters orally administered 75 mg/kg-day BPS (specific method not reported) for 28 days (Kumar et al. 2020).
    - Increased AR-positive staining in the epithelium and stroma of the ventral prostate of adult male gerbils orally administered 40 µg/kg-day BPS (specific method not reported) for 28 days (Silva et al. 2019).
- Thyroid hormone-related proteins THRA and DIO2 were decreased in the testes of adult male mice gavaged with 150 mg/kg-day BPS for 28 days (Sahu and Verma 2023).

- Decreased melatonin receptor expression in the testes of adult hamsters orally administered 75 mg/kg-day BPS (specific method not reported) for 28 days (Kumar et al. 2020).

- Vitellogenin (Vtg) expression was increased in the caudal fin, liver, and whole body of adult male guppies exposed to 1, 10, and 100 µg/L for 21 days (Wang et al. 2017).

Table 4.3.8 BPS effects on endocrine-related genes

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
Steroidogenic genes						
Star	Mice, adult males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Testes	Upregulated	Shi et al. 2018
Star	Mice, F3 adult males (*in utero* exposure)	Oral pipetting	0.5 or 50 µg/kg-day, GD 7-birth	Testes	Upregulated	Shi et al. 2019
Star	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Upregulated	Wang et al. 2022
star	Zebrafish, 120 dpf F1 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Upregulated	Hao et al. 2022
star	Zebrafish, 120 dpf F2 (F0 exposure)	-	100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
Star	Mouse, testicular explant culture	-	10,000 nM (NS), 3 days	Testicular (basal and LH-stimulated)	Downregulated	Eladak et al. 2015
Star	Mouse GC-2 cell line	-	10^{-8}, 10^{-10} M, 48 and 72 hours	Spermatocyte	Upregulated	Sidorkiewicz et al. 2018
Cyp11a1	Mice, F3 adult males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Testes	Upregulated	Shi et al. 2018
Cyp11a1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Upregulated	Wang et al. 2022

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
cyp11a1.1	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
cyp11a1.1	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
Cyp11a1	Mouse GC-2 cell line	-	10^{-8}, 10^{-10} M, 48 and 72 hours	Spermatocyte	Upregulated	Sidorkiewicz et al. 2018
Cyp17a1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Upregulated	Wang et al. 2022
cyp17, cyp17a1	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Downregulated	Ji et al. 2013
cyp17, cyp17a1	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
Cyp17a1	Mouse MA-10 cell line	-	10 µM, 48 hours	Leydig cells	Upregulated	Roelofs et al. 2015
Cyp17a1	Mouse GC-2 cell line	-	10^{-8} M (24, 48, and 72 hours), 10^{-10} M (48 and 72 hours)	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
Cyp19a1	Mice, F3 adult males (*in utero* exposure)	Oral pipetting	0.5, 50 µg/kg-day, GD 7-birth	Testes	Upregulated	Shi et al. 2019
Cyp19a1	Mice, F3 adult males (*in utero* exposure)	Oral pipetting	0.5, 20, 50 µg/kg-day, GD 11-birth	Testes	Upregulated	Shi et al. 2018

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
cyp19a, cyp19a1a	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
cyp19a, cyp19a1a	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
Cyp19a1	Mouse GC-2 cell line	-	10^{-8} M (48 and 72 hours) and 10^{-10} M (48 hours)	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
cyp19b, cyp19a1b	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
hsd3b1	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
hsd3b1	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
hsd17b3	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Upregulated	Hao et al. 2022
hsd17b1	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Downregulated	Ji et al. 2013
Hsd17b3	Mice, adult	Gavage	20 mg/kg-day, 28 days	Testes	Upregulated	Wang et al. 2022
Hsd17b3	Mice, adult	Gavage	200 mg/kg-day, 28 days	Testes	Downregulated	Wang et al. 2022

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
Hsd17b3	Mouse GC-2 cell line	-	10^{-8} M (72 hours), 10^{-10} M (48 and 72 hours)	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
gnrhr1	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
gnrh2	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Upregulated	Hao et al. 2022
gnrhr2	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
gnrh3	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
gnrh3	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Upregulated	Hao et al. 2022
gnrhr4	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Upregulated	Hao et al. 2022
spata4	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
spata17	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Testes	Downregulated	Hao et al. 2022
Srd5a1	Mouse MA-10 cell line	-	10 µM, 48 hours	Leydig cells	Upregulated	Roelofs et al. 2015

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
Steroid receptor genes						
ar	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Upregulated	Hao et al. 2022
esr1	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Upregulated	Hao et al. 2022
esr1	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Downregulated	Hao et al. 2022
esr2b	Zebrafish, 3-4 months old	-	40, 200 µg/L, 21 days	Liver	Downregulated	Park et al. 2022
Esr1	Mouse, GC-2 cell line	-	10^{-8}, 10^{-10} M, 48 and 72 hours	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
Esr2	Mouse GC-2 cell line	-	10^{-8}, 10^{-10} M, 48 and 72 hours	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
Essrg	Mouse GC-2 cell line	-	10^{-8} M (72 hours) and 10^{-10} M (48 and 72 hours)	Spermatocytes	Upregulated	Sidorkiewicz et al. 2018
Other endocrine-related genes						
fshr	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
lhr, lhcgr	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013

Gene	Species	Exposure method	Dose	Tissue	Direction	Reference
hmgra, hmgcra	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
hmgrb, hmgcrb	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Testes	Upregulated	Ji et al. 2013
fshb	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
fshb	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain	Downregulated	Hao et al. 2022
lhb	Zebrafish, 3-4 months old	-	50 µg/L, 21 days	Brain	Upregulated	Ji et al. 2013
lhb	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain (F1 & F2)	Upregulated	Hao et al. 2022
lhb	Zebrafish, 120 dpf F1 and F2 (F0 exposure)	-	1, 100 µg/L, 3 hpf–120 dpf	Brain (F1 & F2)	Downregulated	Hao et al. 2022
vtg1	Zebrafish	-	40, 200 µg/L	Liver	Upregulated	Park et al. 2022
Insl3	Mouse, testicular explant culture	-	10,000 nM (NS), 3 days	Testicular (basal and LH-stimulated)	Downregulated	Eladak et al. 2015

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).
dpf = days post fertilization, GD = gestational day, hpf = hours post fertilization, LH = luteinizing hormone, PND = postnatal day.

Table 4.3.9 BPS effects on endocrine-related proteins

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
Steroidogenic proteins						
HSD3B1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Wang et al. 2022
CYP17A1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Wang et al. 2022
STAR	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Wang et al. 2022
CYP11A1	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Wang et al. 2022
HSD17B3	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Wang et al. 2022
CYP11A1	Rat, PND 35	Drinking water	5 µg/L, 14 days	Testes	Decreased	Jeminiwa et al. 2021
HSD17B1	Rat, PND 35	Drinking water	5 µg/L, 14 days	Testes	Decreased	Jeminiwa et al. 2021
Steroid receptor proteins						
AR	Mice, GD 16 (*in utero* exposure)	Drinking water	2, 200 µg/kg-day, GD 9-16	Mammary	Decreased dose-dependently	Kolla et al. 2019

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
AR	Rats, adult male (*in utero* exposure)	Gavage, GD 4–12	0.4, 4, µg/kg-day	Testes	Decreased	Molangiri et al. 2022
AR	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Decreased	Kumar et al. 2020
ESRRG	Rats, adult male (*in utero* exposure)	Gavage	0.4 µg/kg-day, GD 4–21	Testes	Decreased	Molangiri et al. 2022
AR	Gerbils, adult	Oral (not further specified)	40 µg/kg-day, 28 days	Ventral prostate: epithelium and stroma	Increased	Silva et al. 2019
Other						
THRA	Mice, adult	Gavage	150 mg/kg-day	Testes	Decreased	Sahu and Verma 2023
DIO2	Mice, adult	Gavage	150 mg/kg-day	Testes	Decreased	Sahu and Verma 2023
Melatonin receptor (MT1)	Hamsters	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Decreased	Kumar et al. 2020
Vtg	Guppies, adult	-	1, 10, 100 µg/L	Caudal fin, liver, and whole body	Increased	Wang et al. 2017
DHH	Rat, PND 35	Drinking water	5 µg/L, 14 days	Testes	Increased	Jeminiwa et al. 2021

Protein	System	Exposure method	Dose/Duration	Tissue	Direction	Reference
AMH	Rat, PND 35	Drinking water	5 µg/L, 14 days	Testes	Decreased	Jeminiwa et al. 2021

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).
AR = Androgen receptor, GD = gestational day, PND= postnatal day.

4.3.4 Other Effects

Other mechanistic evidence that may be relevant to BPS-mediated male reproductive toxicity is described below.

Organelle and cytoskeletal effects

Mitochondria

- Mitochondrial function was impaired, as indicated by the colorimetric MTT assay, in the testes of PND 21 male rats treated with 50 µg/L BPS in drinking water for 10 weeks (Darghouthi et al. 2022).
- Dose-dependent mitochondrial depolarization, increase in the mitochondrial permeability transition pore opening, and decreased ATP levels in mouse TM3 Leydig cells incubated with 100, 200, or 400 µM BPS for 48 hours (Zhang W et al. 2022).
- Mitochondrial membrane permeabilization was increased (interpreted by the authors as a sign of early apoptosis) in mouse spermatocyte GC-2 cells incubated with 10^{-10}, 10^{-8}, or 10^{-6} M BPS for 1-3 days (Sidorkiewicz et al. 2018).

There were no effects on the mitochondria, including membrane potential, permeability transition pore, and cellular ATP levels, in human spermatozoa or prostate cells (Castellini et al. 2021; Hyun et al. 2021).

Endoplasmic reticulum

- Several endoplasmic reticulum stress-related genes were upregulated in the PNT1A human prostate cell line incubated with 10 µM (*HSPA5*, *ATF4*, *DDIT3*, and *ERN1*) and 0.1, 1, or 10 µM (*EIF2AK3*) BPS for 48 hours, however, these effects were not observed in PC-3 adenocarcinoma prostate cells (Caglayan and Ozden 2024). Endoplasmic reticulum stress is a condition that may occur due to various upstream events, including oxidative stress and calcium channel disruption, that may lead to an accumulation of mis- and unfolded proteins, activating the unfolded protein response (UPR). Aberrant UPR signaling can lead to altered cell proliferation and apoptosis (Hetz et al. 2020). In a network-based toxicological analysis of BPS-induced prostate injury, core targets of BPS included cell cycle-, apoptosis-, and proliferation-related proteins (Huang 2024).

Lysosomes/autophagy

- In mouse TM3 Leydig cells incubated with 10, 25, or 50 µg/mL for 48 hours, decreased lysosomal activity and cell viability were observed (Jambor et al.

2023). Autophagy is a lysosome-dependent degradation process that has a variety of purposes in testicular cells, including maintenance of testosterone production, sperm cell integrity, and cytoskeletal structure (Yan et al. 2022).

- Autophagy-related protein expression (Beclin1, p62, LC3B-II/LC3B-I ratio) was increased in mouse TM3 Leydig cells incubated with 100, 200, or 400 μM BPS for 48 hours with increased mitochondrial swelling and accumulation of numerous undegraded autophagic vacuoles (Zhang W et al. 2022).

Cytoskeleton

- In C18-4 mouse spermatogonial cells incubated with 50 μM BPS for 1-3 days, cytoskeletal structure was altered at 24, 48, and 72 hours, and nuclear morphology was altered at 72 hours (Liang et al. 2017).
- In a 3D mouse testicular cell co-culture model treated with 100 μM BPS for 24–72 hours, there were cytoskeletal perturbations, including increased F-actin intensity at 100 μM at 24 and 48 hours, and decreased percentage of cells with stretching F-actin filaments at 72 hours, indicative of possible damage to Sertoli cells. There were also nuclear morphology alterations, including increased nuclear area at 100 μM at 24, 48, and 72 hours, and decreased nuclear roundness and smoothness at 50 and 100 μM at the same times (Yin et al. 2020).

Cell membrane and intercellular communication

- In mouse TM3 Leydig cells incubated with 10, 25, or 50 μg/mL for 48 hours, decreased cell membrane integrity and inhibition of gap junction intercellular communication were observed (Jambor et al. 2023).

Blood-testis barrier (BTB) disruption

BPS interrupted the BTB, as demonstrated in adult male rats gavaged with 50 or 100 mg/kg-day BPS for 30 days. Additionally, apical ectoplasmic specialization (ES) was interrupted with grossly disrupted organization and presence of structural proteins in the seminiferous tubules and altered actin-binding proteins. BTB- and ES-associated gene and protein expression were also altered. There were no effects on the immunolocalization of BTB integrity-related junctional proteins (Wu et al. 2021).

Cell cycle and cell proliferation effects

- Proliferating cell nuclear antigen (PCNA)-positive cell frequency, indicative of cell proliferation, was increased in the epithelium and stroma of the ventral prostate

of adult male gerbils orally administered 40 µg/kg-day BPS (specific method not reported) for 28 days (Silva et al. 2019).

- In a 3D mouse testicular cell co-culture model treated with 100 µM BPS for 1-3 days, there was an increased number of cells in the M phase with decreased cell proliferation, cell number, and cell viability (Yin et al. 2020).
- In cultured C18-4 mouse spermatogonial cells incubated with BPS for 1-3 days, there was a disruption of mitotic progression; cells accumulated in G2/M at 10 and 50 µM after 48 hours with a decreased percentage of cells in G2/M at 10 µM after 72 hours and decreased BrdU-positive cells at 50 µM at 24 and 48 hours (Liang et al. 2017).
- In mouse TM3 Leydig cells incubated with 20, 40, or 80 µM BPS for 72 hours, there was an increase in cell cycle arrest at G1/S at 40 or 80 µM with altered cell cycle-related protein expression and decreased cell viability (Li et al. 2023).

One study found no effect on cell cycle progression in mouse spermatocyte cells (Sidorkiewicz et al. 2018).

Altered gene and protein expression and post-translational protein modification of factors involved in sperm development and maturation.

- In the testes of adult male offspring of pregnant rats gavaged with 0.4 or 4.0 µg/kg-day BPS from GD 4-21, *Tex101* and *Spo11* gene expression was downregulated at 0.4 µg/kg-day, and TEX11 protein expression was increased at 0.4 or 4.0 µg/kg-day BPS in testicular tissue. *Tex101* and *Spo11* encode proteins essential to produce fertile spermatozoa and initiate meiotic double-stranded breaks in Sertoli cells, respectively. TEX11 is expressed in germ cells during early spermatogenesis and is required for maintaining male fertility (Molangiri et al. 2022).
- In the testes of male mice administered 0.001, 1 (non-significant), or 100 (non-significant) µg/kg-day BPS in drinking water for eight weeks, several protein modifications were observed, including altered acetylation and phosphorylation of proteins (Řimnáčová et al. 2020).
- In sperm of adult offspring of pregnant rats gavaged with 0.4 or 4 µg/kg-day BPS from GD 4-21, there was a decreased pAKT/AKT ratio (Molangiri et al. 2022). The phosphorylation of AKT upregulates AKT/PI3K (protein kinase B/phosphoinositide 3-kinase) which in turn stimulates sperm motility, capacitation, and the acrosome reaction.

Interaction with StAR (steroidogenic acute regulatory) protein

In a computational docking study, the BPS molecule was found to dock into the ligand-binding pocket of the crystal structure of the StAR protein (an essential steroidogenesis protein), most likely through hydrophobic interactions and hydrogen bond formation with several StAR amino acids (Darghouthi et al. 2022).

Table 4.3.10 Male reproductive toxicity: studies with mechanistic data not already included in Tables 4.2.1 and 4.2.2

Study Design	Outcomes assessed	Major Findings
Caglayan and Ozden 2024 *In vitro*: Human prostate cell lines: PNT1A (post-pubertal normal immortalized with SV40) and PC-3 (adenocarcinoma). Treatment: BPS (purity ≥99%) in 1% DMSO at 0, 0.1, 1, or 10 µM (non-cytotoxic concentrations) for 96 hours. Media was replaced at 48 hours.	Data reported only at 48 hours. Reactive oxygen species (ROS) formation. Endoplasmic reticulum stress-related gene expression. Apoptosis Apoptosis-related gene expression. Apoptosis-related protein expression: CHOP/DDIT3, markers of endoplasmic reticulum stress and unfolded protein response (UPR). Cell proliferation (BrdU assay).	ROS formation: PNT1A and PC-3 cells: BPS treatment had no effect. Endoplasmic reticulum stress-related gene expression: PNT1A cells: Increased *HSPA5, ATF4, DDIT3*, and *ERN1* at 10 µM and *EIF2AK3* at ≥0.1 µM. PC-3 cells: No effect Apoptosis: PTN1A cells: No effect PC-3 cells: Concentration-dependent increase in % cells in late apoptosis: non-significant (NS) at 0.1 µM (+35%) and significant at 1 µM (+64%), and at 10 µM (99%). Apoptosis-related gene expression: No effects in either cell line. Apoptosis-related protein expression: PTN1A cells: Increased CHOP/DDIT3 at 10 µM. PC-3 cells: No effect. No effect on cell proliferation in both cells.
Castellini et al. 2021 *In vitro*: Human spermatozoa from healthy 25- to 30-year-old volunteers with normozoospermia. Treatment: BPS (purity not reported) in 1.2% DMSO at 0, 10, 100, 300 or 400 µM for 4 hours.	Mitochondrial membrane potential. Oxidative stress (mitochondrial superoxide anion). Sperm parameters: viability and motility.	Mitochondrial membrane potential: No effects Oxidative stress: No effects. No effects on sperm parameters.
Eladak et al. 2015 *In vitro*: Fetal Testis Assay in human and mouse testicular tissue explants, 6.3-11.1 gestation weeks (human), and 12.5 days post-conception (mouse). N = 6–10 (human); N = 9–17 (mouse). Treatment: BPS (purity not reported) in ethanol (final concentration not reported) at 0, 10, 100, 1,000 or 10,000	Testosterone (T) secretion on day 1, day 2, and day 3 in both human and mouse explants. Mouse explants: Leydig cell-specific gene expression at 10,000 nM on day 3 only: *Star, Cyp11a1, Hsd3b1, Cyp17a1, Lhcgr,* and *Insl3*. *Insl3* is essential in the development of the gubernaculum for the initial descent of the testes.	Human tissue: Decreased T secretion ≥1000 nM on day 1, day 2, and day 3. Mouse tissue: Decreased T secretion at ≥100 nM on day 1, day 2, and day 3. Decreased (NS) Leydig cell-specific gene expression: *Star* and *Insl3*.

Study Design	Outcomes assessed	Major Findings
nM. Explants were cultured for 24 hours in control medium (day 0) followed by culture with vehicle or BPS for the three subsequent days (day 1, day 2, day 3). (100 nM of BPS = 0.25 µg/L)		
Hyun et al. 2021 *In vitro*: WPMY-1 human prostatic stromal myofibroblast cell line. Treatment: BPS (purity 98%) in 0.5% DMSO (vehicle control) at concentrations 0, 50, 100, 250, and 500 µM for 24 hours.	Mitochondrial reactive oxygen species (ROS) levels. Cellular ATP concentrations measured (ATP assay). Mitochondrial permeability transition pore assay. General toxicity: cell viability.	Note: From the text and figures, it is unclear which concentration(s) was / were assessed in the following assays: Mitochondrial ROS levels: No effect. Mitochondrial permeability transition pore assay opening: No effect. Relative ATP level: No effect. General toxicity: NS concentration-dependent decrease in cell viability ≤ 250 µM, and significant decrease at 500 µM.
Jambor et al. 2019 *In vitro*: Mouse TM3 Leydig cell line. Treatment: BPS (purity and vehicle not reported) at 0, 0.04, 0.2, 1, 2.5, 5, 10, 25, or 50 µg/mL for 24 hours.	T production. General toxicity: cell viability	T production: Decreased (NS) at 50 µg/mL (-19%). Quantitation of T production was not adjusted for viability. Note: Adjusting T secretion for cell viability at the high dose results in a value higher than control. General toxicity: decreased cell viability at ≥10 µg/mL (-15%, -20%, and -39% at 10, 25, and 50 µg/mL, respectively).
Jambor et al. 2023 *In vitro*: Mouse TM3 Leydig cell line. Treatment: BPS (purity >98%) in 0.5% (v/v) ethanol at 0 (no treatment), 0 (vehicle control), 1, 2.5, 5, 10, 25, or 50 µg/mL for 48 hours. Note: Its appears that treated groups were compared to the "non-treated" control rather than the vehicle control (referred in the text as "negative control")	Cell membrane integrity. Lysosomal activity. Oxidative stress (superoxide radical production). Gap junction intercellular communication (GJIC) assay. T secretion. General toxicity: Cell viability.	Cell membrane integrity: Decreased at ≥ 10 µg/mL (-11%, -31%, and -48%, respectively). Lysosomal activity: Decreased at ≥ 10 µg/mL (-16%, -38%, and -69%, respectively). Superoxide radical production: Increased at 5 (+21%) and 10 (+25%) µg/mL. GJIC assay: Inhibited at ≥ 10 µg/mL (-21%, -43%, and -51%, respectively). T secretion: Decreased at 10 (NS; -13%), 25 (-38%), and 50 (-58%) µg/mL. General toxicity: decreased cell viability at ≥ 10 µg/mL (-15 to -42%).
Kose et al. 2020 *In vitro*: RWPE-1 human prostatic epithelial cell line.	Antioxidant measures: GPX1, GR, and SOD activity, GSH levels, and total antioxidant capacity. DNA damage (comet assay and modified comet assay with formamidopyrimidine-DNA glycosylase	Antioxidant measures: Decreased GPX1 activity. Increased GR activity. Increased GSH levels. No effect on SOD activity or total antioxidant capacity level.

Study Design	Outcomes assessed	Major Findings
Treatment: BPS (purity >98%) prepared in 1% (v/v) DMSO stock solution and diluted with culture medium to final concentrations of 0 (vehicle control) and 108 µM (IC20) for 24 hours. IC20 determined in range finding cell viability study, using concentrations of 0 (vehicle control), 50, 100, 200, 300, and 600 µM, for 24 hours. Dose-dependent decrease in cell viability, IC50 dose 380.90 µM, and IC20 dose 108 µM.	(FPG), which detects 8-OH guanine and other purines damaged via oxidation). DNA damage repair gene expression (*TP53, OGG1, APE1, MYH* [*MUTYH*], and *POLB*)	DNA damage: Increased % tail intensity at IC20 with and without FPG Standard comet assay (without FPG): 3.20-fold increase in % tail intensity. FPG comet assay: about 2.6-fold higher levels of tail intensity compared to the standard comet assay (OEHHA estimation from study figure). DNA damage repair gene expression: Decreased *MYH (MUTYH)*. NS decrease in *TP53* and *OGG1*, NS increase in *POLB*, and No effect on *APE1*.
Li et al. 2023 *In vitro*: Mouse TM3 Leydig cell line. Treatment: BPS (purity not reported) in 0.1% DMSO at 0, 20, 40, or 80 µM for 72 hours. Transfection experiments: knockdown (siRNA-mediated) or overexpression of YTHDF1, an N^6-methyladenosine (m^6A) binding protein (reader). m^6A is a common form of methylated adenosine that occurs in mRNA.	Apoptosis: Apoptosis rate (flow cytometry). Apoptosis-related protein expression (BCL2, CASP3, CASP9, and BAX). Cell Cycle: Phase analysis (flow cytometry). Cell cycle-related protein expression (CDK2 and CyclinE1). RNA methylation-related protein expression (YTHDF1, FTO, and METTL3). Total m^6A levels. General toxicity: cell number, morphology, and viability (CCK-8 assay).	Apoptosis: Dose-dependent increase in apoptosis rate at ≥20 µM (about 3.7-fold increase at 80 µM). Apoptosis-related protein expression: Dose-dependent decrease in BCL2 at ≥20 µM. Increased CASP9 at ≥40 µM. Increased CASP3 and BAX at ≥20 µM. Cell cycle Phase analysis: Increased cell cycle arrest at G1/S at 40 and 80 µM. Cell cycle-related protein expression: Decreased CDK2 and CyclinE1 at ≥20 µM. RNA methylation-related protein expression: Decreased YTHDF1 at ≥20 µM. Increased FTO at ≥20 µM. Decreased total m^6A levels at ≥20 µM. General toxicity: decreased cell number with treatment. Cell morphology: Not reported in treated cells. Cell viability: decreased at ≥20 µM with around 85% viability at 80 µM Cell viability for YTHDF1 knockdown and overexpressed cells assessed with 80 µM BPS: Decreased in YTHDF1 knockdown cells. Increased in YTHDF1-overexpressing cells compared to non-transfected BPS-treated cells.

Study Design	Outcomes assessed	Major Findings
Liang et al. 2017 *In vitro*: Mouse spermatogonial cell line (C18-4). Treatment: BPS (98% purity) in 0.05% DMSO at 0, 0.1, 1, 5, 10, 25, or 50 µM for 24, 48, or 72 hours.	Nuclear morphology Marker of DNA synthesis/cell proliferation (BrdU incorporation into DNA). Cell cycle. Cell cytoskeleton Marker of DNA damage (double-strand breaks; DSBs): γH2AX expression. Effect concentration (EC20) evaluation. General toxicity: Cell number and nuclear morphology.	Nuclear morphology: Increased nuclear area and altered nuclear shape (decreased P2A [nuclear perimeter to area ratio] and length/width ratio) at 50 µM at 72 hours. DNA synthesis/cell proliferation: Decreased BrdU-positive cells at 50 µM at 24 and 48 hours. Cell cycle: Accumulation of cells in G2/M at 10 and 50 µM at 48 hours. Decreased percentage of cells in G2/M at 10 µM at 72 hours. Cytoskeletal Structure: Decreased dot-like structures at 50 µM at 72 hours. Increased F-actin at 50 µM at 24, 48, and 72 hours. DNA DSBs: Increased γH2AX-positive cells at 50 µM at 24, 48, and 72 hours. EC20 Evaluations: Lowest EC20 of 4.5 µM for cytoskeleton perturbation at 24 hours. General toxicity: decreased cell number with 50 µM at 24, 48, and 72 hours.
Nguyen M et al. 2022 *In vitro*: Spermatozoa from one bull with known fertility and proven *in vitro* fertilization capability. Treatment: BPS (purity not reported) at 0 (blank), 0 (vehicle), or 0.05 mg/mL of BPS for 4 hours.	Sperm parameters (morphology and motility). ROS levels. Oxidative stress-related gene expression: superoxide dismutase (*SOD1*) and glutathione peroxidases (*GPX4* and *GPX1*). Expression and immunolocalization of oxidative stress-related proteins (SOD1, GPX1, and GPX4).	Sperm parameters: Decreased percentage progressive sperm motility. Increased percentage of immotile sperm. ROS generation: Decreased ROS levels. Oxidative stress-related gene expression: Increased *SOD1* Decreased *GPX4*. Oxidative stress-related protein expression and localization: No effects.
Roelofs et al. 2015 Experiment 1: Recombinant yeast stably expressing human androgen receptor (AR) or glucocorticoid receptors (GR) with enhanced green fluorescent protein (yEGFP). Treatment: BPS (purity >98%) in 0.1% (v/v) DMSO at 0 or 10 pM-1 mM for 24 hours.	Experiment 1: Yeast AR and GR bioassays (agonism and antagonism of the AR and GR). Experiment 2: T secretion. Steroid profiling (10 µM only; metabolomics).	Experiment 1: recombinant yeast No agonist or antagonist activity with human AR or GR. Experiment 2: Mouse MA-10 Leydig cell line T secretion: No effect. Steroid profiling: Increased pregnenolone (+108%) and progesterone (+303%) at 10 µM. Cholesterol biosynthesis-related gene expression: No effects.

Study Design	Outcomes assessed	Major Findings
Experiment 2: *In vitro*: Mouse MA-10 Leydig cell line. Treatment: BPS (purity >98%) in DMSO at 0, 0.01, 0.1, 1, 3, 10, and 30 µM for 48 hours.	Cholesterol biosynthesis-related gene expression (at 10 µM only); *Cyp51*, hmg-coA red (*Hmgcr*), and *Por*. Steroidogenic-related gene expression (at 10 µM only); *Star*, *Cyp11A1*, *Cyp17 (Cyp17a1)*, *3β-hsd1 (Hsd3B1)*, *17β-hsd3 (Hsd17B3)*, *5αred1 (Srd5a1)*, *Ckit*, and *Lhr*. General toxicity: Cell viability (MTT assay).	Steroidogenic-related gene expression: Increased *Cyp17* and *5αred1*. General toxicity: MTT assay not reported
Sidorkiewicz et al. 2018 *In vitro*: GC-2spd(ts) (GC-2) mouse spermatocyte cell line. Treatment: BPS (purity not reported) in 0.1% DMSO, 10^{-14} to 10^{-4} M for 24, 48 or 72 hours.	Cell cycle progression (24 hours). Early apoptosis (10^{-10} - 10^{-6} M BPS; 24 hours exposure only; mitochondrial membrane potential). Steroid receptor-related gene expression at 10^{-10} and 10^{-8} M: estrogen receptors α and β (*Esr1* and *Esr2*), AR (*Ar*), glucocorticoid receptor (*Gpr30*), and estrogen-related receptor gamma (*Errγ [Esrrg]*). Steroidogenic-related gene expression (at 10^{-10} and 10^{-8} M): *Star*, *Cyp11a1*, *Cyp17a1*, *Cyp19a1*, and hydroxysteroid 17-beta dehydrogenase 3 [*Hsd17b3*]). Global DNA methylation (at 10^{-8} M). General toxicity: Cell viability (MTT assay) Lactate dehydrogenase (LDH) cytotoxicity assay with 10^{-8} M BPS.	Cell cycle progression: No effect. Mitochondrial membrane potential: Increased permeabilized membrane at 10^{-10}, 10^{-8}, and 10^{-6} M Steroid receptor-related gene expression: Increased *Esr1* and *Esr2* at 48 and 72 hours at 10^{-10} and 10^{-8} M Increased *Errγ* at ≥48 hours (10^{-10} M) and 72 hours (10^{-8} M) Steroidogenic-related gene expression at 10^{-10} and 10^{-8} M: Increased *Star* and *Cyp11a1* at 48 and 72 hours at both doses. Increased *Hsd17b3* at ≥48 hours (10^{-10} M) and 72 hours (10^{-8} M). Increased *Cyp17a1* at ≥48 hours (10^{-10} M) and ≥24 hours (10^{-8} M). Increased *Cyp19a1* at 48 hours (10^{-10} M) and ≥48 hours (10^{-8} M) Global DNA methylation: Increased percentage of 5-methylcytosine (5-mC) by BPS at 72 hours at 10^{-8} M. General toxicity: Increased cell viability (MTT assay) from 10^{-9} M – 10^{-6} M at 24 hours, with no effects at high doses or later time points. LDH cytotoxicity assay: increased at 48 and 72 hours (at 10^{-8} M BPS).
Ullah et al. 2016 *In vitro*: Short-term incubation of testicular slices with BPS. Both testes from 5 adult SD rats were dissected, decapsulated, cut into five sections of equal weight, and then randomly distributed into culture tubes (presumed one per treatment group) and incubated with BPS in DMEM/F-12 medium.	Oxidative stress: catalase (CAT), peroxidase, SOD, lipid peroxidase (LPO), and total ROS levels. T level (ng/g tissue)	Oxidative stress: Increased (NS) CAT at ≥1 ng/mL (+61 to+105%). Increased (NS) peroxidase at 0.5 and 100 ng/mL (+24% and +39%, respectively). Increased SOD at 100 ng/mL (+152%) Increase LPO at all doses (+76 to +137%; significant at 1 ng/mL only). Increased total ROS at 100 ng/mL (+142%). T level: No effect

Study Design	Outcomes assessed	Major Findings
Treatment: BPS (99% purity) in 0.1% ethanol at 0, 0.5, 1, 10, 100 ng/mL for 2 hours incubation. After incubation, tissues were removed from media, rinsed, and homogenized in phosphate-buffered saline. The supernatant was collected and used for the various assays.		
Ullah et al. 2017 *In vitro*: Sperm from cauda epididymis, 7 adult SD rats total, sperm from the same animal was randomly distributed among groups. Treatment: BPS (99% purity) in 0.01% ethanol at 0, 0.5, 1, 10, or 100 µg/L for 2 hours incubation.	Oxidative stress: SOD, lipid peroxidation marker (thiobarbituric acid reactive substances [TBARS]), and total ROS levels. DNA damage (comet assay).	Oxidative stress: Increased SOD, TBARS, and ROS at 100 µg/L. DNA damage (comet assay): Increased (NS) number of comets/100 cells at 100 µg/L (+18%). Increased tail moment at ≥0.5 µg/L (NS at 0.5 and 10 µg/L) (+50-100%). Increased tail DNA at 100 µg/L (+49%).
Ullah et al. 2018a *In vitro*: Short-term incubation of testicular slices with BPS. Both testes from 7 adult SD rats were dissected, decapsulated, cut into five sections of equal weight, and then randomly distributed into culture tubes and incubated with BPS in culture medium. Treatment: BPS (99% purity) in 0.1-0.5% ethanol at 0, 1, 10, or 100 ng/mL BPS for 2 hours. After incubation, tissues were removed from media, rinsed, and homogenized in phosphate-buffered saline. The supernatant was collected and used for the various assays.	Oxidative stress: CAT, peroxidase, SOD, lipid peroxidase [LPO], and total ROS levels). T level (ng/g tissue).	Oxidative stress: Concentration-dependent increase (NS at 1 and 10 ng/mL) in LPO (+27-80%), Increased total ROS at 100 ng/mL (+54%). T level: No effect.
Ullah et al. 2019a *In vitro*: Sperm harvested from 26 male adult SD rats. Treatment: BPS (99% purity) in 0.1% ethanol at 0, 1, 10, or 100 ng/mL for 2 hours.	Oxidative stress: SOD activity, LPO marker TBARS, and total ROS levels. DNA damage (comet assay).	Oxidative stress: Increased SOD activity, LPO, and ROS at 100 ng/mL. DNA damage: Increased number of comets/100 cells, tail moment, and tail DNA at 100 ng/mL (+14%, +34%, and +17%, respectively).
Wang et al. 2017 Adult male red albino guppies (*Poecilia reticulata*), 20 fish per treatment group.	Vtg (µg/g) in caudal fin, liver tissue, and whole body.	Vtg concentration (µg/g): Increased in caudal fin at all concentrations, Increased in liver tissue at all concentrations,

Study Design	Outcomes assessed	Major Findings
Treatment: BPS (purity not reported) in 0.001% v/v ethanol (solvent control) at concentrations 0, 1, 10, and 100 µg/L for 21 days. Exposure solution was replaced daily. Caudal fin, liver tissue, or whole-body homogenates were analyzed to determine vitellogenin (Vtg) content.		Increased in whole body at all concentrations.
Yin et al. 2020 *In vitro*: Three-dimensional mouse (BALB/c) testicular cell co-culture model: spermatogonial cell line C18-4 [80%], Leydig cell line TM3 [5%], and Sertoli cell line TM4 [15%]. Treatment: BPS (98% purity) in 0.01-0.05% DMSO at 0, 25, 50, or 100 µM for 24, 48, and 72 hours. Cell viability, as determined by neutral red (NR) uptake, was used for concentration-range finding (25 to 400 µM). Cell viability results are reported for the concentrations used in this study (25, 50, and 100 µM).	Nuclear morphology: Nuclear area. Nuclear roundness measured as the ratio of nuclear length to width. Nuclear smoothness measured as the ratio of nuclear perimeter squared to 4π × nuclear area. Nuclear DNA intensity (average, total, and variance). Multinucleated cells (%). Cell number (Hoechst nuclear stain). DNA damage (DSB): number of γH2AX-positive cells. Cytoskeletal changes. Cell cycle analysis. Cell proliferation. Cell viability.	Nuclear morphology: Increased nuclear area at 100 µM at 24, 48, and 72 hours. Decreased nuclear roundness and smoothness at 50 and 100 µM at 24, 48, and 72 hours. No effects on average, total, and variance of DNA intensity. No effects on % of multinucleated cells. Decreased cell number at 100 µM at 24 hours; and at 50 and 100 µM at 48 and 72 hours. No effects on % of γH2AX-staining cells. Cytoskeletal changes: Increased F-actin intensity at 100 µM at 24 and 48 hours. Decreased percentage of cells with stretching F-actin filaments at 100 µM at 72 hours, indicative of possible damage to Sertoli cells. Cell cycle analysis: Increased percentage of cells in M phase at 100 µM at 48 and 72 hours. Cell proliferation: Decreased total number of BrdU-positive cells at 100 µM at 24 and 48 hours. Increase of the 90% quantiles in total intensity of BrdU at 100 µM at 24 and 72 hours. Decreased geometric mean of total intensity of BrdU at 50 and 100 µM at 48 hours. Cell viability: Decreased at 100 µM at 48 and 72 hours.
Zhang W et al. 2022 *In vitro*: Mouse TM3 Leydig cell line. Treatment: BPS (≥98% purity) in 0.1% DMSO at 0, 100, 200 or 400 µM for 48 hours.	Oxidative stress: SOD and CAT activities, and malondialdehyde (MDA) and ROS generation. Mitochondrial function (membrane potential, mitochondrial permeability transition pore [mPTP] opening, and ATP levels). Metabolomics. Apoptosis: Protein expression: BCL2, BAX, and BCL2/BAX	Oxidative stress: Concentration-dependent increase in ROS at ≥100 µM. Increased MDA at ≥100 µM. Decreased SOD and CAT (concentration-dependent) activities at ≥200 µM. Mitochondrial membrane potential: Concentration-dependent mitochondrial depolarization at ≥100 µM. Concentration-dependent increase in mPTP opening at ≥100 µM. Concentration-dependent decrease in ATP levels at ≥100 µM.

Study Design	Outcomes assessed	Major Findings
	ratio. CASP3 activity. Autophagy: Protein expression (LC3B-II/LC3B-I ratio, beclin1 (BECN1), and p62 [SQSTM1]). Ultrastructural analysis (transmission electron microscopy).	Metabolomics: Upregulation of L-malic acid (LMA) and adenosine monophosphate (AMP) at 200 µM Downregulation of thiamine pyrophosphate (TPP), adenosine triphosphate (ATP), beta-D-fructose 6-phosphate (b-F6P), cis-aconitate, phosphoenolpyruvate (PEP), cyclic AMP (cAMP), D-glucose 6-phosphate (G6P) and dihydroxyacetone phosphate (DHAP) at 200 µM. Apoptosis: Decreased BCL2 expression at ≥200 µM. Increased BAX expression at ≥100 µM. Decreased BCL2/BAX ratio at ≥100 µM. Increase in CASP3 activity at ≥200 µM. Autophagy: Increased BECN1 and p62 expression at ≥100 µM, Increased LC3B-II/LC3B-I ratio at ≥200 µM. Increased mitochondrial swelling and accumulation of numerous undegraded autophagic vacuoles.

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APPENDIX A. LITERATURE SEARCH APPROACH ON THE MALE REPRODUCTIVE TOXICITY OF BPS

Searches of the published scientific literature on the developmental and reproductive toxicity (DART) of bisphenol S (BPS) were conducted in January 2022 and updates to the original search were conducted in February 2023, July 2023, and January 2024. The goal was to identify peer reviewed- open source and proprietary journal articles, print and digital books, reports, and gray literature that potentially reported toxicological and epidemiological information on the DART of this chemical.

The searches were conducted using the following three approaches:

- Primary searches in major biomedical databases, conducted by OEHHA librarian Nancy Firchow, MLS
- Searches in other data sources, including authoritative reviews and reports, and databases or web resources, conducted by OEHHA scientists
- Additional focused searches, conducted by OEHHA scientists

In addition to information identified from these searches, OEHHA also considered the following:

- One submission received during the data call-in period (March 4 – April 18, 2022) (https://oehha.ca.gov/proposition-65/comments/comment-submissions-request-relevant-information-reproductive-toxicity)

Primary Search Process

1. *Data Sources*

The biomedical literature databases used as data sources that were searched to find information on BPS are listed below.

PubMed (National Library of Medicine) (https://www.ncbi.nlm.nih.gov/pmc/)

Embase (https://www.embase.com/)

Scopus (https://www.scopus.com/)

SciFinder-n (https://scifinder-n.cas.org/)

Google Scholar (https://scholar.google.com)

2. Search Term Identification

- The US EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard) was used to identify synonyms for BPS. The PubMed MeSH database (https://www.ncbi.nlm.nih.gov/mesh/) was used to find additional synonyms, subject headings and other index terms related to the chemical.

- The PubMed DART filter (https://www.nlm.nih.gov/bsd/pubmed_subsets/dart_strategy.html) was used for developmental and reproductive toxicity-related terminology.

- National Toxicology Program's Standard Search Strings for Literature Database Searches: Appendix to the Draft Handbook for Preparing Report on Carcinogens Monographs (NTP 2015) (https://ntp.niehs.nih.gov/ntp/roc/handbook/rochandbookappendix_508.pdf) was used to identify search strategies for Experimental Animals and ADME concepts.

- Additional strategies for DART and Key Characteristics of Female Reproductive Toxicity and Key Characteristics of Male Reproductive Toxicity were developed by OEHHA.

3. Primary Search Execution

Searches were executed in PubMed, Embase, Scopus, SciFinder^{n}, and Google Scholar in January 2022, February 2023, July 2023, and January 2024.

Three separate searches were done in PubMed, Embase and Scopus. Searches in these databases were divided into evidence streams as:

- Human DART Studies

- Animal DART Studies

- ADME Studies

The basic structure used for each search and search dates are shown in Tables A.1 through A.3.

Table A.1 Human DART studies search structure (PubMed, Embase, Scopus)

Search Dates	Search step	Search Concepts
1/2022 2/2023 7/2023 1/2024	#1	BPS terms
	#2	DART terms (PubMed DART Filter)
	#3	Additional DART terms (OEHHA strategy)
	#4	Key Characteristics of Male Reproductive Toxicity (OEHHA Strategy)
	#5	Key Characteristics of Female Reproductive Toxicity (OEHHA Strategy)
	#6	#2 OR #3 OR #4 OR #5
	#7	#1 AND #6
	#8	Remove animals from #7

Table A.2 Animal DART studies search structure (PubMed, Embase, Scopus)

Search Dates	Search step	Search Concepts
1/2022 1/2023 7/2023 1/2024	#1	BPS terms
	#2	DART terms (PubMed DART Filter)
	#3	Additional DART terms (OEHHA strategy)
	#4	Key Characteristics of Male Reproductive Toxicity terms (OEHHA Strategy)
	#5	Key Characteristics of Female Reproductive Toxicity terms (OEHHA Strategy)
	#6	#2 OR #3 OR #4 OR #5
	#7	#1 AND #6
	#8	Experimental Animals terms (RoC strategy)
	#9	#7 AND #8

Table A.3 ADME studies search structure (PubMed, Embase, Scopus)

Search Date	Search step	Search Concepts
8/2022	#1	BPS terms
	#2	ADME terms (RoC Strategy)
	#3	#1 AND #2

The searches were run first in PubMed. The search terms and syntax were then tailored according to the search features unique to the other databases. For example, Embase uses different subject headings than PubMed, so the Emtree subject heading list was searched to identify equivalent terms to replace the MeSH terms used in the PubMed searches.

Two separate searches were run in SciFinder^{n}. Searches in this database were divided into Human and Animal evidence streams. The basic structure used in each search is shown in Tables A.4 and A.5.

Table A.4 Human DART studies search structure (SciFinder^{n})

Search Dates	Search step	Search Concepts
1/2022	#1	BPS terms
	#2	Limit to Journal Article
	#3	Limit to human concept
	#4	Limit to Database "CA Plus"
	#5	Search within results: DART terms

CAplus (chemical abstract plus) is a database of chemical information that can be accessed via SciFinder-N.

Table A.5 Animal studies search structure (SciFinder^{n})

Search Dates	Search step	Search Concepts
1/2022	#1	BPS terms
	#2	Limit to Journal Article
	#3	Limit to animals concept
	#4	Limit to Database "CA Plus"
	#5	Search within results: DART terms

CAplus, chemical abstract plus.

One search was run in Google Scholar. The basic structure used in the search is shown in Table A.6.

Table A.6 DART studies search structure (Google Scholar)

Search Dates	Search step	Search Concepts
1/2022 1/2023 1/2024	#1	(BPS terms) AND (DART terms)

Results from all databases were uploaded to EndNote, maintaining separate libraries for each of the three concepts searched (human DART studies, animal DART studies, and ADME studies). Duplicates were removed. The results of the primary searches for BPS are shown in Table A.7.

Table A.7 BPS DART studies search results (totals from all search dates)

Search	PubMed Results	Embase Results	Scopus Results	SciFinder^{n} Results	Google Scholar Results	Unique Results After Deduplication
Human DART	780	686	713	131	14	865
Animal DART	506	580	474	102	16	631
ADME	236	489	505	*	*	759

**SciFinder^{n} and Google Scholar were not searched for ADME evidence stream.*

4. Other Data Sources

Additional databases and websites of governmental and other authoritative entities (e.g., US EPA, European Chemicals Agency) were searched for data and reports that may have been missed in the primary literature search. Other relevant studies were identified from citations in individual articles, and through alert services (e.g., ScienceDirect, Google Scholar, etc.).

Literature Screening Process

Use of Health Assessment Workspace Collaborative

The EndNote libraries containing the literature search results (citations) for BPS were uploaded to HAWC (Health Assessment Workspace Collaborative, (https://hawcproject.org). HAWC is a tool used for multi-level screening of literature search results. Using HAWC, the references were screened and tagged.

In Level 1 screening, each citation was first screened by at least one OEHHA scientist, based solely on titles and abstracts, using specific inclusion and exclusion criteria to eliminate studies or articles that did not contain information on BPS and studies of its DART or other key related topics (e.g., pharmacokinetics, mechanisms of action). This initial screen (Level 1) was intended to identify all studies deemed to have a reasonable possibility of containing information relevant to DART that could be useful for the review process, and to further identify (i.e., tag in HAWC) studies relevant to specific aspects of DART (e.g., male reproductive toxicity, female reproductive toxicity, developmental toxicity).

Screening the literature relevant to developmental and male reproductive toxicity

For purposes of identifying the available evidence on the developmental and male reproductive toxicity of BPS, citations identified as having a reasonable possibility of containing information relevant to either endpoint underwent Level 2 screening. In the Level 2 screening of this subset of citations, the full texts were obtained. These full papers were screened independently by one OEHHA scientist, using similar inclusion/exclusion criteria as was used in the Level 1 screening. However, Level 2 reviewers could make more accurate judgements about the relevance of the articles because they were reviewing the full text in addition to the title and abstract. Following Level 2 screening, the tagging of articles according to key topics was updated in HAWC. Level 1 and 2 screenings were repeated as search results were updated, and with additional relevant studies identified from citations in individual articles and alert services (e.g., ScienceDirect, Google Scholar).

Detailed PubMed Literature Search Strategies – Primary Searches

Table A.8 PubMed search strategy for human DART studies

SET #	STRATEGY	CONCEPT GROUP
1	(80-09-1[rn] OR "Bisphenol S"[tiab] OR "bis(4-hydroxyphenyl)sulfone"[nm] OR "bis(4-hydroxyphenyl)sulfone"[tiab] OR "4,4'-Sulfonyldiphenol"[tiab] OR "Phenol, 4,4'-sulfonylbis-"[tiab] OR "BPS-monoglucuronide"[tiab] OR "BPS-1G"[tiab] OR "bisphenol S dicyanate ester"[tiab] OR (bps[tiab] NOT ("bps"[tiab] NOT "bisphenol"[tiab]))))	Chemical Terms
2	(abnormalities, drug-induced [mh] AND (fetus [mh] or pregnancy [mh])) OR ((abnormalities, multiple/chemically induced [mh] OR abnormalities, multiple/epidemiology [mh] OR abnormalities, multiple/etiology[mh] OR abnormalities, multiple/genetics [mh] OR abnormalities, multiple/pathology [mh]) AND (pregnancy [mh] OR fetus [mh]))OR (abnormalities, radiation-induced [mh] AND (fetus [mh] or pregnancy [mh])) OR abortion, habitual/chemically induced [mh] OR abortion, habitual/etiology [mh] OR abortion, spontaneous/chemically induced [mh] OR abortion, spontaneous/etiology [mh] OR (alcoholic intoxication[mh] AND (fetus [mh] or pregnancy [mh])) OR (alcohol drinking [mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR Birth Defects Res B Dev Reprod Toxicol [TA] OR birth weight/drug effects [mh] OR birth weight/radiation effects [mh] OR breast feeding/drug effects [mh] OR (carcinogens, environmental [mh] AND (fetus [mh] or pregnancy [mh])) OR (carcinogens [mh] AND (fetus [mh] OR pregnancy [mh])) OR (cardiovascular abnormalities/ci [mh] AND fetus [mh]) OR (cardiovascular abnormalities/et [mh] AND fetus [mh]) OR (cocaine[mh] AND (fetus [mh] or pregnancy [mh])) OR (congenital abnormalities [mh] AND (fetus [mh] or pregnancy [mh])) OR (dna damage [mh] AND (pregnancy [mh] OR fetus [mh])) OR embryo/de [mh] OR embryo/re [mh] OR embryo loss/ci [mh] OR embryonic and fetal development/drug effects [mh] OR embryonic and fetal development/radiation effects [mh] OR embryonic structures/drug effects [mh] OR embryonic structures/pathology [mh] OR embryonic structures/radiation effects [mh] OR (environmental exposure[mh] AND (pregnancy [mh] OR fetus [mh])) OR fertility/drug effects [mh] OR fertility/radiation effects [mh] OR fetal alcohol syndrome[mh:noexp] OR fetal death/chemically induced [mh] OR fetal death/etiology [mh] OR fetal death/genetics [mh] OR fetal death/pathology [mh] OR fetal diseases/chemically induced [mh] OR fetal diseases/etiology [mh] OR fetal diseases/genetics [mh] OR fetal growth retardation/et [mh] OR fetal growth retardation/ci [mh] OR fetal resorption/chemically induced [mh] OR fetal resorption/etiology [mh] OR fetal resorption/genetics [mh] OR fetus/abnormalities [mh] OR fetus/drug effects [mh] OR fetus/radiation effects [mh] OR (fetus*[tw] AND expos*[tw]) OR (genetic diseases, inborn/CI [mh] AND (fetus [mh] OR pregnancy [mh])) OR germ cells/drug effects [mh] OR germ cells/radiation effects [mh] OR (hazardous substances [mh] AND (fetus [mh] or pregnancy [mh])) OR heavy metal	PubMed Dart strategy

SET #	STRATEGY	CONCEPT GROUP
	poisoning[mh] OR lactation/drug effects [mh] OR lactation/radiation effects [mh] OR (lead [mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR (lead poisoning[mh] AND (fetus [mh] or pregnancy [mh]))OR maternal exposure [mh] OR maternal-fetal exchange/genetics [mh] OR maternal-fetal exchange/drug effects [mh] OR maternal-fetal exchange/radiation effects[mh] OR (mutagens [mh] AND (pregnancy [mh] OR fetus [mh])) OR neonatal abstinence syndrome[mh] OR "neonatal abstinence syndrome"[ti] OR neonatal sepsis [mh] OR ovary/drug effects [mh] OR ovary/radiation effects [mh] OR paternal exposure [mh] OR placenta diseases/chemically induced [mh] OR placenta diseases/etiology [mh] OR placenta/abnormalities [mh] OR placenta/drug effects [mh] OR placenta/radiation effects [mh] OR pregnancy Complications, Infectious/epidemiology [mh] OR pregnancy Complications/ci [mh] OR pregnancy outcome/ge [mh] OR (prenatal*[tw] AND expos*[tw]) OR prenatal exposure delayed effects [mh] OR (protein deficiency[mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR reproduction/drug effects [mh:noexp] OR reproduction/radiation effects [mh] OR rubella/congenital[mh:noexp] OR rubella syndrome, congenital/etiology[mh:noexp] OR (teratogens [mh] AND (pregnancy [mh] OR fetus [mh]))OR Teratology [Journal] OR teratology [mh] OR testis/drug effects [mh] OR testis/radiation effects [mh]	
3	"abortion, spontaneous"[mh] OR "abortion*"[tiab] OR "Acrosome"[mh] OR "Acrosome"[tiab] OR "Adrenarche"[tiab] OR "androgen antagonists"[mh] OR "androgen*"[tiab] OR "androgens"[mh] OR "Androstenedione"[tiab] OR "anogenital distance"[tiab] OR "ano genital distance"[tiab] OR "anovulat*"[tiab] OR "Aspermia"[tiab] OR "atretic follicle*"[tiab] OR "Azoospermia"[tiab] OR "birth defect*"[tiab] OR "birth weight"[mh] OR "birth weight"[tiab] OR "breast feed*"[tiab] OR "breast feeding"[mh] OR "breastfeed*"[tiab] OR "chorionic villi"[tiab] OR "conception*"[tiab] OR "congenital abnormalities"[mh] OR "Congenital"[tiab] OR "corpus luteum"[tiab] OR "cumulus cell*"[tiab] OR "cytotrophoblast*"[tiab] OR "decidua"[tiab] OR "deciduum"[tiab] OR "dna damage"[mh] OR "ductus deferens"[tiab] OR "efferent duct*"[tiab] OR "ejaculat*"[tiab] OR "Embryo"[tiab] OR "Embryoes"[tiab] OR "embryonic and fetal development"[mh] OR "embryonic structures"[mh] OR "Embryonic"[tiab] OR "embryotoxic*"[tiab] OR "endometri*"[tiab] OR "Epididymis"[mh] OR "Epididymis"[tiab] OR "erecti*"[tiab] OR "Estradiol"[tiab] OR "estrogen antagonists"[mh] OR "estrogen receptor modulators"[mh] OR "estrogen*"[tiab] OR "estrogens"[mh] OR "Estrus"[tiab] OR "fallopian tube*"[tiab] OR "fallopian tubes"[mh] OR "fecund*"[tiab] OR "Fertility"[mh] OR "Fertility"[tiab] OR "Fertilization"[tiab] OR "Fetal"[tiab] OR "Fetus"[mh] OR "Fetus"[tiab] OR "foetal"[tiab] OR "foetus"[tiab] OR "follicle stimulating hormone"[tiab] OR "FSH"[tiab] OR "genetic diseases, inborn"[mh] OR "genital diseases, female"[mh] OR "genital diseases, male"[mh] OR "genital*"[tiab] OR "genitalia"[mh] OR "germ cell*"[tiab] OR "germ cells"[mh] OR "gestat*"[tiab] OR "gonad*"[tiab] OR "gonadal disorders"[mh] OR "gonadal hormones"[mh] OR "gonadotropins"[mh] OR "gonads"[mh] OR "graafian follicle*"[tiab] OR "granulosa	Additional DART terms (OEHHA Strategy)

SET #	STRATEGY	CONCEPT GROUP
	cell*"[tiab] OR "human development"[mh] OR "Implantation"[tiab] OR "in utero"[tiab] OR "infant*"[tiab] OR "infant, newborn"[mh] OR "infertil*"[tiab] OR "Inhibin"[tiab] OR "Intrauterine"[tiab] OR "Lactation"[tiab] OR "lactation disorders"[mh] OR "leydig cell*"[tiab] OR "leydig cells"[mh] OR "LH"[tiab] OR "luteal cell*"[tiab] OR "luteinizing hormone"[tiab] OR "maternal exposure"[mh] OR "Maternal"[tiab] OR "Menses"[tiab] OR "menstrua*"[tiab] OR "miscarriage*"[tiab] OR "neonat*"[tiab] OR "Oligospermia"[tiab] OR "oocyte*"[tiab] OR "Oogonia"[tiab] OR "Ova"[tiab] OR "ovarian follicle*"[tiab] OR "Ovarian"[tiab] OR "Ovaries"[tiab] OR "Ovary"[mh] OR "Ovary"[tiab] OR "oviduct*"[tiab] OR "oviducts"[mh] OR "ovulat*"[tiab] OR "Ovum"[mh] OR "Ovum"[tiab] OR "paternal exposure"[mh] OR "Paternal"[tiab] OR "peripubert*"[tiab] OR "pituitary hormones"[mh] OR "placenta*"[tiab] OR "placenta"[mh] OR "placental hormones"[mh] OR "preconception*"[tiab] OR "pre conception*"[tiab] OR "pregnan*"[tiab] OR "pregnancy complications"[mh] OR "pregnancy"[mh] OR "prenatal exposure delayed effects"[mh] OR "prenatal"[tiab] OR "Pre-natal"[tiab] OR "Preterm"[tiab] OR "Pre-term"[tiab] OR "primary follicle*"[tiab] OR "Progesterone"[tiab] OR "progestin*"[tiab] OR "progestins"[mh] OR "Prostate"[mh] OR "Prostate"[tiab] OR "reproduct*"[tiab] OR "reproductive physiological phenomena"[mh] OR "secondary follicle*"[tiab] OR "Semen"[mh] OR "Semen"[tiab] OR "seminal vesicle*"[tiab] OR "seminal vesicles"[mh] OR "Seminal"[tiab] OR "seminiferous epithelium"[tiab] OR "seminiferous tubule*"[tiab] OR "seminiferous tubules"[mh] OR "Seminiferous"[tiab] OR "sertoli cells"[mh] OR "sertoli cell*"[tiab] OR "sexual development"[mh] OR "Sperm"[tiab] OR "spermatid*"[tiab] OR "spermatocyte*"[tiab] OR "spermatogenesis"[tiab] OR "Spermatogonia"[tiab] OR "Spermatozoa"[mh] OR "Spermatozoa"[tiab] OR "Sterile"[tiab] OR "Sterility"[tiab] OR "stillbirth*"[tiab] OR "Stillborn"[tiab] OR "syncytiotrophoblast*"[tiab] OR "teratogen*"[tiab] OR "teratogens"[mh] OR "tertiary follicle*"[tiab] OR "Testes"[tiab] OR "testic*"[tiab] OR "Testis"[mh] OR "Testis"[tiab] OR "Testosterone"[tiab] OR "theca cell*"[tiab] OR "thyroid hormones"[mh] OR "trophoblast*"[tiab] OR "urogenital abnormalities"[mh] OR "urogenital*"[tiab] OR "Uterine"[tiab] OR "Uterus"[mh] OR "Uterus"[tiab] OR "vagina*"[tiab] OR "vas deferens"[mh] OR "vas deferens"[tiab] OR "zygote*"[tiab]	
4		

SET #	STRATEGY	CONCEPT GROUP
	"spermatozoa"[mh] OR "acrosome reaction"[mh] OR "Sperm Capacitation"[mh] OR "sperm transport"[mh] OR "sperm-ovum interactions"[mh] OR "acrosome"[tiab] OR "spermatozoa"[tiab] OR "sperm"[tiab] OR "spermatogonia"[tiab] OR "spermatophore*"[tiab] OR "spermatocyte*"[tiab] OR "spermatid*"[tiab] OR "spermatogenesis"[tiab] OR "capacitation"[tiab] OR (("Germ cells"[mh] OR "germ cell*"[tiab]) AND ("male"[mh] OR "male"[tiab])) OR "Leydig cells"[mh] OR "leydig cell*"[tiab] OR "sertoli cells"[mh] OR "sertoli cell*"[tiab] OR "cytoskeleton"[tiab] OR "gonadal somatic cells"[tiab] OR "follicle-stimulating hormone"[mh] OR "testosterone congeners"[mh] OR "luteinizing hormone"[mh] OR "androgens"[mh] OR "follicle stimulating hormone*"[tiab] OR "FSH"[tiab] OR "luteinizing hormone"[tiab] OR "LH"[tiab] OR "Inhibin"[tiab] OR "testosterone"[tiab] OR "prolactin"[tiab] OR "androgen*"[tiab] OR "gonadotropin releasing hormone"[tiab] OR "GnRH"[tiab] OR "steroidogenic"[tiab] OR "reproductive hormone*"[tiab] OR "reproductive steroid hormone*"[tiab] OR "sex steroid*"[tiab] OR "hypothalamic pituitary gonadal axis"[tiab] OR "hpg axis"[tiab] OR "hypothalamic pituitary adrenal axis"[tiab] OR "hypothalamic pituitary thyroid axis"[tiab] OR "CYP3A4"[tiab] OR "CYP17A1"[tiab] OR "aromatase"[tiab] OR "receptors, gonadotropin"[mh] OR "receptors, pituitary hormone"[mh] OR "receptors, estrogen"[mh] OR "receptors, oxytocin"[mh] OR "receptors, androgen"[mh] OR "hormone receptor*"[tiab] OR "lh receptor*"[tiab] OR "gonadotropin receptor*"[tiab] OR "estrogen receptor*"[tiab] OR "oestrogen receptor*"[tiab] OR "fsh receptor*"[tiab] OR "androgen receptor*"[tiab] OR "testosterone receptor*"[tiab] OR "prolactin receptor*"[tiab] OR "DNA Adducts"[mh] OR "comet assay"[mh] OR "Germ-line mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "sister chromatid exchange"[mh] OR "Mutation"[mh] OR "DNA Repair"[mh] OR "genomic instability"[mh] OR "Aneuploidy"[mh] OR "ames assay"[tiab] OR "ames test"[tiab] OR "bacterial reverse mutation assay"[tiab] OR "clastogen*"[tiab] OR "genetic toxicology"[tiab] OR "hyperploid"[tiab] OR "micronucleus test"[tiab] OR "tetraploid"[tiab] OR "chromosome aberrations"[tiab] OR "mutation*"[tiab] OR "chromosome translocation*"[tiab] OR "dna protein crosslink*"[tiab] OR "dna damag*"[tiab] OR "dna inhibit*"[tiab] OR "Micronuclei"[tiab] OR "Micronucleus"[tiab] OR "Mutagens"[tiab] OR "strand break*"[tiab] OR "unscheduled dna synthes*"[tiab] OR "chromosomal aberration*"[tiab] OR "chromosome aberration"[tiab] OR "chromosomal abnormalit*"[tiab] OR "chromosome abnormalit*"[tiab] OR "chromosome damage*"[tiab] OR "genotoxic*"[tiab] OR "adduct formation"[tiab] OR "dna adduct*"[tiab] OR "dna break*"[tiab] OR "dsdna break*"[tiab] OR ("DNA"[tiab]AND "Crosslink"[tiab]) OR "microsatellite-instability"[tiab] OR "chromosomal-instability"[tiab] OR "binucleation"[tiab] OR "binucleated"[tiab] OR (("comet assay"[tiab] OR "Mutagenic"[tiab] OR "Mutagenicity"[tiab] OR "mutations"[tiab] OR "chromosomal-aberration-test"[tiab] OR "sister chromatid exchange"[tiab] OR "SOS-response"[tiab] OR "polyploid*"[tiab] OR "genomic instability"[tiab] OR "dna repair*"[tiab] OR "aneuploid*"[tiab]) NOT "Medline"[Filter])	Key Characteristics of Male Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	OR "epigenesis, genetic"[mh] OR "epigenomics"[mh] OR "DNA methylation"[mh] OR "gene silencing"[mh] OR "histone deacetylases"[mh] OR "RNA Interference"[mh] OR "microRNAs"[mh] OR "rna, small interfering"[mh] OR "cpg islands/genetics"[mh] OR "cpg island methylator"[tiab] OR "cpg island methylation"[tiab] OR "epigenotype"[tiab] OR "epimutation*"[tiab] OR "methylation associated silencing"[tiab] OR "histone tail modifications"[tiab] OR "histone tail modification"[tiab] OR "chromatin organization"[tiab] OR "chromatin packag*"[tiab] OR "histone modification"[tiab] OR "histone retention"[tiab] OR "epigenetic*"[tiab] OR "epigenomic*"[tiab] OR "RNA Interference"[tiab] OR "noncoding rna"[tiab] OR "ncRNA"[tiab] OR "gene activation"[tiab] OR "proteasome"[tiab] OR "DNA methylation"[tiab] OR "Methylation"[Title] OR "CIMP"[tiab] OR "Free Radicals"[mh] OR "Reactive Oxygen Species"[mh] OR "Oxidative stress"[mh] OR "Electron Transport"[mh] OR "free radical*"[tiab] OR "oxygen radical*"[tiab] OR "Oxidative stress"[tiab] OR "redox balance"[tiab] OR "oxidative damage*"[tiab] OR "Reactive Oxygen Species"[tiab] OR "reactive nitrogen species"[tiab] OR "superoxide radical*"[tiab] OR "hydroxyl radical"[tiab] OR "glutathione deplet*"[tiab] OR "oxidative protein damage"[tiab] OR "C-reactive protein"[mh] OR "eosinophils"[mh] OR ("fibrinogen"[tiab]AND "Inflammation"[tiab]) OR "chronicinflammation"[tiab] OR "chronically inflamed"[tiab] OR "acute inflammat*"[tiab] OR "infiltrating leukocyt*"[tiab] OR "inflammatory-leukocyte"[tiab] OR "inflammatory-leukocytes"[tiab] OR "leukocyte infiltrat*"[tiab] OR "pro-inflammatory"[tiab] OR "proinflammatory"[tiab] OR "macrophage-recruitment"[tiab] OR "macrophage inflammatory proteins"[tiab] OR "macrophage colony stimulating factor*"[tiab] OR "urethritis"[tiab] OR "prostatitis"[tiab] OR "seminal vesiculitis"[tiab] OR "epididymitis"[tiab] OR "orchitis"[tiab]	
5	("hypothalamic pituitary ovarian axis"[tiab] OR "hpo axis"[tiab] OR "gonadal hormones"[mh] OR "pituitary hormones"[mh] OR "gonadotropin releasing hormone"[mh] OR "follicle stimulating hormone"[mh] OR "testosterone"[mh] OR "receptors, gonadotropin"[mh] OR "receptors, pituitary hormone"[mh] OR "receptors, estrogen"[mh] OR "receptors, oxytocin"[mh] OR "estrogens"[mh] OR "estradiol"[mh] OR "estriol"[mh] OR "estrone"[mh] OR "luteinizing hormone"[mh] OR "androgens"[mh] OR "hydroxyprogesterones"[mh] OR "Dehydroepiandrosterone"[mh] OR "Androstenedione"[mh] OR "Androstenediol"[mh] OR "Dihydrotestosterone"[mh] OR "androgen receptor*"[tiab] OR "estradiol receptor*"[tiab] OR "estrogen receptor*"[tiab] OR "follicle stimulating hormone"[tiab] OR "fsh receptor*"[tiab] OR "gonadotropin releasing hormone"[tiab] OR "hormone receptor*"[tiab] OR "lh receptor*"[tiab] OR "luteinizing hormone"[tiab] OR "oestrogen receptor*"[tiab] OR "ovarian hormone*"[tiab] OR "ovarian steroid*"[tiab] OR "oxytocin receptor*"[tiab] OR "plasma membrane receptor*"[tiab] OR "prolactin receptor*"[tiab] OR "reproductive hormone*"[tiab] OR "sex hormone*"[tiab] OR "testosterone receptor*"[tiab] OR "activin"[tiab] OR	Key Characteristics of Female Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	"estradiol"[tiab] OR "estriol"[tiab] OR "estrogen"[tiab] OR "estrone"[tiab] OR "FSH"[tiab] OR "gnrh"[tiab] OR "gonadotropin*"[tiab] OR "gonadotropin receptor*"[tiab] OR "hcg"[tiab] OR "inhibin"[tiab] OR "LH"[tiab] OR "LHRH"[tiab] OR "oestriol"[tiab] OR "oestradiol"[tiab] OR "oestrogen"[tiab] OR "oestrone"[tiab] OR "Oxytocin"[tiab] OR "progesterone"[tiab] OR "prolactin"[tiab] OR "Steroidogenic"[tiab] OR "testosterone"[tiab] OR "Pregnenolone"[tiab] OR "17alpha hydroxy 6 methylene progesterone"[Supplementary Concept] OR "Dehydroepiandrosterone"[tiab] OR "DHEA"[tiab] OR "DHEAS"[tiab] OR "Androstenedione"[tiab] OR "Androstenediol"[tiab] OR "Dihydrotestosterone"[tiab] OR "steroidogenic acute regulatory protein"[Supplementary Concept] OR "steroidogenic acute regulatory protein"[tiab] OR "star protein"[tiab] OR "cholesterol side chain cleavage enzyme"[mh] OR "cholesterol side chain cleavage enzyme"[tiab] OR "cholesterol desmolase"[tiab] OR "cytochrome p 450 scc"[tiab] OR "P450scc"[tiab] OR "CYP11A"[tiab] OR "CYP11A1"[tiab] OR "17alpha hydroxylase"[tiab] OR "17,20 lyase"[tiab] OR "P450c17"[tiab] OR "CYP17"[tiab] OR "aromatase"[mh] OR "aromatase"[tiab] OR "cytochrome p450 family 19"[mh] OR "cytochrome p450 family 19"[tiab] OR "P450arom"[tiab] OR "CYP19"[tiab] OR "3 or 17 beta hydroxysteroid dehydrogenase"[Supplementary Concept] OR "3beta hydroxysteroid dehydrogenase"[tiab] OR "3beta hsd"[tiab] OR "17beta hydroxysteroid dehydrogenase"[tiab] OR "17beta hsd*"[tiab] OR "5alpha-reductase"[tiab] OR ("DNA Adducts"[mh] OR "Comet Assay"[mh] OR "Germ-line mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "Sister-chromatid exchange"[mh] OR "Mutation"[mh] OR "Ames-Assay"[tiab] OR "Ames-test"[tiab] OR "Bacterial-Reverse-Mutation-Assay"[tiab] OR "clastogen*"[tiab] OR "dna repair*"[tiab] OR "Genetic-toxicology"[tiab] OR "hyperploid"[tiab] OR "micronucleus-test"[tiab] OR "tetraploid"[tiab] OR "Chromosome-aberrations"[tiab] OR "DNA-damage"[tiab] OR "mutation*"[tiab] OR "chromosome-translocations"[tiab] OR "dna protein crosslink*"[tiab] OR "dna damag*"[tiab] OR "dna inhibit*"[tiab] OR "Micronuclei"[tiab] OR "Micronucleus"[tiab] OR "Mutagens"[tiab] OR "strand break*"[tiab] OR "unscheduled dna synthes*"[tiab] OR "chromosomal aberration*"[tiab] OR "chromosome aberration*"[tiab] OR "chromosomal abnormalit*"[tiab] OR "chromosome abnormalit*"[tiab] OR "genotoxic*"[tiab] OR "adduct-formation"[tiab] OR "dna adduct*"[tiab] OR "dna break*"[tiab] OR "dsdna break*"[tiab]) OR ("epigenesis, genetic"[mh] OR "epigenomics"[mh] OR "DNA methylation"[mh] OR "gene silencing"[mh] OR "histone deacetylases"[mh] OR "RNA Interference"[mh] OR "microRNAs"[mh] OR "rna, small interfering"[mh] OR "cpg islands/genetics"[mh] OR "cpg island methylator"[tiab] OR "cpg island methylation"[tiab] OR "epigenotype"[tiab] OR "epimutation*"[tiab] OR "methylation associated silencing"[tiab] OR "histone tail modifications"[tiab] OR "histone tail modification"[tiab] OR "chromatin organization"[tiab] OR "chromatin packag*"[tiab] OR "histone modification"[tiab] OR "histone retention"[tiab] OR "epigenetic*"[tiab] OR "epigenomic*"[tiab] OR	

SET #	STRATEGY	CONCEPT GROUP
	"RNA Interference"[tiab] OR "noncoding rna"[tiab] OR "ncRNA"[tiab] OR "gene activation"[tiab] OR "proteasome"[tiab] OR "DNA methylation"[tiab] OR "Methylation"[Title] OR "CIMP"[tiab]) OR ("mitochondria"[mh] OR "oxidative phosphorylation"[mh] OR "mitochrondria*"[tiab] OR "oxidative phosphorylation"[tiab] OR "oxidative damage"[tiab] OR "fatty acid beta oxidation"[tiab] OR "calcium buffering"[tiab] OR "ca2 buffering"[tiab] OR "mitochondrial dna mutation*"[tiab] OR "mtdna mutation*"[tiab] OR "mtDNA"[tiab]) OR ("Free Radicals"[mh] OR "Reactive Oxygen Species"[mh] OR "Oxidative stress"[mh] OR "Electron Transport"[mh] OR "free radical*"[tiab] OR "oxygen radical*"[tiab] OR "Oxidative stress"[tiab] OR "redox balance"[tiab] OR "oxidative damage*"[tiab] OR "Reactive Oxygen Species"[tiab] OR "reactive nitrogen species"[tiab] OR "superoxide radical*"[tiab] OR "hydroxyl radical"[tiab] OR "glutathione deplet*"[tiab] OR "oxidative protein damage"[tiab]) OR ("cytotoxicity, immunologic"[mh] OR "Immunologic Factors"[mh] OR "Immunomodulation"[mh] OR "B-Cell Activation Factor Receptor"[mh] OR "Antigenic Modulation"[mh] OR "B-Cell Activating Factor"[mh] OR "Immunologic Factors"[Pharmacological Action] OR "b-cell-activation"[tiab] OR "immune surveillance"[tiab] OR "immune suppress*"[tiab] OR "immunostimulant"[tiab] OR "immune-activation"[tiab] OR "immunodeficien*"[tiab] OR "somatic-hypermutation"[tiab] OR "immune-activation"[tiab] OR "immune-system-activation"[tiab] OR "Chronic-antigenic-stimulation"[tiab] OR "immunosuppress*"[tiab] OR "immune dysregulation"[tiab]) OR ("signal transduction"[mh] OR "signal transduction"[tiab] OR "signal pathway*"[tiab] OR "signaling pathway*"[tiab] OR "ion channel"[tiab] OR "signaling system*"[tiab] OR "cell signal*"[tiab] OR "cellular signal*"[tiab] OR "intracellular signal*"[tiab] OR "signal cascade*"[tiab] OR "signaling cascade*"[tiab] OR "second messenger*"[tiab] OR "calcium signal*"[tiab]) OR ("cell communication"[mh] OR "gap junctions"[mh] OR "connexins"[mh] OR "connexins"[Supplementary Concept] OR "cell communication*"[tiab] OR "cellular communication*"[tiab] OR "intracellular communication*"[tiab] OR "cell interaction*"[tiab] OR "gap junction*"[tiab] OR "connexin*"[tiab]) OR ("Apoptosis"[mh] OR "cytotoxicity, immunologic"[mh] OR "Caspases"[mh] OR "autophagy"[mh] OR "necrosis"[mh] OR "Autolysis"[mh] OR "Angiogenesis Modulating Agents"[mh] OR "Angiogenesis Inducing Agents"[Pharmacological Action] OR "Angiogenesis Inducing Agents"[mh] OR "neovascularization, pathologic"[mh] OR "Cell Proliferation"[mh] OR "homeostasis"[mh] OR "Cyclin-Dependent Kinases"[mh] OR "Cyclin-Dependent Kinase Inhibitor Proteins"[mh] OR "Mitogens"[mh] OR "Mitogens"[Pharmacological Action] OR "cell hypoxia"[mh] OR "angiogenic"[tiab] OR "Apoptosis"[tiab] OR "autophagy"[tiab] OR "Caspases"[tiab] OR "cell cycle control*"[tiab] OR "cell cycle arrest"[tiab] OR "cell hypoxia"[tiab] OR "Cell Proliferation"[tiab] OR "cellular-energetics"[tiab] OR "cellular-hypoxia"[tiab] OR "cellular proliferation"[tiab] OR "cellular replication*"[tiab] OR "Cytogenesis"[tiab] OR "Cytogenic"[tiab] OR "Cytotoxin"[tiab] OR "hepatocellular-proliferation"[tiab] OR "hyperplasia"[tiab] OR	

SET #	STRATEGY	CONCEPT GROUP
	"hypoxic cell*"[tiab] OR "mitogenesis"[tiab] OR "mitotic checkpoint*"[tiab] OR "Neoplasia"[tiab] OR "p53 delet*"[tiab] OR "p53 inactivat*"[tiab] OR "p53 inhibit*"[tiab] OR "prb delet*"[tiab] OR "prb inactivat*"[tiab] OR "prb inhibit*"[tiab] OR "programmed cell death"[tiab] OR ("Rb"[All Fields] AND "p16ink4a inactiv*"[tiab]) OR "retinoblastoma-protein"[tiab] OR "senescence"[tiab] OR "senescent"[tiab] OR "survivin"[tiab]) OR "microtubules"[mh] OR "spindle apparatus"[mh] OR "microtubule organizing center"[mh] OR "microtubule*"[tiab] OR "spindle formation"[tiab] OR "spindle apparatus"[tiab] OR "meiotic spindle*"[tiab] OR "mitotic spindle*"[tiab])	
6	#2 OR #3 OR #4 OR #5	Combine DART concept groups
7	#1 AND #6	Combine Chemical + DART
8	#7 NOT (animals[mh] NOT humans[mh])	Remove animal studies **FINAL**

Table A.9 PubMed search strategy for animal DART studies

SET #	STRATEGY	CONCEPT GROUP
1	(80-09-1[rn] OR "Bisphenol S"[tiab] OR "bis(4-hydroxyphenyl)sulfone"[nm] OR "bis(4-hydroxyphenyl)sulfone"[tiab] OR "4,4'-Sulfonyldiphenol"[tiab] OR "Phenol, 4,4'-sulfonylbis-"[tiab] OR "BPS-monoglucuronide"[tiab] OR "BPS-1G"[tiab] OR "bisphenol S dicyanate ester"[tiab] OR (bps[tiab] NOT ("bps"[tiab] NOT "bisphenol"[tiab]))	Chemical Terms
2	(abnormalities, drug-induced [mh] AND (fetus [mh] or pregnancy [mh])) OR ((abnormalities, multiple/chemically induced [mh] OR abnormalities, multiple/epidemiology [mh] OR abnormalities, multiple/etiology[mh] OR abnormalities, multiple/genetics [mh] OR abnormalities, multiple/pathology [mh]) AND (pregnancy [mh] OR fetus [mh]))OR (abnormalities, radiation-induced [mh] AND (fetus [mh] or pregnancy [mh])) OR abortion, habitual/chemically induced [mh] OR abortion, habitual/etiology [mh] OR abortion, spontaneous/chemically induced [mh] OR abortion, spontaneous/etiology [mh] OR (alcoholic intoxication[mh] AND (fetus [mh] or pregnancy [mh])) OR (alcohol drinking [mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR Birth Defects Res B Dev Reprod Toxicol [TA] OR birth weight/drug effects [mh] OR birth weight/radiation effects [mh] OR breast feeding/drug effects [mh] OR (carcinogens, environmental [mh] AND (fetus [mh] or pregnancy [mh])) OR (carcinogens [mh] AND (fetus [mh] OR pregnancy [mh])) OR (cardiovascular abnormalities/ci [mh] AND fetus [mh]) OR (cardiovascular abnormalities/et [mh] AND fetus [mh]) OR (cocaine[mh] AND (fetus [mh] or pregnancy [mh])) OR (congenital abnormalities [mh] AND (fetus [mh] or pregnancy [mh])) OR (dna damage [mh] AND (pregnancy [mh] OR fetus [mh])) OR embryo/de [mh] OR embryo/re [mh] OR embryo loss/ci [mh] OR embryonic and fetal development/drug effects [mh] OR embryonic and fetal development/radiation effects [mh] OR embryonic structures/drug effects [mh] OR embryonic structures/pathology [mh] OR embryonic structures/radiation effects [mh] OR (environmental exposure[mh] AND (pregnancy [mh] OR fetus [mh])) OR fertility/drug effects [mh] OR fertility/radiation effects [mh] OR fetal alcohol syndrome[mh:noexp] OR fetal death/chemically induced [mh] OR fetal death/etiology [mh] OR fetal death/genetics [mh] OR fetal death/pathology [mh] OR fetal diseases/chemically induced [mh] OR fetal diseases/etiology [mh] OR fetal diseases/genetics [mh] OR fetal growth retardation/et [mh] OR fetal growth retardation/ci [mh] OR fetal resorption/chemically induced [mh] OR fetal resorption/etiology [mh] OR fetal resorption/genetics [mh] OR fetus/abnormalities [mh] OR fetus/drug effects [mh] OR fetus/radiation effects [mh] OR (fetus*[tw] AND expos*[tw]) OR (genetic diseases, inborn/CI [mh] AND (fetus [mh] OR pregnancy [mh])) OR germ cells/drug effects [mh] OR germ cells/radiation effects [mh] OR (hazardous substances [mh] AND (fetus [mh] or pregnancy [mh])) OR heavy metal poisoning[mh] OR lactation/drug effects [mh] OR lactation/radiation effects [mh]	PubMed DART strategy

SET #	STRATEGY	CONCEPT GROUP
	OR (lead [mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR (lead poisoning[mh] AND (fetus [mh] or pregnancy [mh]))OR maternal exposure [mh] OR maternal-fetal exchange/genetics [mh] OR maternal-fetal exchange/drug effects [mh] OR maternal-fetal exchange/radiation effects[mh] OR (mutagens [mh] AND (pregnancy [mh] OR fetus [mh])) OR neonatal abstinence syndrome[mh] OR "neonatal abstinence syndrome"[ti] OR neonatal sepsis [mh] OR ovary/drug effects [mh] OR ovary/radiation effects [mh] OR paternal exposure [mh] OR placenta diseases/chemically induced [mh] OR placenta diseases/etiology [mh] OR placenta/abnormalities [mh] OR placenta/drug effects [mh] OR placenta/radiation effects [mh] OR pregnancy Complications, Infectious/epidemiology [mh] OR pregnancy Complications/ci [mh] OR pregnancy outcome/ge [mh] OR (prenatal*[tw] AND expos*[tw]) OR prenatal exposure delayed effects [mh] OR (protein deficiency[mh:noexp] AND (fetus [mh] or pregnancy [mh])) OR reproduction/drug effects [mh:noexp] OR reproduction/radiation effects [mh] OR rubella/congenital[mh:noexp] OR rubella syndrome, congenital/etiology[mh:noexp] OR (teratogens [mh] AND (pregnancy [mh] OR fetus [mh]))OR Teratology [Journal] OR teratology [mh] OR testis/drug effects [mh] OR testis/radiation effects [mh]	
3	"abortion, spontaneous"[mh] OR "abortion*"[tiab] OR "Acrosome"[mh] OR "Acrosome"[tiab] OR "Adrenarche"[tiab] OR "androgen antagonists"[mh] OR "androgen*"[tiab] OR "androgens"[mh] OR "Androstenedione"[tiab] OR "anogenital distance"[tiab] OR "ano genital distance"[tiab] OR "anovulat*"[tiab] OR "Aspermia"[tiab] OR "atretic follicle*"[tiab] OR "Azoospermia"[tiab] OR "birth defect*"[tiab] OR "birth weight"[mh] OR "birth weight"[tiab] OR "breast feed*"[tiab] OR "breast feeding"[mh] OR "breastfeed*"[tiab] OR "chorionic villi"[tiab] OR "conception*"[tiab] OR "congenital abnormalities"[mh] OR "Congenital"[tiab] OR "corpus luteum"[tiab] OR "cumulus cell*"[tiab] OR "cytotrophoblast*"[tiab] OR "decidua"[tiab] OR "deciduum"[tiab] OR "dna damage"[mh] OR "ductus deferens"[tiab] OR "efferent duct*"[tiab] OR "ejaculat*"[tiab] OR "Embryo"[tiab] OR "Embryoes"[tiab] OR "embryonic and fetal development"[mh] OR "embryonic structures"[mh] OR "Embryonic"[tiab] OR "embryotoxic*"[tiab] OR "endometri*"[tiab] OR "Epididymis"[mh] OR "Epididymis"[tiab] OR "erecti*"[tiab] OR "Estradiol"[tiab] OR "estrogen antagonists"[mh] OR "estrogen receptor	Additional DART terms (OEHHA Strategy)

SET #	STRATEGY	CONCEPT GROUP
	modulators"[mh] OR "estrogen*"[tiab] OR "estrogens"[mh] OR "Estrus"[tiab] OR "fallopian tube*"[tiab] OR "fallopian tubes"[mh] OR "fecund*"[tiab] OR "Fertility"[mh] OR "Fertility"[tiab] OR "Fertilization"[tiab] OR "Fetal"[tiab] OR "Fetus"[mh] OR "Fetus"[tiab] OR "foetal"[tiab] OR "foetus"[tiab] OR "follicle stimulating hormone"[tiab] OR "FSH"[tiab] OR "genetic diseases, inborn"[mh] OR "genital diseases, female"[mh] OR "genital diseases, male"[mh] OR "genital*"[tiab] OR "genitalia"[mh] OR "germ cell*"[tiab] OR "germ cells"[mh] OR "gestat*"[tiab] OR "gonad*"[tiab] OR "gonadal disorders"[mh] OR "gonadal hormones"[mh] OR "gonadotropins"[mh] OR "gonads"[mh] OR "graafian follicle*"[tiab] OR "granulosa cell*"[tiab] OR "human development"[mh] OR "Implantation"[tiab] OR "in utero"[tiab] OR "infant*"[tiab] OR "infant, newborn"[mh] OR "infertil*"[tiab] OR "Inhibin"[tiab] OR "Intrauterine"[tiab] OR "Lactation"[tiab] OR "lactation disorders"[mh] OR "leydig cell*"[tiab] OR "leydig cells"[mh] OR "LH"[tiab] OR "luteal cell*"[tiab] OR "luteinizing hormone"[tiab] OR "maternal exposure"[mh] OR "Maternal"[tiab] OR "Menses"[tiab] OR "menstrua*"[tiab] OR "miscarriage*"[tiab] OR "neonat*"[tiab] OR "Oligospermia"[tiab] OR "oocyte*"[tiab] OR "Oogonia"[tiab] OR "Ova"[tiab] OR "ovarian follicle*"[tiab] OR "Ovarian"[tiab] OR "Ovaries"[tiab] OR "Ovary"[mh] OR "Ovary"[tiab] OR "oviduct*"[tiab] OR "oviducts"[mh] OR "ovulat*"[tiab] OR "Ovum"[mh] OR "Ovum"[tiab] OR "paternal exposure"[mh] OR "Paternal"[tiab] OR "peripubert*"[tiab] OR "pituitary hormones"[mh] OR "placenta*"[tiab] OR "placenta"[mh] OR "placental hormones"[mh] OR "preconception*"[tiab] OR "pre conception*"[tiab] OR "pregnan*"[tiab] OR "pregnancy complications"[mh] OR "pregnancy"[mh] OR "prenatal exposure delayed effects"[mh] OR "prenatal"[tiab] OR "Pre-natal"[tiab] OR "Preterm"[tiab] OR "Pre-term"[tiab] OR "primary follicle*"[tiab] OR "Progesterone"[tiab] OR "progestin*"[tiab] OR "progestins"[mh] OR "Prostate"[mh] OR "Prostate"[tiab] OR "reproduct*"[tiab] OR "reproductive physiological phenomena"[mh] OR "secondary follicle*"[tiab] OR "Semen"[mh] OR "Semen"[tiab] OR "seminal vesicle*"[tiab] OR "seminal vesicles"[mh] OR "Seminal"[tiab] OR "seminiferous epithelium"[tiab] OR "seminiferous tubule*"[tiab] OR "seminiferous tubules"[mh] OR "Seminiferous"[tiab] OR "sertoli cells"[mh] OR "sertoli cell*"[tiab] OR "sexual development"[mh] OR "Sperm"[tiab] OR "spermatid*"[tiab] OR "spermatocyte*"[tiab] OR "spermatogenesis"[tiab] OR "Spermatogonia"[tiab] OR "Spermatozoa"[mh] OR "Spermatozoa"[tiab] OR "Sterile"[tiab] OR "Sterility"[tiab] OR "stillbirth*"[tiab] OR "Stillborn"[tiab] OR "syncytiotrophoblast*"[tiab] OR "teratogen*"[tiab] OR "teratogens"[mh] OR "tertiary follicle*"[tiab] OR "Testes"[tiab] OR "testic*"[tiab] OR "Testis"[mh] OR "Testis"[tiab] OR "Testosterone"[tiab] OR "theca cell*"[tiab] OR "thyroid hormones"[mh] OR "trophoblast*"[tiab] OR "urogenital abnormalities"[mh] OR "urogenital*"[tiab] OR "Uterine"[tiab] OR "Uterus"[mh] OR "Uterus"[tiab] OR "vagina*"[tiab] OR "vas deferens"[mh] OR "vas deferens"[tiab] OR "zygote*"[tiab]	

SET #	STRATEGY	CONCEPT GROUP
4	"spermatozoa"[mh] OR "acrosome reaction"[mh] OR "Sperm Capacitation"[mh] OR "sperm transport"[mh] OR "sperm-ovum interactions"[mh] OR "acrosome"[tiab] OR "spermatozoa"[tiab] OR "sperm"[tiab] OR "spermatogonia"[tiab] OR "spermatophore*"[tiab] OR "spermatocyte*"[tiab] OR "spermatid*"[tiab] OR "spermatogenesis"[tiab] OR "capacitation"[tiab] OR (("Germ cells"[mh] OR "germ cell*"[tiab]) AND ("male"[mh] OR "male"[tiab])) OR "Leydig cells"[mh] OR "leydig cell*"[tiab] OR "sertoli cells"[mh] OR "sertoli cell*"[tiab] OR "cytoskeleton"[tiab] OR "gonadal somatic cells"[tiab] OR "follicle-stimulating hormone"[mh] OR "testosterone congeners"[mh] OR "luteinizing hormone"[mh] OR "androgens"[mh] OR "follicle stimulating hormone*"[tiab] OR "FSH"[tiab] OR "luteinizing hormone"[tiab] OR "LH"[tiab] OR "Inhibin"[tiab] OR "testosterone"[tiab] OR "prolactin"[tiab] OR "androgen*"[tiab] OR "gonadotropin releasing hormone"[tiab] OR "GnRH"[tiab] OR "steroidogenic"[tiab] OR "reproductive hormone*"[tiab] OR "reproductive steroid hormone*"[tiab] OR "sex steroid*"[tiab] OR "hypothalamic pituitary gonadal axis"[tiab] OR "hpg axis"[tiab] OR "hypothalamic pituitary adrenal axis"[tiab] OR "hypothalamic pituitary thyroid axis"[tiab] OR "CYP3A4"[tiab] OR "CYP17A1"[tiab] OR "aromatase"[tiab] OR "receptors, gonadotropin"[mh] OR "receptors, pituitary hormone"[mh] OR "receptors, estrogen"[mh] OR "receptors, oxytocin"[mh] OR "receptors, androgen"[mh] OR "hormone receptor*"[tiab] OR "lh receptor*"[tiab] OR "gonadotropin receptor*"[tiab] OR "estrogen receptor*"[tiab] OR "oestrogen receptor*"[tiab] OR "fsh receptor*"[tiab] OR "androgen receptor*"[tiab] OR "testosterone receptor*"[tiab] OR "prolactin receptor*"[tiab] OR "DNA Adducts"[mh] OR "comet assay"[mh] OR "Germ-line mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "sister chromatid exchange"[mh] OR "Mutation"[mh] OR "DNA Repair"[mh] OR "genomic instability"[mh] OR "Aneuploidy"[mh] OR "ames assay"[tiab] OR "ames test"[tiab] OR "bacterial reverse mutation assay"[tiab] OR "clastogen*"[tiab] OR "genetic toxicology"[tiab] OR "hyperploid"[tiab] OR "micronucleus test"[tiab] OR "tetraploid"[tiab] OR "chromosome aberrations"[tiab] OR "mutation*"[tiab] OR "chromosome translocation*"[tiab] OR "dna protein crosslink*"[tiab] OR "dna damag*"[tiab] OR "dna inhibit*"[tiab] OR "Micronuclei"[tiab] OR "Micronucleus"[tiab] OR "Mutagens"[tiab] OR "strand break*"[tiab] OR "unscheduled dna synthes*"[tiab] OR "chromosomal aberration*"[tiab] OR "chromosome aberration"[tiab] OR "chromosomal abnormalit*"[tiab] OR "chromosome abnormalit*"[tiab] OR "chromosome damage*"[tiab] OR "genotoxic*"[tiab] OR "adduct formation"[tiab] OR "dna adduct*"[tiab] OR "dna break*"[tiab] OR "dsdna break*"[tiab] OR ("DNA"[tiab]AND "Crosslink"[tiab]) OR "microsatellite-instability"[tiab] OR "chromosomal-instability"[tiab] OR "binucleation"[tiab] OR "binucleated"[tiab] OR (("comet assay"[tiab] OR "Mutagenic"[tiab] OR "Mutagenicity"[tiab] OR "mutations"[tiab] OR "chromosomal-aberration-test"[tiab] OR "sister chromatid exchange"[tiab] OR "SOS-response"[tiab] OR "polyploid*"[tiab] OR "genomic	Key Characteristics of Male Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	instability"[tiab] OR "dna repair*"[tiab] OR "aneuploid*"[tiab]) NOT "Medline"[Filter]) OR "epigenesis, genetic"[mh] OR "epigenomics"[mh] OR "DNA methylation"[mh] OR "gene silencing"[mh] OR "histone deacetylases"[mh] OR "RNA Interference"[mh] OR "microRNAs"[mh] OR "rna, small interfering"[mh] OR "cpg islands/genetics"[mh] OR "cpg island methylator"[tiab] OR "cpg island methylation"[tiab] OR "epigenotype"[tiab] OR "epimutation*"[tiab] OR "methylation associated silencing"[tiab] OR "histone tail modifications"[tiab] OR "histone tail modification"[tiab] OR "chromatin organization"[tiab] OR "chromatin packag*"[tiab] OR "histone modification"[tiab] OR "histone retention"[tiab] OR "epigenetic*"[tiab] OR "epigenomic*"[tiab] OR "RNA Interference"[tiab] OR "noncoding rna"[tiab] OR "ncRNA"[tiab] OR "gene activation"[tiab] OR "proteasome"[tiab] OR "DNA methylation"[tiab] OR "Methylation"[Title] OR "CIMP"[tiab] OR "Free Radicals"[mh] OR "Reactive Oxygen Species"[mh] OR "Oxidative stress"[mh] OR "Electron Transport"[mh] OR "free radical*"[tiab] OR "oxygen radical*"[tiab] OR "Oxidative stress"[tiab] OR "redox balance"[tiab] OR "oxidative damage*"[tiab] OR "Reactive Oxygen Species"[tiab] OR "reactive nitrogen species"[tiab] OR "superoxide radical*"[tiab] OR "hydroxyl radical"[tiab] OR "glutathione deplet*"[tiab] OR "oxidative protein damage"[tiab] OR "C-reactive protein"[mh] OR "eosinophils"[mh] OR ("fibrinogen"[tiab]AND "Inflammation"[tiab]) OR "chronicinflammation"[tiab] OR "chronically inflamed"[tiab] OR "acute inflammat*"[tiab] OR "infiltrating leukocyt*"[tiab] OR "inflammatory-leukocyte"[tiab] OR "inflammatory-leukocytes"[tiab] OR "leukocyte infiltrat*"[tiab] OR "pro-inflammatory"[tiab] OR "proinflammatory"[tiab] OR "macrophage-recruitment"[tiab] OR "macrophage inflammatory proteins"[tiab] OR "macrophage colony stimulating factor*"[tiab] OR "urethritis"[tiab] OR "prostatitis"[tiab] OR "seminal vesiculitis"[tiab] OR "epididymitis"[tiab] OR "orchitis"[tiab]	
5	("hypothalamic pituitary ovarian axis"[tiab] OR "hpo axis"[tiab] OR "gonadal hormones"[mh] OR "pituitary hormones"[mh] OR "gonadotropin releasing hormone"[mh] OR "follicle stimulating hormone"[mh] OR "testosterone"[mh] OR "receptors, gonadotropin"[mh] OR "receptors, pituitary hormone"[mh] OR "receptors, estrogen"[mh] OR "receptors, oxytocin"[mh] OR "estrogens"[mh] OR "estradiol"[mh] OR "estriol"[mh] OR "estrone"[mh] OR "luteinizing hormone"[mh] OR "androgens"[mh] OR "hydroxyprogesterones"[mh] OR "Dehydroepiandrosterone"[mh] OR "Androstenedione"[mh] OR "Androstenediol"[mh] OR "Dihydrotestosterone"[mh] OR "androgen receptor*"[tiab] OR "estradiol receptor*"[tiab] OR "estrogen receptor*"[tiab] OR "follicle stimulating hormone"[tiab] OR "fsh receptor*"[tiab] OR "gonadotropin releasing hormone"[tiab] OR "hormone receptor*"[tiab] OR "lh receptor*"[tiab] OR "luteinizing hormone"[tiab] OR "oestrogen receptor*"[tiab] OR "ovarian hormone*"[tiab] OR "ovarian steroid*"[tiab] OR "oxytocin receptor*"[tiab] OR "plasma membrane receptor*"[tiab] OR "prolactin receptor*"[tiab] OR "reproductive hormone*"[tiab] OR "sex	Key Characteristics of Female Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	hormone*"[tiab] OR "testosterone receptor*"[tiab] OR "activin"[tiab] OR "estradiol"[tiab] OR "estriol"[tiab] OR "estrogen"[tiab] OR "estrone"[tiab] OR "FSH"[tiab] OR "gnrh"[tiab] OR "gonadotropin*"[tiab] OR "gonadotropin receptor*"[tiab] OR "hcg"[tiab] OR "inhibin"[tiab] OR "LH"[tiab] OR "LHRH"[tiab] OR "oestriol"[tiab] OR "oestradiol"[tiab] OR "oestrogen"[tiab] OR "oestrone"[tiab] OR "Oxytocin"[tiab] OR "progesterone"[tiab] OR "prolactin"[tiab] OR "Steroidogenic"[tiab] OR "testosterone"[tiab] OR "Pregnenolone"[tiab] OR "17alpha hydroxy 6 methylene progesterone"[Supplementary Concept] OR "Dehydroepiandrosterone"[tiab] OR "DHEA"[tiab] OR "DHEAS"[tiab] OR "Androstenedione"[tiab] OR "Androstenediol"[tiab] OR "Dihydrotestosterone"[tiab] OR "steroidogenic acute regulatory protein"[Supplementary Concept] OR "steroidogenic acute regulatory protein"[tiab] OR "star protein"[tiab] OR "cholesterol side chain cleavage enzyme"[mh] OR "cholesterol side chain cleavage enzyme"[tiab] OR "cholesterol desmolase"[tiab] OR "cytochrome p 450 scc"[tiab] OR "P450scc"[tiab] OR "CYP11A"[tiab] OR "CYP11A1"[tiab] OR "17alpha hydroxylase"[tiab] OR "17,20 lyase"[tiab] OR "P450c17"[tiab] OR "CYP17"[tiab] OR "aromatase"[mh] OR "aromatase"[tiab] OR "cytochrome p450 family 19"[mh] OR "cytochrome p450 family 19"[tiab] OR "P450arom"[tiab] OR "CYP19"[tiab] OR "3 or 17 beta hydroxysteroid dehydrogenase"[Supplementary Concept] OR "3beta hydroxysteroid dehydrogenase"[tiab] OR "3beta hsd"[tiab] OR "17beta hydroxysteroid dehydrogenase"[tiab] OR "17beta hsd*"[tiab] OR "5alpha-reductase"[tiab] OR ("DNA Adducts"[mh] OR "Comet Assay"[mh] OR "Germ-line mutation"[mh] OR "Mutagenesis"[mh] OR "Mutagenicity tests"[mh] OR "Sister-chromatid exchange"[mh] OR "Mutation"[mh] OR "Ames-Assay"[tiab] OR "Ames-test"[tiab] OR "Bacterial-Reverse-Mutation-Assay"[tiab] OR "clastogen*"[tiab] OR "dna repair*"[tiab] OR "Genetic-toxicology"[tiab] OR "hyperploid"[tiab] OR "micronucleus-test"[tiab] OR "tetraploid"[tiab] OR "Chromosome-aberrations"[tiab] OR "DNA-damage"[tiab] OR "mutation*"[tiab] OR "chromosome-translocations"[tiab] OR "dna protein crosslink*"[tiab] OR "dna damag*"[tiab] OR "dna inhibit*"[tiab] OR "Micronuclei"[tiab] OR "Micronucleus"[tiab] OR "Mutagens"[tiab] OR "strand break*"[tiab] OR "unscheduled dna synthes*"[tiab] OR "chromosomal aberration*"[tiab] OR "chromosome aberration*"[tiab] OR "chromosomal abnormalit*"[tiab] OR "chromosome abnormalit*"[tiab] OR "genotoxic*"[tiab] OR "adduct-formation"[tiab] OR "dna adduct*"[tiab] OR "dna break*"[tiab] OR "dsdna break*"[tiab]) OR ("epigenesis, genetic"[mh] OR "epigenomics"[mh] OR "DNA methylation"[mh] OR "gene silencing"[mh] OR "histone deacetylases"[mh] OR "RNA Interference"[mh] OR "microRNAs"[mh] OR "rna, small interfering"[mh] OR "cpg islands/genetics"[mh] OR "cpg island methylator"[tiab] OR "cpg island methylation"[tiab] OR "epigenotype"[tiab] OR "epimutation*"[tiab] OR "methylation associated silencing"[tiab] OR "histone tail modifications"[tiab] OR "histone tail modification"[tiab] OR "chromatin	

SET #	STRATEGY	CONCEPT GROUP
	organization"[tiab] OR "chromatin packag*"[tiab] OR "histone modification"[tiab] OR "histone retention"[tiab] OR "epigenetic*"[tiab] OR "epigenomic*"[tiab] OR "RNA Interference"[tiab] OR "noncoding rna"[tiab] OR "ncRNA"[tiab] OR "gene activation"[tiab] OR "proteasome"[tiab] OR "DNA methylation"[tiab] OR "Methylation"[Title] OR "CIMP"[tiab]) OR ("mitochondria"[mh] OR "oxidative phosphorylation"[mh] OR "mitochrondria*"[tiab] OR "oxidative phosphorylation"[tiab] OR "oxidative damage"[tiab] OR "fatty acid beta oxidation"[tiab] OR "calcium buffering"[tiab] OR "ca2 buffering"[tiab] OR "mitochondrial dna mutation*"[tiab] OR "mtdna mutation*"[tiab] OR "mtDNA"[tiab]) OR ("Free Radicals"[mh] OR "Reactive Oxygen Species"[mh] OR "Oxidative stress"[mh] OR "Electron Transport"[mh] OR "free radical*"[tiab] OR "oxygen radical*"[tiab] OR "Oxidative stress"[tiab] OR "redox balance"[tiab] OR "oxidative damage*"[tiab] OR "Reactive Oxygen Species"[tiab] OR "reactive nitrogen species"[tiab] OR "superoxide radical*"[tiab] OR "hydroxyl radical"[tiab] OR "glutathione deplet*"[tiab] OR "oxidative protein damage"[tiab]) OR ("cytotoxicity, immunologic"[mh] OR "Immunologic Factors"[mh] OR "Immunomodulation"[mh] OR "B-Cell Activation Factor Receptor"[mh] OR "Antigenic Modulation"[mh] OR "B-Cell Activating Factor"[mh] OR "Immunologic Factors"[Pharmacological Action] OR "b-cell-activation"[tiab] OR "immune surveillance"[tiab] OR "immune suppress*"[tiab] OR "immunostimulant"[tiab] OR "immune-activation"[tiab] OR "immunodeficien*"[tiab] OR "somatic-hypermutation"[tiab] OR "immune-activation"[tiab] OR "immune-system-activation"[tiab] OR "Chronic-antigenic-stimulation"[tiab] OR "immunosuppress*"[tiab] OR "immune dysregulation"[tiab]) OR ("signal transduction"[mh] OR "signal transduction"[tiab] OR "signal pathway*"[tiab] OR "signaling pathway*"[tiab] OR "ion channel"[tiab] OR "signaling system*"[tiab] OR "cell signal*"[tiab] OR "cellular signal*"[tiab] OR "intracellular signal*"[tiab] OR "signal cascade*"[tiab] OR "signaling cascade*"[tiab] OR "second messenger*"[tiab] OR "calcium signal*"[tiab]) OR ("cell communication"[mh] OR "gap junctions"[mh] OR "connexins"[mh] OR "connexins"[Supplementary Concept] OR "cell communication*"[tiab] OR "cellular communication*"[tiab] OR "intracellular communication*"[tiab] OR "cell interaction*"[tiab] OR "gap junction*"[tiab] OR "connexin*"[tiab]) OR ("Apoptosis"[mh] OR "cytotoxicity, immunologic"[mh] OR "Caspases"[mh] OR "autophagy"[mh] OR "necrosis"[mh] OR "Autolysis"[mh] OR "Angiogenesis Modulating Agents"[mh] OR "Angiogenesis Inducing Agents"[Pharmacological Action] OR "Angiogenesis Inducing Agents"[mh] OR "neovascularization, pathologic"[mh] OR "Cell Proliferation"[mh] OR "homeostasis"[mh] OR "Cyclin-Dependent Kinases"[mh] OR "Cyclin-Dependent Kinase Inhibitor Proteins"[mh] OR "Mitogens"[mh] OR "Mitogens"[Pharmacological Action] OR "cell hypoxia"[mh] OR "angiogenic"[tiab] OR "Apoptosis"[tiab] OR "autophagy"[tiab] OR "Caspases"[tiab] OR "cell cycle control*"[tiab] OR "cell cycle arrest"[tiab] OR "cell hypoxia"[tiab] OR "Cell Proliferation"[tiab] OR "cellular-	

SET #	STRATEGY	CONCEPT GROUP
	energetics"[tiab] OR "cellular-hypoxia"[tiab] OR "cellular proliferation"[tiab] OR "cellular replication*"[tiab] OR "Cytogenesis"[tiab] OR "Cytogenic"[tiab] OR "Cytotoxin"[tiab] OR "hepatocellular-proliferation"[tiab] OR "hyperplasia"[tiab] OR "hypoxic cell*"[tiab] OR "mitogenesis"[tiab] OR "mitotic checkpoint*"[tiab] OR "Neoplasia"[tiab] OR "p53 delet*"[tiab] OR "p53 inactivat*"[tiab] OR "p53 inhibit*"[tiab] OR "prb delet*"[tiab] OR "prb inactivat*"[tiab] OR "prb inhibit*"[tiab] OR "programmed cell death"[tiab] OR ("Rb"[All Fields] AND "p16ink4a inactiv*"[tiab]) OR "retinoblastoma-protein"[tiab] OR "senescence"[tiab] OR "senescent"[tiab] OR "survivin"[tiab]) OR "microtubules"[mh] OR "spindle apparatus"[mh] OR "microtubule organizing center"[mh] OR "microtubule*"[tiab] OR "spindle formation"[tiab] OR "spindle apparatus"[tiab] OR "meiotic spindle*"[tiab] OR "mitotic spindle*"[tiab])	
6	#2 OR #3 OR #4 OR #5	Combine DART concept groups
7	#1 AND #6	Combine Chemical + DART
8	("Animals, Genetically Modified"[mh] OR "Animals, Inbred Strains"[mh] OR "Chimera"[mh] OR "Animals, Laboratory"[mh] OR animals[mh:noexp]) OR (animal-stud*[tiab] OR wood-mouse[tiab] OR murinae[tiab] OR muridae[tiab] OR cricetinae[tiab] OR rodentia[tiab] OR rodent[tiab] OR rodents[tiab] OR ferrets[tiab] OR ferret[tiab] OR polecat*[tiab] OR mustela-putorius[tiab] OR cavia[tiab] OR callithrix[tiab] OR marmoset*[tiab] OR chinchilla*[tiab] OR jird[tiab] OR jirds[tiab] OR merione[tiab] OR meriones[tiab] OR cats[tiab] OR cat[tiab] OR felis[tiab] OR canis[tiab] OR sheep[tiab] OR sheeps[tiab] OR goats[tiab] OR goat[tiab] OR capra[tiab] OR saguinus[tiab] OR tamarin*[tiab] OR leontopithecus[tiab] OR ape[tiab] OR apes[tiab] OR pan-paniscus[tiab] OR bonobo*[tiab] OR pan-troglodytes[tiab] OR gibbon*[tiab] OR siamang*[tiab] OR nomascus[tiab] OR symphalangus[tiab] OR chimpanzee*[tiab] OR orangutan*[tiab] OR horse[tiab] OR horses[tiab] OR equus[tiab] OR cow[tiab] OR cows[tiab] OR chicken[tiab] OR chickens[tiab] OR wistar[tiab] OR balb[tiab] OR C57[tiab] OR C57bl[tiab] OR quail[tiab] OR long-evans[tiab] OR guppy[tiab] OR medaka[tiab] OR zebrafish[tiab] OR flying-fox[tiab] OR Fruit-bat[tiab] OR non-human-primate*[tiab] OR capuchin*[tiab] OR rhesus[tiab] OR macaque*[tiab] OR cattle[tiab] OR bovine[tiab] OR pigs[tiab] OR pig[tiab] OR swine[tiab] OR swines[tiab] OR piglet*[tiab] OR Sprague-Dawley[tiab] OR vervet*[tiab]) OR ((mice[tiab] OR mouse[tiab] OR murine[tiab] OR rats[tiab] OR rat[tiab] OR hamster[tiab] OR hamsters[tiab] OR guinea-pig*[tiab] OR gerbil*[tiab] OR rabbits[tiab] OR rabbit[tiab] OR dogs[tiab] OR dog[tiab] OR monkey[tiab] OR monkeys[tiab] OR pongo-pygmaeus[tiab] OR	Experimental Animals (RoC modified)

SET #	STRATEGY	CONCEPT GROUP
	sow[tiab] OR sows[tiab] OR boar[tiab] OR boars[tiab]) NOT medline[sb]) OR ((in vitro[tiab] OR in vitro techniques[mh] OR cell line*[tiab]) AND animals[mh:noexp])	
9	#7 AND #8	Combine Chemical + DART + Animals

Table A.10 PubMed search strategy for ADME

SET #	STRATEGY	CONCEPT GROUP
1	(80-09-1[rn] OR "Bisphenol S"[tiab] OR "bis(4-hydroxyphenyl)sulfone"[nm] OR "bis(4-hydroxyphenyl)sulfone"[tiab] OR "4,4'-Sulfonyldiphenol"[tiab] OR "Phenol, 4,4'-sulfonylbis-"[tiab] OR "BPS-monoglucuronide"[tiab] OR "BPS-1G"[tiab] OR "bisphenol S dicyanate ester"[tiab] OR (bps[tiab] NOT ("bps"[tiab] NOT "bisphenol"[tiab])))	Chemical Terms
2	(("Volume of Distribution"[tiab] OR "Toxicokinetics"[mh] OR "tissue distribut*"[tiab] OR "Renal Elimination"[mh] OR "protein bound"[tiab] OR "protein bind*"[tiab] OR "plasma protein"[tiab] OR "Pharmacokinetics"[mh] OR "Metabolism"[mh] OR "kinetic"[tiab] OR "Intestinal Elimination"[mh] OR "Hepatobiliary Elimination"[mh] OR "Hepatobiliary"[tiab] OR "enterohepatic"[tiab] OR "entero-hepatic"[tiab] OR "Distribution volume"[tiab] OR "cellular clearance"[tiab] OR "cell clearance"[tiab] OR "Biotransformation"[tiab] OR "bioavailability"[tiab] OR "ADME"[tiab] OR "absorptive"[tiab] OR "PBPK"[tiab] OR "toxicodynamic*"[tiab] OR ("Skin"[tiab] AND "absorption"[tiab]) OR ("Oral"[tiab] AND "absorption"[tiab]) OR ("Injection"[tiab] AND "absorption"[tiab]) OR ("Gavage"[tiab] AND "absorption"[tiab]) OR ("Dietary"[tiab] AND "absorption"[tiab]) OR ("Dermal"[tiab] AND "absorption"[tiab])) OR (("urine"[tiab] OR "Urination"[tiab] OR "toxicokinetic*"[tiab] OR "Pharmacokinetic*"[tiab] OR "Metabolite*"[tiab] OR "metabolism"[tiab] OR "Metabolic*"[tiab] OR "feces"[tiab] OR "fecal"[tiab] OR "excretion"[tiab] OR "defecation"[tiab] OR "biliary"[tiab] OR "Bile"[tiab]) NOT Medline[sb]))	ADME Terms
3	#1 AND #2	Combine Chemical + ADME terms

APPENDIX B. SUMMARY OF EPIDEMIOLOGIC STUDIES OF MALE REPRODUCTIVE TOXICITY

Table B.1 BPS: Summary of epidemiologic studies of male reproductive toxicity.

Study/ Design	Date of Collection/ Sample size	LOD (ng/mL)/ Adjustment for <LOD	% samples with BPS detected	Matrix – Median BPS (ng/mL)	Range (IQR) (ng/mL)	Results
Semen Quality						
Benson et al. 2021 Cross-sectional	2017–2019 n = 556	0.03 No mention	72	Urine, 0.06	0.03, 0.17	No significant results for sperm parameters, except for lower ejaculate volume in 3rd versus 1st quartile.
Chen et al. 2022 Cross-sectional	2013 n = 984	0.02 LOD÷√2	95.83	Urine, 0.38 average of 2 samples	0.17, 0.81	Decreased sperm progressive and total motility, inverted U curves.
Ghayda et al. 2019 Cross-sectional	2011–2017 n = 158	0.1 LOD÷√2	76	Urine, 0.30	0.20, 0.90	Lower semen volume, and sperm concentration. Among men with BMI ≥25 kg/m^2, BPS was associated with lower sperm concentration, total count, and motility. These men also had significantly higher BPS levels (compared to men with BMI <25 kg/^{2}).
Jeseta et al. 2024 Cross-sectional	2019-2021 n = 306	0.002 Values < LOQ were imputed using repeated imputations	77.4	Seminal plasma, 0.024	0.01, 0.07 Approximated from figure	Lower ejaculation volume, lower total sperm count, and lower sperm concentration.
Smarr et al. 2018 Cross-sectional	2005–2009 n = 339	0.018† No mention	75†	Seminal plasma, 0.11	0.02, 0.28	No significant results for sperm parameters.

Study/ Design	Date of Collection/ Sample size	LOD (ng/mL)/ Adjustment for <LOD	% samples with BPS detected	Matrix – Median BPS (ng/mL)	Range (IQR) (ng/mL)	Results
Reproductive Hormones						
Hu et al. 2022 Cross-sectional	2013–2016 n = 605	0.1 LOD÷√2	88.3	Urine, 1.16 (Included males and females, all ages)	0.70, 2.09	Lower E2, TT, FAI, TT/E2, higher SHBG only in boys 6-11. No significant association in boys 12-19.
Wang et al. 2021 Cross-sectional	2013–2016 n = 662	0.1 LOD÷√2	88.4	Urine, 0.3	0.6	Lower E2 concentration, lower TT and FAI in 4th versus 1st quartile.
Zeng et al. 2022 Cross-sectional	2013 n = 462	0.02–0.10 LOD÷√2	~95	Urine, 0.33 Average of 2 samples	0.18, 0.80	Lower E2, E2/T (3rd quartile) and SHBG. An inverse association with FSH was significant only among men with BMI ≥24 kg/m^2. (P interaction = 0.03).
Zhang C et al.2022 Cross-sectional	2013–2016 n = 1,575	0.1 LOD÷√2	92.32	Urine, 0.50	0.20, 1.10	Lower FT. Higher SHBG. Although E2 was not significantly associated with BPS by quartile, a significant interaction was seen with BMI.
Time to Pregnancy						
Buck Louis et al. 2018 Cohort	2005–2009 n = 339	0.018 Values < LOD/LOQ were not substituted	75	Seminal plasma, 0.11 ("not measured in urine")	0.2, 0.28	No significant change in time to pregnancy.
Offspring Reproductive Development						
Blaauwendraad et al. 2022 Cohort	2004–2005 n = 506	0.15 LOD÷√2	68.5 (TM1) 29.7 (TM2) 20 (TM3)	Urine, 0.2 (TM1) < LOD (TM2) < LOD (TM3)	< LOD, 0.7 (TM1) < LOD, < LOD (TM2) < LOD, < LOD (TM3)	No associations with male offspring reproductive development.

Abbreviations: BMI (body mass index); E2 (estradiol); FAI (free androgen index); FSH (follicle stimulating hormone); FT (free testosterone); IQR (interquartile range); LOD (limit of detection); LOQ (limit of quantification); SHBG (sex hormone-binding globulin); TT (total testosterone); TM (trimester)

† Not reported in paper. Estimates were pulled from Buck Louis et al. (2018), which used data from the same study population.

APPENDIX C. ANTIOXIDANT MOLECULES AND ENZYME ACTIVITY ALTERED BY BPS

Table C1. Antioxidant molecules and enzyme activity altered by BPS.

Enzyme	System	Exposure method	Dose / Duration	Tissue	Direction	Reference
GSH	Human, RWPE-1 cell culture	-	108 µM, 24 hours	Prostate	Increased	Kose et al. 2020
GSH	Human, RWPE-1 cell culture	-	108 µM, 24 hours	Prostate	Decreased	Kose et al. 2020
GR	Human, RWPE-1 cell culture	-	108 µM, 24 hours	Prostate	Decreased	Kose et al. 2020
Peroxidase (GPX)	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Dai et al. 2021
Peroxidase	Rat, adult (*in utero* exposure)	Drinking water	25, 50 µg/L, GD 1-21	Testes	Decreased	Ullah et al. 2019b
Peroxidase (GPX)	Rat, PND 21	Drinking water	50 µg/L, 10 weeks	Testes	Increased	Darghouthi et al. 2022
Peroxidase	Rat, PND 23	Drinking water	25, 50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2018b

Enzyme	System	Exposure method	Dose / Duration	Tissue	Direction	Reference
Peroxidase	Rat, PND 22	Drinking water	5, 50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2021
Peroxidase	Rat, adult	Gavage	5, 25 (NS), 50 (NS) mg/kg-day, 28 days	Testes	Decreased	Ullah et al. 2018a
Peroxidase	Rat, adult	Oral (not further specified)	5 (NS), 25 (NS), 50 (NS) µg/kg-day, 28 days	Testes	Decreased	Ullah et al. 2016
Peroxidase	Testicular explant culture, adult rats	-	0.5, 100 ng/mL, 2 hours	Testes	Increased	Ullah et al. 2016
Peroxidase (GPX1)	Human, RWPE-1 cell culture	-	108 µM, 24 hours	Prostate	Decreased	Kose et al. 2020
SOD	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Decreased	Kumar et al. 2020
SOD	Mice, adult	Gavage	2, 20, 200 mg/kg-day, 28 days	Testes	Decreased	Dai et al. 2021
SOD	Rat, adult	Oral (not further specified)	5, 25, 50 µg/kg-day, 28 days	Testes	Decreased	Ullah et al. 2016
SOD	Rat, PND 22	Drinking water	50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2021

Enzyme	System	Exposure method	Dose / Duration	Tissue	Direction	Reference
SOD	Rat, PND 23	Drinking water	50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2018b
SOD	Rat, adult (*in utero* exposure)	Drinking water	50 µg/L, GD 1-21	Testes	Decreased	Ullah et al. 2019b
SOD	Mouse, TM3 Leydig cell culture	-	200, 400 µM, 48 hours	TM3 Leydig cells	Decreased	Zhang W et al. 2022
SOD	Testicular explant culture, adult rats	-	100 ng/mL, 2 hours	Testes	Increased	Ullah et al. 2016
SOD	Sperm culture, adult rats	-	100 µg/L 2 hours	Sperm	Increased	Ullah et al. 2019a; Ullah et al. 2017
CAT	Hamster, adult	Oral (not further specified)	75 mg/kg-day, 28 days	Testes	Decreased	Kumar et al. 2020
CAT	Rat, PND 22	Drinking water	5, 50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2021
CAT	Rat, adult	Oral (not further specified)	5 (NS), 25 (NS), 50 (NS) µg/kg-day, 28 days	Testes	Decreased	Ullah et al. 2016

Enzyme	System	Exposure method	Dose / Duration	Tissue	Direction	Reference
CAT	Rat, adult	Gavage	5, 25 (NS), 50 mg/kg-day, 28 days	Testes	Decreased	Ullah et al. 2018a
CAT	Rat, PND 23	Drinking water	50 µg/L, 48 weeks	Testes	Decreased	Ullah et al. 2018b
CAT	Rat, adult (*in utero* exposure)	Drinking water	50 µg/L, GD 1-21	Testes	Decreased	Ullah et al. 2019b
CAT	Mouse, TM3 Leydig cell culture	-	200, 400 µM, 48 hours	TM3 Leydig cells	Decreased	Zhang W et al. 2022
CAT	Testicular explant culture, adult rats	-	1, 10, 100 ng/mL, 2 hours	Testes	Increased	Ullah et al. 2016

Note: All effects reported in this table are statistically significant unless otherwise noted ($p \leq 0.05$).
CAT = catalase, GD = gestation day, GPX = glutathione peroxidase, GR = glutathione reductase, GSH = glutathione, PND = post-natal day, SOD = superoxide dismutase.