OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Proposition 65

Evidence on the Female 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

Proposition 65[1] requires the publication of a list of chemicals “known to the state” to cause cancer or reproductive toxicity. 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 Developmental and Reproductive Toxicant Identification Committee (DARTIC) advises and assists OEHHA, and adds 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.

This document presents evidence relevant to the evaluation of the female reproductive toxicity of this chemical. On December 12, 2023, the DARTIC is scheduled to deliberate on the female reproductive toxicity of bisphenol S. The Committee will be convened in 2024 to consider the male reproductive toxicity and developmental toxicity of this chemical.

OEHHA developed this document as part of the hazard identification materials that are provided to the DARTIC to assist it in its deliberations on whether or not bisphenol S should be listed under Proposition 65. The original papers and reports discussed in the document are provided to the DARTIC.

OEHHA is holding a public comment period on this hazard identification document. 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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[1] The Safe Drinking Water and Toxic Enforcement Act of 1986 (California Health and Safety Code section 25249.5 *et seq.*).

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LIST OF ABBREVIATIONS

Abbreviation	Full name
β	beta estimate
Δ	change / delta
µg	micrograms
µM	micromolar
5-HT	5-hydroxytryptamine (serotonin)
5meC	5-methylcytosine
5α-R	5α-reductase
ADME	Absorption, distribution, metabolism, excretion
AGI	anogenital index
AMH	anti-müllerian hormone
AMHRII	anti-müllerian hormone receptor
AR	androgen receptor
BCS	body condition score
BKMR	Bayesian kernel machine regression
BMI	body mass index
Bmp15	bone morphogenetic protein 15
BPA	bisphenol A
BPAF	bisphenol AF
BPAP	bisphenol AP
BPB	bisphenol B
BPF	bisphenol F
BPP	bisphenol P
BPS	bisphenol S
BPZ	bisphenol Z
CARE	California Regional Exposure Study
CAS	chemical abstracts service
CAT	catalase
CHDS	Canadian House Dust Study
CI	confidence interval
CL	corpus luteum / corpora lutea
CMC	carboxymethylcellulose
COC	cumulus-oocyte complex

Abbreviation	Full name
CompTox	computational toxicology
CRH	corticotropin-releasing hormone
CX	connexins
CYP	cytochrome P450 enzyme
CYP11A1	P450 side chain cleavage [P450scc]
CYP19A1	P450 aromatase
DART	developmental and reproductive toxicity
DEG	differentially expressed genes
DHEA	dehydroepiandrosterone
DHT	dihydrotestosterone
dL	deciliters
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DOR	diminished ovarian reserve
DPF	days post-fertilization
DPP	days post-partum
DSBs	double strand breaks
E1	estrone
E2	estradiol
ECHA	European Chemicals Agency
EDC	endocrine disrupting chemical
EE	ethinyl estradiol
EGF-R	epidermal growth factor 1
EPA	Environmental Protection Agency
ER	estrogen receptor
ERK	extracellular signal regulated kinase
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
Esr1	estrogen receptor alpha
Esr2	estrogen receptor beta
EU	European Union
Exp	experiment
FAI	free androgen index
FDA	Food and Drug Administration
FF	follicular fluid

Abbreviation	Full name
fM	femtomolar
FPG	fasting plasma glucose
FR	fecundability ratio
FSH	follicular stimulating hormone
FT	free testosterone
FT3	free triiodothyronine
FT4	free thyroxine
g	gram
GD	gestational day
Gdf9	growth differentiation factor 9
GDM	gestational diabetes mellitus
GJIC	gap junction intercellular communication
GM	geometric mean
GSI	gonadosomatic index
GV	germinal vesicle
HAWC	Health Assessment Workspace Collaborative
HDL	high-density lipoprotein
hpf	hours post-fertilization
HPG	hypothalamic-pituitary-gonadal
HR	hazard ratio
HSD	hydroxysteroid dehydrogenases
HSD3B1	3-beta-hydroxysteroid dehydrogenase
HSI	hepatic somatic index
ICR	Institute of Cancer Research
IGF-1	insulin-like growth factor 1
ip	intraperitoneal
IQR	inter quartile range
IVF	in vitro fertilization
KC	key characteristic
kg	kilogram
L	liter
LD	lactation day
LH	luteinizing hormone
ln	natural logarithm
LOD	limit of detection

Abbreviation	Full name
LOQ	limit of quantification
m	meter
MAPK	mitogen-activated protein kinase
MARBLES	Markers of Autism Risk in Babies - Learning Early Signs
MDA	malondialdehyde
mg	milligram
MI	metaphase I
MII	metaphase II
mL	milliliter
mmol	millimole
mRNA	messenger ribonucleic acid
MVH	mouse vasa homolog
n	sample size
ng	nanogram
NHANES	National Health and Nutrition Examination Survey
nM	nanomolar
nmol	nanomole
NS	non-significant
NTP	National Toxicology Program
OEHHA	Office of Environmental Health Hazard Assessment
OR	odds ratio
P	progesterone
p	p-value
P450scc	P450 side chain cleavage
PAS	periodic acid-schiff
PBS	phosphate buffered saline
PCNA	proliferating cell nuclear antigen
PCOS	polycystic ovary syndrome
PC-PLC	phosphatidyl choline specific phospholipase C
PCR	polymerase chain reaction
pg	picograms
PG	plasma glucose
PIP	posterior inclusion probability
PI-PLC	phosphatidylinositol-specific phospholipase C
PKA	protein kinase A

Abbreviation	Full name
PKC	protein kinase C
pM	picomolar
pmol	picomoles
PND	postnatal day
PR	progesterone receptor
PRL	prolactin
PrlR	prolactin receptor
PROTECT	Puerto Rico Testsite for Exploring Contamination Threats
Q1	quartile 1
Q2	quartile 2
Q3	quartile 3
Q4	quartile 4
r	correlation coefficient
RBC	red blood cell
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
RF	restricted feed
RoC	report on carcinogens
sc	subcutaneous
Scp3	synaptonemal complex protein 3
SD	standard deviation / Sprague-Dawley
SERM	selective estrogen receptor modulator
SG	specific gravity
SHBG	sex hormone binding globulin
SIRT-1	silent information regulator 1
SOD	superoxide dismutase
STAR	steroidogenic acute regulatory protein
STAT5	signal transducer and activator of transcription 5
T	testosterone
T1	tertile 1
T2	tertile 2
T3	triiodothyronine / tertile 3
T4	thyroxine
TAC	total antioxidant capacity
TBARS	thiobarbituric acid reactive substances

Abbreviation	Full name
TBBPA	tetrabromobisphenol A
TC	total cholesterol
TCBPA	tetrachlorobisphenol A
TEB	terminal end buds
TM1	trimester 1
TM2	trimester 2
TM3	trimester 3
Tmax	time to get to maximum concentration
TNF-α	tumor necrosis factor-α
TSH	thyroid stimulating hormone
TT	total testosterone
TT3	total triiodothyronine
TT4	total thyroxine
TUNEL	terminal deoxynucleotidyl transferase dUTP nick end labelling
UL	uterine leiomyomata
URM	unexplained recurrent miscarriage
US	United States
VEGF	vascular endothelial growth factor
VTG	vitellogenin
WF	well fed
WHO	World Health Organization

SUMMARY

Introduction

This document presents evidence relevant to the evaluation of the female 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. BPS is also used as a color developer in thermal paper and is in protective coatings inside some food cans. 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 is detected in approximately two-thirds of Californians tested. Data from other 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. For purposes of preparing this document, OEHHA has focused on literature relevant to female reproductive toxicity (last comprehensive search, July 2023).

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.

Studies in Humans

OEHHA identified 23 epidemiologic studies of effects of BPS on the female reproductive system. The female reproductive outcomes that were assessed in more than one study were gestational diabetes, thyroid hormones, sex steroid hormones, and polycystic ovary syndrome.

BPS was typically not the sole chemical exposure of interest and methodological challenges related to valid measurement of BPS in observational studies should be considered. Most studies measured BPS levels in a single urine spot sample, which may be insufficient to assess exposure considering BPS' short half-life. Also, the levels of detection (LOD) for BPS varied widely across studies, from 0.002 to 0.20 ng/mL. The percentage of samples with BPS detected for each study was similarly varied, ranging from 14.8% to 97.7%, and the sample median BPS levels were generally low. Imprecise exposure assessment resulting in non-differential misclassification of exposure would likely bias the estimate of any association of risk towards the null.

Evidence for associations of BPS exposure and gestational diabetes (GDM) was mixed across three studies and seemed to vary by population subgroup. Positive associations between BPS and odds of GDM were observed among pregnant individuals with pre-pregnancy body mass index over 23 kilograms per square meter (kg/m^2), among those carrying a female fetus, and among those identifying as non-Asian/Pacific Islanders (data from two different studies). A third study did not find significant associations with BPS exposure and onset of GDM but did report increases in screening measures of GDM, including fasting, 1- and 2-hour plasma glucose with higher BPS exposure levels in pregnancies with a female fetus.

Four prospective cohort studies and one nested case-control study investigated the associations between urinary BPS levels and thyroid hormone levels and showed mixed results. There were observations that BPS was associated with higher levels of total thyroxine (TT4), free triiodothyronine (FT3) or total thyroid stimulating hormone (TSH) and lower levels of total triiodothyronine (TT3) or free thyroxine (FT4) at various timepoints over pregnancy. However, study findings related to specific thyroid hormones did not overlap and the studies conducted many statistical tests, varying times of BPS and thyroid hormone assessment, with no adjustment for multiple comparisons.

One study of sex steroid hormones during pregnancy reported an association of BPS with lower corticotropin-releasing hormone levels, while another study reported no associations with kisspeptin.

BPS exposures were not associated with changes in sex steroid hormones in young girls within one cohort and two cross-sectional studies. The only significant finding reported was the association between BPS exposure and a higher total testosterone (TT)/estradiol (E2) ratio in female adolescents. In adult women, one study reported a positive association between BPS and TT, with no differences in levels of luteinizing hormone, follicle stimulating hormone, E2, or prolactin.

Two case-control studies reported higher odds of polycystic ovary syndrome in women with higher BPS levels.

Other female reproductive outcomes were examined in one study each. Findings suggested BPS exposure was associated with a decrease in gestational weight gain over pregnancy, decreases in anti-müllerian hormone, higher odds of diminished ovarian reserve, and delayed onset of menstruation. Associations between BPS and unexplained recurrent miscarriage varied by age, such that BPS was associated with higher odds of unexplained recurrent miscarriage among women at 30 years and older and lower odds among women younger than 30 years old. Conflicting evidence was given for uterine fibroids where within the same study higher BPS levels were associated with a significant lower risk for developing uterine fibroids, but among women with existing fibroids, higher levels of BPS were associated with increases in fibroid volume growth. There were no associations between BPS exposure and endometriosis, placental angiogenic/hemodynamic function measures, and gestational hypertension disorders.

BPS was not significantly associated with fecundability, though there was a suggested association of longer time to pregnancy with higher BPS exposure among women with inadequate folic acid intake. There were no observed effects on infertility, defined as not conceiving after one year of trying.

Studies in Animals

BPS has been evaluated for its effects on the female reproductive system in 43 *in vivo* animal studies, including 18 studies in mice, 13 in rats, three in ewes, one in gerbils, one in hamsters, five in zebrafish, one in chickens, and one in *C. elegans*. Most mammals were exposed to BPS orally by gavage, diet or drinking water, chickens were

exposed by gavage, zebrafish were exposed in tank water, and *C. elegans* were exposed directly via the nematode growth medium. Exposures varied by life stage (e.g., gestational, pubertal, adult) and duration (ranging from a few hours in studies assessing the effects of BPS on hormone levels to 90 days in a repeated-dose oral toxicity study).

Ovarian weight and histology

Effects of BPS on ovarian weight and gonadosomatic index were mixed and only apparent in a few studies. In the studies that reported changes in ovarian weight, the direction of the effect appeared to be related to the magnitude of the dose administered.

Seven studies in mice, three of four studies in rats, and one study in hamsters reported that BPS exposure was consistently associated with effects on ovarian follicle development, including alterations in the timing of germ cell nest breakdown and decreases in the numbers of primary, secondary, antral follicles, and corpora lutea (CL). Gestational BPS exposure of mice was observed to accelerate meiotic progression, defined by a smaller percentage of oocytes at earlier stages of meiosis prophase I and a larger percentage of oocytes at later stages. Neonatal BPS exposure was observed to increase the number of atretic cystic follicles in rats and pubertal BPS exposure was associated with dose-dependent decreases in primary, preantral, and antral follicles in mice. Exposure to adult rats was observed to decrease numbers of antral and atretic follicles. BPS exposure to adult mice was associated with atrophy in immature follicles and increased numbers of atretic follicles, an effect also reported in BPS exposed adult golden hamsters. Exposure to adult chickens with 50 µg/kg-day for three months was observed to decrease the number of preovulatory follicles. One study examined follicle development in ewes and reported no effects.

Across five mouse studies, the other BPS-related effects observed were damaged oocyte structure, increased incidence of spindle damage and spindle malformations, and increased numbers of abnormal oocytes at doses as low as 0.001 µg/kg-day. In zebrafish, observed effects of BPS included increased numbers of perinucleolar oocytes, decreased numbers of cortical alveolar oocytes, and decreased percentages of late vitellogenic and spawning oocytes at 1, 10, and 100 µg/L for 14 days, increased numbers of regressed oocytes, perinucleolar oocytes, and yolk vesicle oocytes at 8, 40, and 200 µg/mL, and fewer oocytes at the primary growth stage and more oocytes at the full-grown stage at 1 and 100 µg/L. In *C. elegans*, one study reported that BPS exposure was associated with a dose-dependent irregular distribution of nuclei along the distal to proximal axis, nuclear loss, and increased apoptosis.

Disruptions to follicle and oocyte maturation were often reported alongside observations of downstream effects on ovulation, measured by changes in the number of CL. Specifically, three rat studies and one golden hamster study reported associations

between BPS exposure and reductions of CL and one rat study reported no changes in the number of CL with BPS exposure.

Uterine weight and histology

In rats, BPS was observed to reduce relative uterine weights in two studies with doses ranging from 5 to 50 mg/kg-day and to increase uterine weight in two studies with doses ranging from 40 mg/kg-day to 500 mg/kg-day. Four other studies, also in mice and rats, reported no effects on uterine weight. Across three studies, BPS exposure was associated with changes in uterine histology, including narrowing of the uterine cavity, increased numbers of uterine glands, reduced endometrial area, atrophied lamina propria myometrium and plasma layer in adult ICR mice, increased numbers of glands in the endometrium and thickness of the myometrium in adult C57BL/6J mice, and increased squamous metaplasia in Wistar rats. In estrogen challenge studies, BPS at 20 mg/kg-day was observed to further increase the uterine weight of ethinyl estradiol (EE) treated rats, and in mice, treatment with BPS + EE was observed to increase uterine epithelial height, whereas treatment with only BPS had no observed effect on uterine tissue organization.

Endocrine effects

BPS exposure was observed to decrease gonadotropin levels [luteinizing hormone (LH) and follicle-stimulating hormone (FSH)] in rats and mice, and down-regulate expression of the gonadotropin releasing hormone *gnrh3* and *fshβ* genes in zebrafish brain tissue.

BPS exposure to rats, mice, ewes, and zebrafish was associated with changes in progesterone (P) levels that were mixed in direction depending on the dose, time of exposure, and time of assessment.

In mice and rats, increases in serum testosterone (T) levels were observed after BPS exposure during pregnancy or early in life and decreases in T levels were observed after BPS exposure later in life (postnatal day [PND] 35 or at eight months of age). No effects of BPS exposure were observed on plasma T in zebrafish.

Three studies in mice, one in rats, and one study in ewes reported that BPS exposure was associated with decreased E2 levels in various body fluids (e.g., serum, plasma, oviduct fluid, urine). Also, in mice, BPS exposure was associated with fewer granulosa cell layers – the cells producing E2. Two studies in mice reported increased serum E2 levels with BPS exposure during gestation, or by subcutaneous injection every three days from birth to PND 60. In zebrafish, BPS exposure was associated with increases in E2 (in plasma or whole-body) and the E2/T ratio, decreases in estrogen receptor alpha

(ERα) (and vitellogenin) mRNA levels, and non-significant changes in ER beta (ERβ) mRNA levels.

In the mammary gland of female mice exposed to BPS during gestation and via lactation (through PND 20), an increase in progesterone receptor (PR) expression was observed at puberty, no effects on ERα or PR expression were observed at PND 24, and a decrease in expression of ERα without effects on PR was observed in adulthood. Increased mammary gland expression of ERα at PND 31, but not PR, was reported in mice exposed to BPS during gestation and for a shorter time after birth (to PND 2). In the ovary and uterus of mice, non-significant increases in the expression of ERα and ERβ (uterus only) were observed at PND 22 after exposure during gestation and lactation.

Puberty onset

BPS exposure was observed to delay puberty onset in mice exposed during gestation at 20 µg/kg-day or on the first 10 days of life, and in rats exposed perinatally at 60 mg/kg-day but not at other doses. Puberty onset was observed to occur 1-2 days earlier in mice exposed during gestation at 0.5 µg/kg-day. Four other studies, with similar dosing strategies, reported no effects on pubertal timing.

Estrous cycle

BPS exposure was associated with estrous cycle irregularity in mice and rats exposed prenatally, postnatally, and as adults. The most frequently observed effect was lengthening of the estrous cycle, and specifically, more days spent in diestrus. Only one study in mice reported no effects on estrus cyclicity.

Mammary gland development

The effects observed with BPS exposure on mammary gland development varied by timing of outcome assessment. Gestational and lactational exposure to BPS was observed to accelerate the development of terminal end buds (TEBs) in pre-pubertal mice (2 studies), have no effect on TEBs in pubertal mice (2 studies), and be associated with retention of TEBs in adult mice (2 studies). Other effects observed with BPS exposure on the mammary gland were increased numbers of alveolar buds and incidence of intraductal hyperplasia, increased incidence of mixed cell inflammation, non-neoplastic lesions, and lobuloalveolar hyperplasia at 14 months of age, decreased ductal area at PND 31, and increased mammary gland development score at multiple times and doses. In addition to these mammary gland effects, BPS exposure during pregnancy and lactation was observed to alter dam nursing behaviors.

Reproductive performance – including fertilization and implantation

Five studies examined BPS-related effects on fertility rats and mice, of which three studies observed effects. One reported reduced fertility in rats, one reported non-significant decreases in SD rats with 300 mg/kg-day in rats, and a third reported decreased fertilization at 10 µg/kg-day and increased fertilization at 100 µg/kg-day in pubertal mice.

Treatment of adult rats with BPS was observed to decrease the mean number of implantation sites in one study, while no effects were reported in one rat and one mouse study, both with shorter durations of exposure.

An increase in the rate of post-implantation loss was observed in one study of mice exposed to BPS for 14 weeks from premating to lactation. However, no differences in the rate of post-implantation loss were observed in rats treated with BPS for a shorter duration (gestational day 6 to 19) or in mice treated with a lower dose (200 µg/kg-day for 2 weeks prior to mating through gestational day 12.5). Two studies reported decreased numbers of live pups born, one in mice and the other in rats, while three separate studies in mice and one in rats reported no effects on litter size.

In zebrafish, BPS exposure was associated with a decrease in the number of eggs during a 7-day spawning period, a lower hatching rate, an increase in time to hatching of embryos, and altered spawning behavior, i.e., debilitated female shoaling. In *C. elegans* BPS exposure was associated with a dose dependent increase in embryonic lethality and a decrease in brood size.

Sex ratio

In zebrafish, exposure to BPS from day post fertilization 2 to 75 at doses of 10 and 100 µg/L was observed to skew the sex ratio towards female. No effects on the sex ratio were observed in rodent models.

Mechanistic Considerations

Numerous *in vitro* studies, as well as multiple *in vivo* studies, provide mechanistic evidence on the potential for BPS to cause female reproductive toxicity.

With respect to adverse effects on whole ovaries, BPS exposure was associated with alterations in α-tubulin assembly, decreased numbers of tubulin filaments that was associated with spindle disorganization, and chromosome misalignment in oocytes.

BPS interfered with theca cell gap junction intercellular communication in ovarian theca cells from sheep and primary human ovarian theca cells obtained from women at reproductive age. It is hypothesized that BPS can modulate gap junction intercellular communication through activation of kinase and kinase-independent pathways.

Gestational exposure to BPS in mice was observed to alter ovarian differentiation and reduce oocyte quality, while in a human cell line *in vitro* exposure to BPS resulted in increased cell proliferation that is thought to be mediated by a member of the mitogen-activated protein kinase family pathway.

BPS exposure was associated with epigenetic effects, including an observed increase in DNA methylation in oocytes in one study in mice and an observed decrease in trimethyl-histone H3 (Lys4) (H3K4me3) methylation in *in vitro* fertilized embryos (from oocytes collected from BPS exposed mice).

Some studies reported that BPS altered the expression of genes related to ovarian function. In mature female mice, BPS exposure was associated with changes in hub genes (*Synp*, *Fam46c*, *Cpeb1*) that have been linked to mammary gland and breast neoplasms, polycystic ovary syndrome, hypercholesterolemia, ovarian neoplasm, ovarian and breast carcinoma, endometriosis, and estrogen resistance.

BPS exposure was associated with alterations to the endocrine system, including effects on the hypothalamus pituitary gonadal (and liver in zebrafish) axis. Decreases in gonadotropin levels were reported in two studies in rat and one in mice and changes in gonadal steroid levels (P, T, and E2) were reported in several *in vivo* and *in vitro* studies. Alterations in hormone receptor mediated events, such as changes in estrogen receptor expression and/or activity were also observed in several cell types.

Key Characteristics of Female Reproductive Toxicants

Exogenous agents that cause female reproductive toxicity frequently exhibit one or more of the key characteristics of female reproductive toxicants (See Table 5.1 in Section 5). The available mechanistic evidence suggests that BPS exhibits several of these key characteristics (KCs), including KC 1: alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism; KC 2: chemical or metabolite is genotoxic; KC 8: alters direct cell-cell interactions; and KC 10: alters microtubules and associated structures.

1. INTRODUCTION

1.1 Identity of bisphenol S (BPS)

Bisphenol S, or “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) registration number for BPS is 80-09-1.

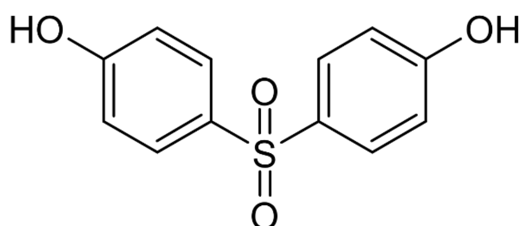

Figure 1 Structure of BPS

1.2 Uses, occurrence, and exposure

Use and production

BPS, a BPA analog, is used to make plastics and is part of polyethersulfone (PES) plastic, which is used to make hard plastic items and synthetic fibers for clothing and other textiles. BPS may also be used to make colors last longer in some fabrics. It is a common replacement for BPA in some types of paper receipts and is also in protective coatings inside some food cans. It is used in coatings of some nonstick pans, as well as in some baby bottles and parts of electronics, like screens for mobile phones (Biomonitoring California 2020).

The US nationally aggregated production volume range for BPS in 2019 was between 1,000,000 and 10,000,000 pounds per year, as reported by the US Environmental Protection Agency (US EPA) for the entities required to report and appears to be largely or completely 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 Chem Analyst (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 such as blood and urine (Fan et al. 2021; Pelch K et al. 2017).

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

- 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 (CHDS). The median concentration was 0.242 micrograms per gram (µg/g), and the range was <0.017 to 35.1 (Fan et al. 2021).
- BPS was detected in 75% of receipts submitted by volunteers from several US locations, with the majority from southeast Michigan (Ecology Center 2019).
- 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 packaged with thermal labels on plastic film were found with BPS with a mean of 147 ng/g, and a maximum level of 1140 ng/g, exceeding the European Union specific migration limit (50 ng/g ww) (Xu et al. 2023).
- 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, b)

Exposure

Human biomonitoring studies indicate that exposure to BPS is widespread. The table and figures 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% CL, 0.27-0.34)	0.342	2.42	60	76.7%
CARE-2	Adults	NC*	0.233	2.25	151	64.90%
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)
CL: 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. This is the 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. The 95th percentile BPS level was 2.8 nanograms per milliliter (ng/mL, equivalent to micrograms per liter (µg/L) 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	No. 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 National Health and Nutrition Examination Survey (NHANES). Urinary BPS concentration levels were reported in summary form for 1808 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 0.37µg/L for adults and 0.29 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 MARBLES studies, as well as two additional studies: LIEFECODES (pregnant individuals in Massachusetts, 2007-2009), and 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.

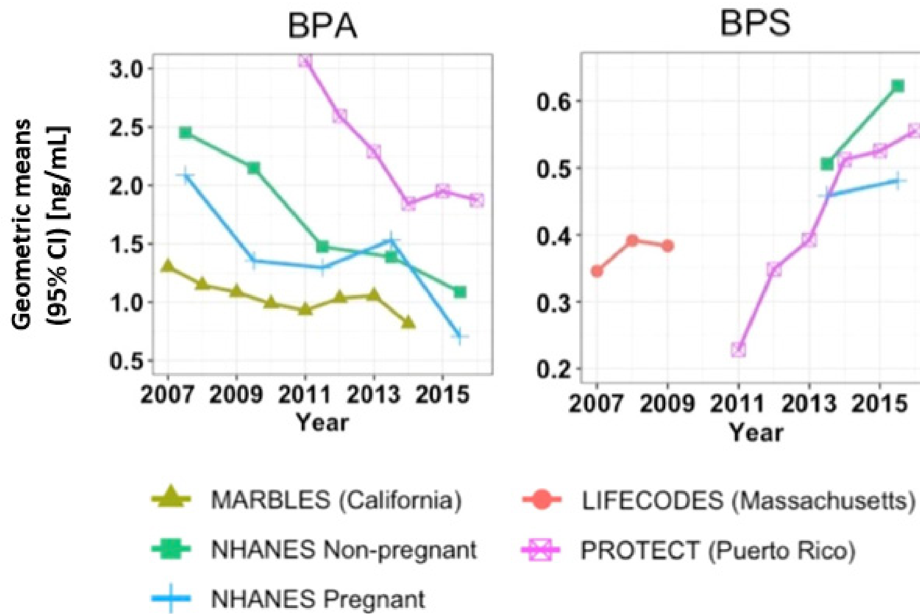

Figure 2 BPS and BPA in pregnant women measured in urine samples collected between 2007 and 2015 in four biomonitoring studies. (Source: Kim et al. 2021 [adapted])

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

Several characteristics for each study related to exposure magnitude and assessment influence the study's ability to detect an association if one exists. 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 of BPS), and (ii) most 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).

Imprecise exposure assessment resulting in non-differential misclassification of exposure would likely bias the estimate of any association of risk towards the null, that is, toward not detecting an effect even if one were present.

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 female 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-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 (e) of Article 57 of the REACH Regulation" (ECHA 2022)

2. OVERVIEW OF SYSTEMATIC LITERATURE REVIEW APPROACH

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, and July 2023. 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)

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 updates to the searches. A total of 1,400 references were identified in the searchers 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 female reproductive toxicity of BPS, citations identified as having a reasonable possibility of containing information

relevant to female reproductive toxicity 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).

One hundred and twenty-four 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 gender differences.

3.1 Absorption

In general, BPS can be absorbed following oral, inhalation or dermal exposures (Kumar et al. 2018). BPS is rapidly absorbed in humans, on the order of hours through oral exposures and seconds through dermal exposures, with higher oral bioavailability of BPS compared to bisphenol A (BPA) (Khmiri et al. 2020). For example, in humans serum concentrations of total and unconjugated d4-BPS increased rapidly within one hour after a single oral administration (Oh et al. 2018). In dermal absorption studies in humans, exposure to BPS from thermal papers and receipts showed slower dermal absorption (Liu and Martin 2019; Reale et al. 2021). In humans the average BPS post exposure BPS measurement in hand wipes was 3.9 µg and the time to get the maximum concentration in urine (Tmax) ranged from 14 – 26 hours. It was reported that 78% of urine samples had detectable BPS after two days. BPS was not detectable in serum collected over 7.5 hours (Liu and Martin 2019). *In vitro* absorption in frozen human skin (800 µm) skin after 24 h of exposure, 73% of BPS remain in skin swab0.4% of BPS was absorbed at 2h (Reale et al. 2021).

In one study the half-life of BPS in human serum was 4.06 and 6.81 hours for unconjugated and total BPS, respectively, after a single oral administration (Oh et al. 2018) and in another study the half-life in human plasma was 7.9 and 9.3 hours for unconjugated and conjugated BPS, respectively, after a single oral dose (Khmiri et al. 2020).

Studies in rats and mice administered a single gavage dose of [^{14}C]BPS have also found that BPS is well absorbed (Waidyanatha et al. 2018; Waidyanatha et al. 2020). There was a slower absorption in females rats compared to males after a single oral BPS exposure 110 mg/kg (Waidyanatha et al. 2020). The Tmax was 5.88 hours for female and 1.17 hour for male (Waidyanatha et al. 2018; Waidyanatha et al. 2020). Another study conducted in female Wistar rats reported that BPS was rapidly absorbed and detected in plasma following a single oral dose of 30 or 300 mg/kg [^{14}C]BPS, with 87% of the lower dose and 96% of the higher dose absorbed within one hour (BASF 2019b).

The plasma half-life of unconjugated BPS following a single oral dose was estimated to be 2.86–4.21 hours in mice and 5.77–11.9 hours in rats (Waidyanatha et al. 2020).

3.2 Distribution

Once absorbed into the circulation, BPS is distributed throughout the body (Oh et al. 2018; Khmiri et al. 2020). BPS has been detected in human cord blood, amniotic fluid, breast milk, semen, and skin (Niu et al. 2021).

As shown in single dose gavage studies in rats and mice, extensive biliary excretion occurs within the first two hours of dosing, with significant re-uptake from the small intestine (i.e., enterohepatic recirculation) (Waidyanatha et al. 2018; Karrer et al. 2018). Similar findings were reported in another study in rats (BASF 2019b). In the rat BPS has been detected in gastrointestinal track tissues, liver, kidney, heart, spleen, lung, and muscle following gavage administration (Mao et al. 2022). In the BASF (2019b) study in female Wistar rats, the tissue distribution (ordered from highest to lowest levels of radiolabel without considering the highest levels in the GI tract with its content) after 1 hour in the 300 mg/kg dose group was: brain, plasma, liver, thyroid, pancreas, lung, skin, and carcass, and in the 30 mg/kg dose group the distribution was: liver, kidney, thyroid, brain, bone, adipose tissue, and muscle. The maximum plasma concentration measured in this study was at 1 hour in the 300 mg/kg dose group and at 4 hours in the 30 mg/kg dose group (BASF 2019b). 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).

Maternal-fetal placental transfer

As reviewed by Abrantes-Soares et al. 2022, there is maternal-fetal transfer of BPS via the placenta in humans, with detection in cord blood and amniotic fluid (Abrantes-Soares et al. 2022; Liu et al. 2017; Pan et al. 2020), and in animals (e.g., Gingrich et al. 2018; Grandin et al. 2018; Mao et al. 2020).

Breast milk transfer

BPS has been measured in human breast milk in the US and other populations around the world (Iribarne-Durán et al. 2022; Luo et al. 2021).

3.3 Metabolism

BPS undergoes metabolism via sulfation, glucuronidation and hydroxylation to form BPS-sulfate, BPS-glucuronide, and hydroxylated bisphenol S (Waidyanatha et al. 2018). Figure 3 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, and mice (Mao et al. 2022; 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). 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 (Waidyanatha et al. 2018).

Considerable inter-individual variation in metabolism of BPS was observed in a study conducted in women, with the percentage of a single administered dose recovered in urine as conjugated BPS during the first 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 conjugated BPS during the first 48 hours was lower in women (59-77%) compared to men (67-104%) (Oh et al. 2018).

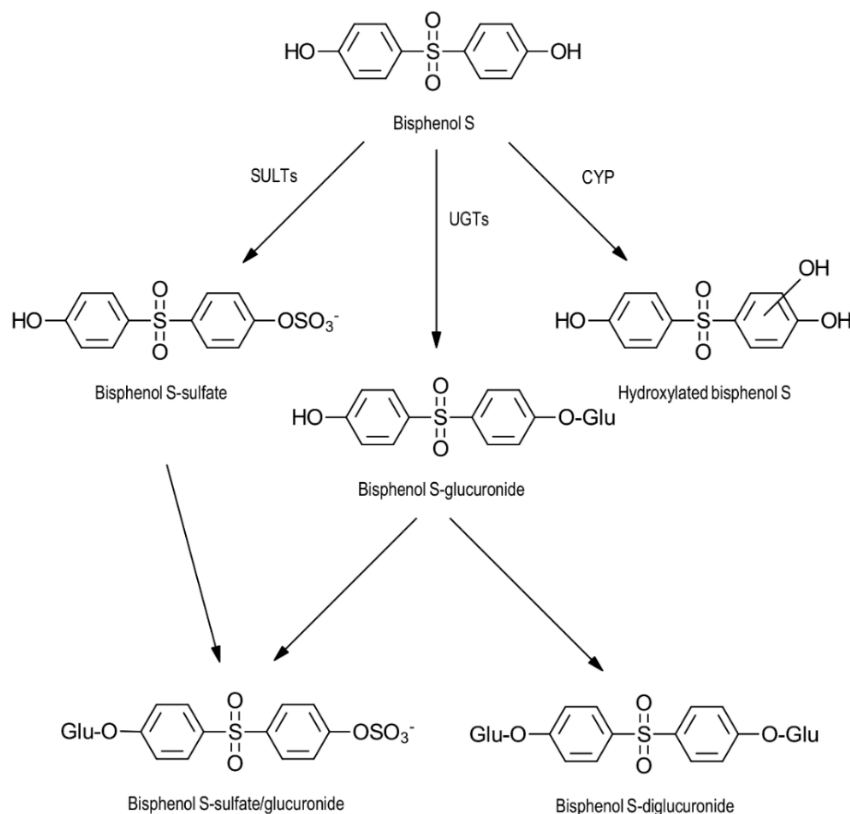

Figure 3 Proposed metabolism of BPS in rodents. Abbreviations: SULTs sulfotransferases; UGTs = uridine 5′-diphospho-glucuronosyl-transferase; CYP= cytochrome P450 enzyme (Adapted from Waidyanatha et al. (2018))

3.4 Excretion

BPS is excreted in the urine, feces and breast milk. In humans, BPS is excreted primarily via urine. In one study, the total urinary excretion of a single oral dose of BPS was 92% in men and 70% in women after 48 hours, with an estimated half-life of elimination of 6.93 hours, representing metabolism and excretion (Oh et al. 2018). Another study in women estimated a similar half-life of elimination for a single oral dose of BPS of around 7.9-9.3 hours and reported that urinary excretion was almost complete after 72 hours (Khmiri et al. 2020).

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 a study conducted in female Wistar rats, 2% of a single oral 30 mg/kg dose of BPS was reported to be excreted in expired air (BASF 2019b). In rats receiving an oral BPS dose of 30 mg/kg 51% was excreted in the urine and 43% in the feces, and in rats that received an oral BPS dose of 300 mg/kg 39.02% was excreted in the urine and 55.71% in the feces (BASF 2019b).

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

4. FEMALE REPRODUCTIVE TOXICITY

Development of the female reproductive system starts *in utero* when fetal follicle and oocyte formation and development begin. At birth, the life-long reserve of ovarian follicles is established, and oocytes have started miotic division. Oocytes remain arrested in meiosis prophase I until follicle development is resumed during puberty, a physiologic stage that brings up a series of coordinated events to prepare the body for reproduction. There are several steps in female reproduction that are possible targets for environmental or chemical insult. These steps include development of the reproductive tract (e.g., the ovaries, oviducts, uterus, cervix, and vagina), early oocyte and follicle development, oocyte and follicle maturation in preparation for ovulation, oocyte fertilization within the oviduct, transport of the embryo to the uterus, implantation, and formation of the placenta.

Female reproductive biology is under tight control of the endocrine system. In addition to the intrinsic regulation present in the organism, environmental factors may influence the reproductive process by altering the ovarian physiology and the endocrine balance necessary for reproductive success. Endocrine effects can occur through a variety of mechanisms, including effects on hormone synthesis or metabolism, hormone transport and binding to proteins in the blood, and cell receptor mediated events, such as receptor transactivation and alterations in intracellular signaling. Endocrine-disrupting chemicals, such as bisphenols, have been recognized to interfere with some of these processes.

In this section, the available evidence on the effects of BPS on female 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. This is followed by an integrated summary of the evidence on the female reproductive toxicity of BPS. Some studies presented here included examination of the effects of prenatal BPS exposure through the pregnant mother on offspring outcomes, so summary of these studies may appear in the developmental toxicity section also.

4.1 Studies in humans

OEHHA identified 23 epidemiologic studies of possible effects of BPS on the female reproductive system for inclusion. These were cohort, case-control, or cross-sectional studies that assessed individual-level exposure to BPS and several different female reproductive outcomes. Included studies evaluated associations between BPS exposure and female reproductive outcomes utilizing multivariable epidemiological methods, reported original data analyses with details about methods, and were published in peer-reviewed journals. Two studies were excluded because of the lack of multivariable methods. One was a comparative correlation study of women with polycystic ovary syndrome (PCOS) by (Šimková et al. 2020) and the other was a study measuring levels of BPS in breast cancer patients and survivors by (Segovia-Mendoza et al. 2022) that reported only correlations or mean levels without adjustment. Abstracts from conferences, opinions, and reviews were also excluded.

Female reproductive outcomes that were assessed in more than one study included gestational diabetes mellitus (GDM), thyroid hormones during pregnancy, sex steroid hormones during pregnancy, sex steroid hormones in young girls, and PCOS. All studies reported BPS exposure as nanograms per milliliter (ng/mL) BPS measured in urine (most common) or serum. As discussed in the exposure section, Appendix B Table B.1 presents the limit of detection (LOD), percent of samples with BPS detected, and sample matrices for each study.

The studies are ordered by outcome in the following discussion. Key elements of each study are presented in Table 4.1. Statistically significant results ($\alpha = 0.05$) in all the tables are shown in bold along with p-values and confidence intervals (CIs). In the summary presented below, all results are statistically significant unless otherwise noted.

Estimation of an unbiased association between BPS and reproductive outcomes requires adjustment for potential confounding variables. Covariates included in study adjustment sets are listed in Table 4.1. The most frequently adjusted variables were age, body mass index (BMI), parity and/or gravidity, exposure to smoking, and markers of socioeconomic status (e.g., household income, occupation, maternal education). Most adjustment sets were comprehensive, though bias due to unmeasured or residual confounding is possible in observational research.

All but one of the studies included measurement of additional bisphenols, mainly bisphenol A (BPA) and bisphenol F (BPF). The correlations between BPS and other bisphenols varied across studies. In general, most studies showed positive correlations between BPS and other bisphenols in the range of 0.10 to 0.30. Four studies controlled for exposure to other bisphenols using mutual adjustment in multivariable models. Nine studies conducted analyses using Bayesian kernel machine regression (BKMR) models, which can estimate the contributions of BPS to the joint effects of a chemical mixture. It is important to note that BPS was typically not the sole exposure of interest. This may suggest that publication bias for BPS is low, as pressure to publish or not publish based on statistical significance or findings related specifically to BPS are unlikely.

4.1.1 Gestational diabetes mellitus

Evidence for associations of BPS exposure and development of GDM was mixed, specific to effect modification, and based on three studies. One of the three studies was a nested case-control study from a prospective birth cohort in Guangxi Province, China (Tang et al. 2021). Overall, pregnant women with BPS levels in the middle tertile of exposure had 1.77 times higher odds of GDM (odds ratio (OR) 1.77; 95% CI: 1.01, 3.13) compared with women in the lowest tertile of exposure. The OR for the highest versus lowest tertile was similar but not statistically significant (OR 1.68, 95% CI: 0.95, 2.99). There was effect modification by BMI and fetus sex such that associations were stronger for pregnant women with pre-pregnancy BMI at or above 23 kilograms per meters squared (kg/m^2) (OR 3.89; 95% CI: 1.29, 13.20) and for pregnancies with a female fetus (OR 2.74; 95% CI: 1.07, 7.47) when comparing the highest to lowest tertiles of BPS exposure (Tang et al. 2021). Within a nested case-control study from a cohort in California, pregnant women in the highest tertile of BPS exposure during the first trimester had 2.12 times higher odds of GDM (OR 2.12; 95% CI: 1.00, 4.50) compared to those in the lowest tertile of exposure. Effect modification was seen as participants identifying as non-Asian/Pacific Islander (race/ethnicities of white, black, Hispanic or other) who had BPS exposure in the highest tertile during the first trimester had 4.6 times higher odds of developing GDM (OR 4.60; 95% CI: 1.55, 13.70) compared to non-Asian/Pacific Islanders in the lowest exposure tertile (Zhu et al. 2022). No elevation in risk of GDM was observed in participants identifying as Asian or Pacific Islander; the study did not report evaluating effect modification by BMI. Zhu et al (2022) also analyzed BPS exposure as the area-under-the-curve across the first and second trimesters to incorporate both concentration and timing of exposure into one index and findings were consistent; among non-Asian/Pacific Islanders, higher GDM risk was observed with higher cumulative exposure across first and second trimesters (Zhu et al. 2022). The third study in a cohort in Wuhan, China did not report significant associations with BPS exposure and onset of GDM, although the authors reported

increases in screening measures of GDM (increased fasting, 1- and 2-hour plasma glucose) with increased BPS exposure among women with female fetuses (Zhang et al. 2019).

4.1.2 Thyroid hormones during pregnancy

Four prospective cohort studies and one nested case-control study investigated the association between urinary BPS levels and thyroid hormone levels, which are supportive of pregnancy, including thyroid stimulating hormone (TSH), total triiodothyronine (TT3), free triiodothyronine (FT3), total thyroxine (TT4), and free thyroxine (FT4). Derakhshan et al. (2021) analyzed data collected prior to the 18th week of pregnancy from a prospective birth cohort in the Netherlands and reported that for every unit increase in natural-log (ln) BPS, serum TT4 levels increased by 0.97 (95% CI: 0.03, 1.91) (Derakhshan et al. 2021). Huang et al. (2022) analyzed data collected during the first and second trimesters from a prospective birth cohort in Guangxi, China, and reported that first trimester TT3 levels were lower by percent change (%Δ) of -10.90 (95% CI: -18.16, -2.99) for those with BPS measurements in the middle tertile compared to the lowest tertile (Huang et al. 2022). Analyses of second trimester data found FT3 increased by %Δ of 6.07 (95% CI: 1.20, 11.17) for those with BPS measurements in the highest tertile compared to the lowest tertile, with no significant effect on TT3 (Huang et al. 2022). No associations between urinary BPS and thyroid hormones were observed in prospective birth cohorts in Puerto Rico (Aker et al. 2019) or Sweden (Derakhshan et al. 2019). A nested case-control study of pregnant women in Boston reported that detectable levels of BPS were associated with changes in FT4 (Aker et al. 2018). Authors originally recruited participants as a nested case-control study and incorporated inverse probability weighting in analyses to account for the over selection of preterm births and to allow the sample to be representative of the original cohort. Analyses proceeded in a cohort study design. This study had very low detection frequency of BPS (26.2%), thus exposure was modeled as a binary variable of above or below the LOD. Aker et al. (2018) observed that in women with BPS above the LOD (compared to below LOD), FT4 was decreased at less than 15 weeks gestational age (%Δ = -9.63, 95% CI: -18.10, -0.33). Additionally, for women with term births (>37 weeks gestation) and BPS above the LOD, TSH was increased at less than 15 weeks gestational age (%Δ = 11.77, 95% CI: 0.08, 24.8). There were no significant associations observed at later gestational ages (Aker et al. 2018).

4.1.3 Sex steroid hormones and related proteins during pregnancy

One prospective cohort study in Puerto Rico analyzed data on urinary BPS levels and serum levels of corticotropin-releasing hormone (CRH), estriol, progesterone,

testosterone (T), and sex hormone binding globulin (SHBG) during pregnancy (Aker et al. (2019). Increased levels of BPS were associated with a decrease in CRH per interquartile range (%Δ = -11.35, 95% CI: -18.71, -3.33), with the strongest associations observed at 16 to 20 weeks. There were no statistically significant associations reported for other outcomes (Aker et al. 2019). One prospective cohort study in China reported no significant associations between urinary BPS and total kisspeptin levels in pregnant women, as assessed during the third trimester (Wang et al. 2022). Kisspeptin is a family of peptides involved in a number of reproductive processes, including the secretion of gonadotrophins and the regulation of fertility and puberty onset.

4.1.4 Sex steroid hormones and related proteins in young girls

One prospective cohort study in China reported no association between gestational exposure to BPS (measured in third trimester maternal urine) and levels of kisspeptin in girls 6 years of age (Wang et al. 2022).Two cross-sectional studies, both using data from the same National Health and Nutrition Examination Survey (NHANES) survey cycles (2013-2014, 2015-2016), investigated associations between urinary levels of BPS and serum estradiol (E2), total testosterone (TT), TT/E2, SHBG, and free androgen index (FAI; TT/SHBG) in female participants ages 6-19 years (Hu et al. 2022; Wang et al. 2021). No statistically significant associations were reported by Hu et al. (2022), while Wang et al. (2021) reported an increase in TT/E2 between the first and second quartiles of BPS exposure in female adolescents. Differences in findings between the studies may be due to the analysis of BPS exposure as continuous by Hu et al. (2022) versus as categorical (in quartiles) by Wang et al (2021). When Wang et al (2021) did analyze the data treating BPS as a continuous variable using restricted cubic spline models, there was evidence to suggest potential non-linear associations between BPS and two outcomes, FAI and SHBG.

4.1.5 Sex steroid hormones in women

A case-control study in China analyzed associations between BPS and hormones in women without PCOS (i.e., women in the study control group) and reported that per unit increases in creatinine-adjusted BPS were positively associated with T levels (β = 0.07, 95% CI: 0.02, 0.12). There were no differences observed for luteinizing hormone, follicle stimulating hormone, E2, and prolactin (Zhan et al. 2023).

4.1.6 Polycystic ovary syndrome

Two case-control studies investigated the association between PCOS and BPS levels. One study in China reported women with higher levels of urinary BPS had higher odds

of PCOS compared to women with lower levels of BPS, when BPS was analyzed as a continuous variable and by quartiles ($p < 0.001$ for trend) (Zhan et al. 2023). Another study in Poland estimated associations between per unit increases in ln BPS and PCOS odds within each tertile of BPS. Within the lowest exposure tertile, per unit increases in ln BPS were associated with higher odds of PCOS (OR 1.12, 95% CI: 1.03, 3.71). Per unit increases in ln BPS were also associated with higher odds of PCOS in the middle exposure tertile (OR 1.29, 95% CI: 0.79, 6.89) and in the highest exposure tertile (OR 1.33, 95% CI: 0.58, 4.88), though these estimates were not statistically significant (Jurewicz et al. 2020). This study had reporting limitations, namely, they only reported associations estimated within each tertile of BPS exposure and did not report the number of PCOS cases within each stratum (Jurewicz et al. 2020).

4.1.7 Other female reproductive outcomes

Weight Gain During Pregnancy

A prospective cohort study from Rotterdam, Netherlands assessed associations between BPS levels measured during early pregnancy and gestational weight gain over pregnancy and reported that for every unit increase in ln BPS, there was a 261-gram reduction in gestational weight gain over the total pregnancy (95% CI: -466, -56) (Philips et al. 2020).

Recurrent Miscarriage

One case-control study of unexplained recurrent (≥ two) miscarriage (URM) in China reported higher odds of URM with BPS exposure levels in quartile two (OR 1.27, 95% CI: 1.22, 2.22) and quartile three (OR 1.37, 95% CI: 1.02, 1.85) versus in quartile one (Ao et al. 2022). There were no differences between quartile four and quartile one (OR 0.92, 95% CI: 0.69, 1.23). Secondary analyses showed that associations between BPS and URM may vary by age. Specifically, there were higher odds of URM observed in women aged 30 or older in the middle tertile of BPS exposure compared to the lowest tertile (OR 1.62, 95% CI: 1.13, 2.33) and lower odds of URM observed in women less than 30 years old in the highest tertile compared to the lowest tertile (OR 0.65, 95% CI: 0.44, 0.97) (Ao et al. 2022).

Uterine Fibroids

In a case-cohort study of Black women followed over the course of 60 months, higher urinary BPS levels were associated with a significant lower risk for developing uterine leiomyomata (fibroids) (Hazard ratio 0.93 (95% CI: 0.87, 0.99) (Wesselink et al. 2021). Growth of a subset of uterine fibroids detected during the study was assessed via transvaginal ultrasound at 20 months (n = 67) and 60 months (n = 145), with measurements conducted on as many as six fibroids per participant. The authors

reported that a 50% increase in urinary BPS was associated with a 4.1% increase in fibroid volume growth over 18 months (95% CI: 0.3%, 8.0%) (Wesselink et al. 2021).

Fecundability/Fertility

A cross-sectional study in China at an infertility clinic reported associations between urinary BPS measurements and decreases in anti-müllerian hormone (β = -0.287, 95% CI: -0.505, -0.070) (Zhang et al. 2023). Zhang et al. (2023) also reported higher odds of diminished ovarian reserve among individuals with high versus low levels of urinary BPS (OR 6.85, 95% CI: 1.24, 37.82). A prospective cohort study in the Netherlands found no association between first trimester urinary BPS levels and time to pregnancy (fecundability), though there was a suggested association of longer time to pregnancy with per unit increases in ln BPS exposure among women with inadequate folic acid intake (fecundability ratio 0.94, 95% CI: 0.87, 1.01) (Philips et al. 2018). A cross-sectional study of NHANES data by Zhan et al. (2022) found no association between urinary BPS levels and infertility in women 18 to 45 years of age.

Onset of Menstruation

A prospective birth cohort study in Rotterdam, Netherlands examined associations between average gestational exposure to BPS and offspring reproductive development at ages 10 and 13 years. BPS was associated with delayed onset of menstruation by 0.17 years, on average, among female offspring (β = 0.17, 95% CI: 0.02, 0.31) (Blaauwendraad et al. 2022).

Placental Function and Hypertension During Pregnancy

Urinary levels of BPS were not associated with placental angiogenic markers, placental hemodynamic function, or hypertensive end points, in a prospective cohort in the Netherlands (Philips et al. 2019).

Endometriosis

No statistically significant associations were reported between urinary BPS levels and endometriosis (Peinado et al. 2020).

Thyroid Levels in Adult Women

Urinary BPS levels from adult women were not associated with changes in thyroid hormones (TT4, TT3, FT4, FT3, and TSH) in a cross-sectional study from Beijing, China (Yue et al. 2023a).

Table 4.1.1 BPS: Epidemiologic studies of female reproductive toxicity.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Gestational Diabetes Mellitus (GDM)					
Tang et al. 2021 Guangxi Providence, China 2015-2021 Nested case-control study, hospital-based Guangxi Zhuang Birth Cohort (GZBC) prospective birth cohort n = 500; n = 100 cases, matched 4:1 on age and fetal sex Mean ages for cases (± Standard Deviation [SD]: 30.62 ± 6.46 years Mean ages for controls (±SD): 30.6 ± 6.41 years Inclusion criteria: singleton pregnancy, Zhuang population (mother or father), enrolled at ≤ 12 weeks gestation	Serum samples Limit of Detection (LOD) 0.046 % < LOD: 17.8 Values below the LOD were calculated as LOD÷√2 Geometric mean, cases: 0.103 Geometric mean, controls: 0.092 Percentiles: 25th: 0.05 50th: 0.097 75th: 0.107 95th: 0.83 Timing of samples: Samples taken in first trimester	One step GDM screening via blood: oral glucose tolerance test at 24-28 weeks gestation. Criteria for GDM based on meeting one of three criteria: Fasting plasma glucose (FPG) > 5.1 millimoles per liter (mmol/L); 1 hour plasma glucose (1 h-PG) > 10.0 mmol/L; 2 hour plasma glucose (2 h-PG) > 8.5 mmol/L.	Odds of GDM, BPS tertiles **Tertile 2 (T2) vs Tertile 1 (T1), odds ratio (OR): 1.77 (95% Confidence Interval [CI]: 1.01, 3.13)** Tertile 3 (T3) vs T1, OR: 1.68 (95% CI: 0.95, 2.99) ***P* trend: 0.049** Female fetus: T2 vs T1, OR: 2.34 (95% CI: 0.95, 6.18) **T3 vs T1, OR: 2.74 (95% CI: 1.07, 7.47)** ***P* trend = 0.03** No association with male fetus: T2 vs T1, OR: 1.61 (95% CI: 0.74, 3.55) T3 vs T1, OR: 1.45 (95% CI: 0.67, 3.20) *P* trend = 0.28 High body mass index (BMI) (≥ 23 kg/m^2): T2 vs T1, OR: 1.72 (95% CI: 0.55, 5.80) **T3 vs T1, OR: 3.89 (95% CI: 1.29, 13.20)** ***P* trend = 0.03** No association at BMI < 23 kg/m^2: T2 vs T1, OR 1.16 (95% CI: 0.55, 2.42) T3 vs T1, OR: 0.84 (95% CI: 0.40, 1.74) *P* trend = 0.78 BPS consistently showed increased risk of GDM through Bayesian Kernel Machine Regression (BKMR) and g-computation analysis (Posterior inclusion probability (PIP) for BPS = 0.99). Showed an inverted U shape dose-response.	Models adjusted for: area of residence, BMI, passive smoking during pregnancy, parity, gravidity, regular exercise. Matched on maternal age and fetal sex. Effect modification: BMI, fetal sex BKMR models and quantile-based g-computation used to estimate effects of BPS as part of a mixture of chemicals. Other Bisphenols: Bisphenol A (BPA), Bisphenol B (BPB), Bisphenol F (BPF), Tetrabromobisphenol A (TBBPA)	Approach used to classify BPS tertiles was not clearly described and ranges of BPS included in each tertile were not reported. BPA negatively associated with GDM in main analyses and in BKMR/quantile g-computation models. Correlations between BPS and other bisphenols: TBBPA (r = 0.31), BPF (r = 0.32), BPA (r = -0.27), BPB (r = -0.03).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Zhang et al. 2019 Wuhan, China 2013-2015 Prospective cohort n = 1,841 Mean age (±SD): 28.58 ± 3.27 years Inclusion criteria: singleton pregnancy, enrolled at < 16 weeks gestation, no family history of diabetes, no diabetes before pregnancy	Urine spot samples Adjusted with specific gravity (SG) LOD: 0.2 % < LOD: 10 Values below the LOD were calculated as LOD÷√2 Geometric mean (95% CI), SG adjusted: 0.36 (0.33,0.38) Percentiles: 25th: 0.14 50th: 0.31 75th: 0.81 95th: 5.51 Timing of samples: Samples collected between weeks 8-18 gestational age, on average at 13 weeks	One step GDM screening via blood: oral glucose tolerance test at 24-28 weeks gestation Criteria for GDM based on meeting one of three criteria: FPG > 5.1 mmol/L; 1 h-PG > 10.0 mmol/L; 2 h-PG > 8.5 mmol/L Screening measures: FPG, 1 h-PG, 2 h-PG, Sum of PG z-scores	Odds of GDM, BPS tertiles: No significant association between BPS tertiles and GDM in full or BMI-stratified analyses (ORs ranged from 0.83 to 1.66) GDM screening measures: For continuous, per unit increase in ln BPS: **FPG [β = 0.03 (95% CI: 0.01, 0.04)] ($p < 0.01$)** Sum PG z-score [β = 0.07 (95% CI: −0.00, 0.14)] ($p = 0.05$) No associations with 1 h-PG or 2 h-PG Significant interaction with fetal sex for FPG, 1 h PG, Sum PG z-score ($p < 0.05$) Female fetus: For continuous, per unit increase in ln BPS: **FPG [β = 0.04 (95% CI: 0.02, 0.06)] ($p < 0.01$)** **1 h-PG [β = 0.11 (95% CI: 0.04, 0.17)] ($p <0.01$)** **Sum PG z-score [β = 0.19 (95% CI: 0.08, 0.30)] ($p < 0.01$)** Similar results when exposure was categorized into tertiles for pregnancies with female fetus No associations in pregnancies with male fetus	Models adjusted for: maternal age, education level, passive smoking, pre-pregnancy BMI, parity, infant sex. GDM models additionally adjusted for other bisphenols. Effect modification: fetal sex Other Bisphenols: BPA, BPF, Bisphenol AF (BPAF)	BPA had a U-shaped exposure pattern for FPG and PG z-scores, but no association with linear models. Ranges of BPS values included in each tertile were not reported. Correlations between BPS and other bisphenols: BPA (r = 0.14), BPF (r = 0.11), BPAF (r = 0.20). BPAF was associated with an increased risk of GDM in women of normal range pre-pregnancy BMI.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Zhu et al. 2022 Northern California, USA 2015-2017 Nested case-control study within the Kaiser Permanente Northern California – Pregnancy Environment and Lifestyle Study (PETALS) prospective birth cohort n = 111 and n = 222 controls Mean ages (±SD): 31.2 ± 4.6 Inclusion criteria: singleton pregnancy, <11 weeks gestation, no evidence of recognized pre-existing cancer, diabetes, or hepatic diseases	Urine Spot Samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.1 % < LOD: Trimester 1. Cases: 16.2, controls: 15.8 Trimester 2. Cases: 11.8, controls: 18.3 Values below the LOD were calculated as LOD÷√2 Median (Interquartile range [IQR] 25th, 75th): Trimester 1: cases: 0.6 (0.3,1.3), controls: 0.4(0.2,0.9) Trimester 2: cases: 0.5 (0.2,1.2), controls: 0.4 (0.2,1.1) Timing of samples (gestational weeks ± SD): Visit 1: 14.0 ± 2.3 Visit 2: 20.5 ± 2.4	One step GDM screening 24-28 weeks gestation: oral glucose tolerance test or fasting glucose test ≥ 5.1 mmol/L	First trimester BPS tertiles T2 (IQR: 0.4-0.6 ng/mL) vs T1 (IQR 0.1-0.3 ng/mL), OR: 1.86 (95% CI: 0.94, 3.67) **T3 (IQR: 1.0-2.7 ng/mL) vs T1, OR: 2.12 (95% CI:1.00, 4.50)** Significant interaction with race/ethnicity ($p < 0.001$) Non-Asian/Pacific Islanders: T2 vs T1, OR: 1.43 (95% CI: 0.48, 4.27) **T3 vs T1, OR: 4.60 (95% CI: 1.55, 13.70)** No associations among Asian/Pacific Islanders: BPS area-under-the-curve first and second trimester: T2 (IQR: 78.8-111.2 ng/mL) vs T1 (IQR: 19.7-51.0 ng/mL), OR: 1.59 (95% CI: 0.67, 3.77) **T3 (IQR: 189.8, 443.7 ng/mL) vs T1, OR: 2.49 (95% CI: 1.01, 6.17)** No significant associations when BPS was analyzed per IQR increase. BPS showed an inverted u-shaped association with GDM risk through cubic splines for both first trimester BPS and area-under-the-curve Contribution to BPS to mixture in BKMR models was low to moderate (PIPs ranged from 0.24 to 0.57)	Models adjusted for: maternal age at childbirth, race/ethnicity, pre-pregnancy BMI, creatinine. Effect modification: race/ethnicity Other Bisphenols: BPA, BPF BKMR models used to estimate effects of BPS as part of a mixture of chemicals.	Non-Asians/Pacific Islander individuals had higher risk of GDM with higher levels of BPA exposure. Authors stated "…we calculated the area-under-the-curve as a proxy of overall exposure to phenols in early to mid-pregnancy, which showed overall consistent results with time-specific analysis." Correlations between BPS and other bisphenols not reported. IQR of each BPS tertile reported, but specific bounds were not.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Thyroid Hormones During Pregnancy					
Aker et al. 2018 Boston, MA 2006-2008 Nested case-control study within the LIFECODES Cohort* n = 439, n = 130 preterm births and n = 352 controls Mean ages: 31.8 years (27% 35+) Inclusion criteria: singleton, non-anomalous fetus, deliver at participating hospital, ≥ 18 years, < 15 weeks, no pre-existing thyroid conditions such as thyroid cancer, Graves' disease and hyper- or hypothyroidism	Urinary spot samples Adjusted with SG LOD: 0.4** %< LOD: 73.8** Values below the LOD were calculated as LOD/√2 Geometric mean: <LOD Timing of samples - gestation week (median and range): Visit 1: 9.64 (5.43-19.1) Visit 2: 17.9 (14.9-32.1) Visit 3: 26.0 (22.9-36.3) Visit 4: 35.1 (33.1-38.3)	Pregnancy thyroid hormones via blood plasma at gestational visits 1-4: free thyroxine (FT4), total thyroxine (TT4), total triiodothyronine (TT3), and thyroid stimulating hormone (TSH), TT3/TT4 ratio. Outcomes analyzed as percent change (%Δ)	Analyzed as values above or below the LOD since very few were above the LOD: Non-significant increase for TSH Non-significant decrease for FT4, TT4, TT3, TT3/TT4 ratio Stratified by gestational age: **FT4 at <15 weeks gestational age %Δ = –9.63 (95% CI: –18.10, –0.33) (p = 0.04)** No associations observed at other gestational ages. Among births > 37 weeks **TSH at <15 weeks gestational age %Δ = 11.77 (95% CI: 0.08, 24.8) (p = 0.05)** No associations observed at other gestational ages	Models adjusted for: SG, study visit, BMI at the first study visit, insurance provider, maternal age and gestational age at time of sample collection. Stratification: gestational age (< 15, 15-21, 21-30, > 30 weeks) Sensitivity analysis limited to births > 37 weeks	Benzophenone-3, 2,4-dichlorophenol, and 2,5-dichlorophenol were also analyzed, and weakly correlated with BPS (range of correlation coefficients 0.08 to 0.11). * Authors originally recruited participants as a nested case-control study and incorporated inverse probability weighting in analyses to account for the over selection of preterm births and to allow the sample to be representative of the original cohort. Analyses proceeded in a cohort study design. **Mistakes were noted in the methods section where the BPS LOD is given as 0.1 ng/mL in text and 0.4 ng/mL in Table 2 of the study. The percentage of samples with BPS above the LOD was given as <25% in text and given as 26.2% in Table 2. Many statistical tests were run with no adjustment for multiple comparisons.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Derakhshan et al. 2019 Sweden 2007-2010 Prospective cohort study Swedish Environmental Longitudinal, Mother and child, Asthma and allergy (SELMA) cohort n = 1,996 Mean ages (±SD): 30.9 ± 4.9 years Inclusion criteria: singleton pregnancy, no pre-existing thyroid issues	Urine spot samples Adjusted with creatinine LOD: 0.03 % < LOD: 19.8 Reported concentrations were used for values below LOD Percentiles: 5th: 0.03 50th: 0.08 95th: 0.88 Timing of samples (gestation week): median 10 range 6-14	Thyroid hormones in serum measured at first prenatal visit: TSH, FT4, FT3, TT4, and TT3, TT4/TT3 ratio, FT4/FT3 ratio	For continuous, per unit increase in ln BPS No associations of BPS with thyroid hormones Non-significant increase for TSH, FT4, FT3, FT4/FT3 ratio, TT4, TT4/TT3 ratio Non-significant decrease for TT3	Models adjusted for: maternal age, thyroid peroxidase antibodies, thyroglobulin antibodies, human chorionic gonadotropin, urinary creatinine, smoking status by cotinine levels, BMI, education, ethnicity, and parity. Other Bisphenols: BPA, BPF	BPA associated with reduced FT4/FT3 ratio, TT4 (non-linear relationship) and TT4/TT3 ratio. BPA had significant interactions with timing of FT3, FT4/FT3, and TT4/TT3 between weeks 7 and 12 of gestation. BPF was associated with higher FT3. Correlations between BPS and other bisphenols: range 0.17 to 0.33 (exact correlation coefficients not provided).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Derakhshan et al. 2021 Rotterdam, Netherlands 2002-2006 Prospective Cohort Study Generation R Study n = 1,267 Mean ages (±SD): 30.5 ± 4.8 years Inclusion criteria: singleton pregnancy, ≤ 18 weeks gestation	Urine spot samples (creatinine adjusted) LOD: 0.05 % < LOD: 33.3 early pregnancy, 70.2 middle pregnancy, 81.1 late pregnancy Values below the LOD were calculated as LOD÷√2 Percentiles (early pregnancy, middle pregnancy): 5th: < LOD, < LOD 50th: 0.34, 0.24 95th: 8.83, 1.69 Timing of samples (gestation week): Early: <18, middle: 18-25, late: >25	Maternal thyroid function via serum <18 weeks gestation: TSH (mU/L), FT4 (pmol/L), TT4 (nmol/L)	For continuous, per unit increase in ln BPS (early pregnancy): **TT4 [β = 0.97 (95% CI: 0.03 1.91)] (p = 0.04)** Non-significant increase for FT4, non-significant decrease for TSH There was a significant interaction **(p = 0.001)** for FT4 and ln-TSH (i.e., BPS associated with an attenuation of the association between FT4 and ln-TSH)	Models adjusted for: week of gestation, maternal age, thyroid peroxidase antibodies, human chorionic gonadotropin, urinary creatinine, BMI, education, ethnicity, smoking status, and parity. Other Bisphenols: BPA, BPF	No associations between BPA and outcomes. Correlations between BPS and other bisphenols: BPA (r = 0.19), BPF (r = 0.30) at time 1; BPA (r = -0.08) at time 2; estimates at time 3 (late pregnancy) not reported due to > 80% below the limit of detection. Percentiles of BPS at time 3 (> 25 weeks) not reported.
Huang et al. 2022 Guangxi Providence, China 2015-2018 Prospective birth cohort Guangxi Zhuang Birth Cohort (GZBC) prospective cohort n = 446 Mean ages (±SD): 27.21 ± 5.44 years Inclusion criteria:	Serum samples LOD: 0.046 % < LOD: 13.7 Values below the LOD were calculated as LOD÷√2 Geometric mean: 0.091 Percentiles: 25th: 0.096 50th: 0.097 75th: 0.104	Thyroid hormone testing for: TT3, TT4, FT3, FT4, and TSH in first or second trimester. Analyzed as %Δ	3-level BPS variable (tertiles) First trimester thyroid hormone: **T2 vs T1, TT3 %Δ = –10.90 (95% CI: –18.16, –2.99)** T3 vs T1, TT3 %Δ = -4.76 (95% CI: -12.40, 3.54) No associations with TT4, FT3, FT4, TSH Second trimester thyroid hormones: T2 vs T1, FT3 %Δ = 3.26 (95% CI: -1.43, 8.17) **T3 vs T1, FT3 %Δ = 6.07 (95% CI: 1.20, 11.17)** No associations with TT3, TT4, FT4, TSH Male fetus: T2 vs T1, FT3 %Δ = 3.46 (95% CI: -1.00, 8.12) **T3 vs T1, FT3 %Δ = 6.53 (95% CI: 2.07, 11.19)**	Models adjusted for: maternal age, pre-pregnancy BMI, drinking before pregnancy, passive smoking during pregnancy, gravidity, parity, and fetus sex. Effect modification: fetal sex Other Bisphenols: BPA, BPB, BPF, TBBPA BKMR models used to estimate effects of BPS as part of a mixture of chemicals.	No clear patterns were found for the other BPs. For first trimester hormone measurements, BPA associated with higher FT3, BPB lower T4 and FT4 and higher TSH, BPF associated with higher TSH, and TBBPA associated with lower T3. Second trimester hormone measurements, BPB associated with higher FT4, BPF associated with higher T4 and FT4 and lower TSH,

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
< 13 weeks gestational age, singleton pregnancy, Zhuang population (mother or father), no assisted fertilization, no thyroid disease during pregnancy	95th: 0.198 Timing of samples: Samples taken in first trimester		No associations in pregnancies with female fetus In restricted cubic spline and BKMR models, there was an inverted-U shaped dose-response curve between BPS and second trimester FT4, non-linear p-value of 0.046.		and TBBPA associated with higher T4 and FT4. Mixture analyses with FT3 showed non-linear effects of BPs. Ranges of BPS values included in each tertile were not reported. Correlations between BPS and other bisphenols: BPA (r = –0.09), BPF (r = 0.15), BPB (r = –0.01), TBBPA (r = 0.18)

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Sex Steroid Hormones and Related Proteins During Pregnancy					
Aker et al. 2019 Puerto Rico 2012-2017 Prospective cohort study Puerto Rico Testsite for Exploring Contamination Threats (PROTECT) Cohort n = 602 Mean ages (±SD): 26.51 ± 5.66 years Inclusion criteria: singleton fetus, 14 ± 2 weeks gestation, 18 to 40 years, no oral contraceptive use within 3 months prior to getting pregnant, no *in vitro* fertilization, no known medical health conditions (diabetes, hypertension, etc.)	Urinary spot samples Adjusted with SG LOD: 0.1 % < LOD: 3.4 (16 to 20 weeks)), 8.6 (24 to 28 weeks) Values below the LOD were calculated as LOD÷√2 Geometric mean ± geometric standard deviation: 0.54 ± 3.15 (16 to 20 weeks), 0.54 ± 5.2 (24 to 28 weeks) Percentiles (16 to 20 weeks, 24 to 28 weeks): 25th: 0.23, 0.23 50th: 0.50, 0.47 75th: 1.07, 1.06 95th: 4.01, 4.23 Timing of samples Visit 1: 16 to 20 weeks Visit 2: 20 to 24 weeks Visit 3: 24 to 28 weeks	Reproductive and pregnancy hormones via serum at visits 1 and 3: estriol, progesterone, testosterone, sex-hormone-binding globulin (SHBG), corticotropin-releasing hormone (CRH), TT3, TT4, FT4, TSH, TT3/TT4 Analyzed as %Δ	Per IQR increase in BPS **%Δ CRH = –11.35, (95% CI: –18.71, – 3.33) (p < 0.01)** The association with CRH in linear mixed models was driven by findings at 16-20 weeks: **%Δ CRH = –16.15, (95% CI: –26.6, –4.2) (p <0.01)** The association with CRH was weaker and non-significant at 24-28 weeks: %Δ CRH = –7.61, (95% CI: –16.3, 2.01) (p = 0.12) Other outcomes: Non-significant increase for testosterone, TT3, TT4, TT3/TT4 Non-significant decrease for SHBG, progesterone, estriol, progesterone/estriol, TSH, FT4	Models adjusted for: SG, study visit, BMI at the first study visit, maternal age, the number of hours of second-hand smoke exposure per day, and socio-economic status. Other Bisphenols: BPA, BPF	At 24-28 weeks, BPA was associated with lower testosterone, higher FT4 and T3: %Δ = 4.33, 95% CI: 0.11, 8.55. At 16-20 weeks, BPF was associated with higher FT4. 2,4-dichlorophenol, 2,5-dichlorophenol, benzophenone-3, triclosan were also analyzed, and weakly correlated with BPS (range of correlation coefficients: –0.04 to 0.04). Correlations between BPS and other bisphenols: BPF (r = 0.11), BPA (r = 0.17).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Wang et al. 2022 Shanghai, China 2012 Prospective birth cohort Shanghai-Minhang Birth Cohort Study (S-MBCS) n = 528 mother:child pairs n = 195 mother:girl child pairs Mean ages (±SD): 28.5 ± 3.4 years Inclusion criteria: pregnant women 12-16 gestational weeks and receiving care at the Minhang Maternal and Child Health Hospital in Shanghai	Maternal urine spot samples Adjusted with creatinine LOD: 0.004 % < LOD: 75.8% Values below the LOD were calculated as LOD÷2 (imputed in BKMR models) Categorized into < LOD, LOD to median, and ≥ median Geometric mean (95% CI) creatinine corrected, expressed as µg/g: 0.02 (0.01, 0.02) Percentiles (creatinine corrected, expressed as µg/g): 25th: < LOD 50th: 0.01 75th: 0.03 Timing of samples: third trimester	Total urinary kisspeptin (ng/L), including kisspeptin 54, 14, 13, and 10, assessed in mothers during the third trimester	No significant association between third trimester urinary BPS and kisspeptin levels in pregnant women. Male fetus: LOD-Median vs < LOD [β = –17.93 (95% CI: –78.23, 42.37)] ≥ Median vs < LOD [β = 8.74 (95% CI: –61.42, 78.90)] Female fetus: LOD-Median vs < LOD [β = –14.92 (95% CI: –87.79, 57.96)] ≥ Median vs < LOD [β = –56.89 (95% CI: –134.85, 21.07)]	Models adjusted for: maternal creatinine concentrations, household income, maternal education, maternal age at menarche, maternal age, maternal pre-pregnancy BMI, parity, gestational age at maternal urine collection, and fetus sex. Effect modification: fetal sex Other Bisphenols: BPA, BPF, BPAF, tetrachlorobisphenol A (TCBPA) BKMR models used to estimate effects of BPS as part of a mixture of chemicals.	2nd tertile maternal BPA and above median maternal BPF associated with decreased maternal kisspeptin levels in third trimester for women with a male fetus. Correlations between BPS and other bisphenols: BPA (r = 0.095), BPF (r = 0.143), BPAF (r = 0.036), TCBPA (r = -0.063). Study also reviewed in the next section, “Sex Steroid Hormones and Related Proteins in Young Girls”.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Sex Steroid Hormones and Related Proteins in Young Girls					
Hu et al. 2022 United States Cross-sectional study National Health and Nutrition Examination Survey (NHANES) 2013-2016 n = 1,179 participants, males and females, with data on urinary bisphenols, serum sex hormones and covariates 6–19-year-olds Mean age, females (±SD): 12.31 ± 3.93 years 6–11-year-olds were classified as children (females n = 273) 12–19-year-olds Were classified as adolescents (females n = 301) Puberty was defined as: Total testosterone (TT) ≥50 ng/dL in boys, estradiol (E2) ≥20 pg/mL in girls Inclusion criteria: systematic sample of US population, ages 6 - 19	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.1 % < LOD: 11.7 Total for population (males and females): Geometric mean: 0.52 <u>Percentiles:</u> 25th: 0.19 50th: 0.37 75th: 0.74 95th: 2.91	Serum sex steroid hormone levels: TT (ng/dL), E2 (pg/mL), SHBG (nmol/L), TT/E2, Free androgen index (FAI=TT/SHBG)	No significant associations between BPS and sex steroid hormone levels for either prepubertal or pubertal females. Prepubertal: non-significant increase E2, TT, SHBG, FAI and non-significant decrease TT/E2 Pubertal: non-significant increase SHBG, TT/E2 and non-significant decrease E2, TT, FAI	Models adjusted for: age, BMI, race/ethnicity, poverty index ratio, serum cotinine level, survey period, session of blood sampling, NHANES cycle. Other Bisphenols: BPA BKMR models used to estimate effects of BPS as part of a mixture of chemicals.	For all individuals: BKMR methods showed U-shaped exposure for E2, linear for TT, inverted U-shape for SHBG, U-shape for FAI, and linear for TT/E2 when reviewed for both prepubertal and pubertal individuals. No associations found with BPA for prepubertal and pubertal females. Authors had an error in the paper interchanging the BPA and BPS descriptive statistics. Correlations between BPS and other bisphenols: BPA (r = 0.33). Authors also examined associations between parabens and sex steroid hormone levels and thus limited their sample to participants with both bisphenols and parabens measured, resulting in a slightly different analytic sample than Wang 2021 (another NHANES study).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Wang et al. 2021 United States 2013-2016 Cross-sectional study NHANES 2 consecutive survey cycles (2013-2014 and 2015-2016) n = 1,317 participants, males (n = 662) and females (n = 655), with data on urinary bisphenols, serum sex hormones and covariates 6–19-year-olds mean age: 13 years 6–11-year-olds were classified as children (females n = 274) 12–19-year-olds were classified as adolescents (females n = 381) Inclusion criteria: systematic sample of US population, ages 6-19	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.1 % < LOD: 11.6 Median (IQR): Total population: 0.3 (0.6) Females: Children 0.28 (0.5) Adolescent 0.4 (0.7)	Serum sex steroid hormone levels: TT (ng/dL), E2 (pg/mL), SHBG (nmol/L), TT/E2, FAI=TT/SHBG	Female adolescents (ages 6 – 19): **Q2 vs Q1, higher TT/E2 ratio [No values given; results presented in graphs]** No associations with TT, E2 A non-linear, inverted U-shaped, dose-response was observed for BPS and SHBG (**p for non-linearity = 0.013**) Suggestion of non-linear association with FAI (p for non-linearity = 0.076) No associations in female children (ages 6-11)	Models adjusted for: age, race/ethnicity, ratio of family income to poverty, BMI categories, urinary creatinine, serum cotinine, session time of venipuncture, survey cycle, BPA, BPF. Other Bisphenols: BPA, BPF	Correlations between BPS and other bisphenols: BPA (r = 0.33), BPF (r = 0.15), BPA was negatively associated with FAI and E2, while being positively associated with SHBG and TT/E2. BPF associated with lower TT in female adolescents and non-linear associations with FAI and SHBG

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Wang et al. 2022 Shanghai, China 2012 Prospective birth cohort Shanghai-Minhang Birth Cohort Study (S-MBCS) n = 528 mother:child pairs n = 195 mother:girl child pairs Maternal mean ages (±SD): 28.5 ± 3.4 years Girls mean ages: 6 years Inclusion criteria: pregnant women 12-16 gestational weeks and receiving care at the Minhang Maternal and Child Health Hospital in Shanghai	Maternal urine spot samples Adjusted with creatinine LOD: 0.004 % < LOD: 75.8% Values below the LOD were calculated as LOD÷2 Categorized into < LOD, LOD to median, and ≥ median Geometric mean (95% CI) creatinine corrected, expressed as µg/g: 0.02 (0.01, 0.02) Percentiles (creatinine corrected), expressed as µg/g): 25th: < LOD 50th: 0.01 75th: 0.03 Timing of samples: third trimester	Total urinary kisspeptin, including kisspeptin 54, 14, 13, and 10, assessed in mothers during the third trimester and in girls at age 6.	No significant association between maternal BPS levels and kisspeptin levels in girls at 6 years of age LOD-Median vs < LOD β = –0.35 (95% CI -45.14, 44.44) ≥ Median vs < LOD β = 23.22 (95% CI –23.23, 69.68)	Models adjusted for: maternal creatinine concentrations, household income, maternal education, maternal age at menarche, maternal age, maternal pre-pregnancy BMI, parity, children's BMI z-score. Other Bisphenols: BPA, BPF, BPAF, TCBPA BKMR models used to estimate effects of BPS as part of a mixture of chemicals.	2nd tertile maternal BPA and above LOD maternal BPF associated with increased kisspeptin in girls at age 6. Correlations between BPS and other bisphenols: BPA (r = 0.095), BPF (r = 0.143), BPAF (r = 0.036), TCBPA (r = -0.063). Study also reviewed in the previous section, "Sex Steroid Hormones and Related Proteins During Pregnancy".

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Polycystic Ovary Syndrome (PCOS)					
Jurewicz et al. 2020 Poland 2017 Case-control study, medical practice-based n = 357 (199 cases, 158 controls) Mean case ages (±SD): 26.6 ± 5.5 years Mean control ages (±SD): 32.1 ± 6.9 years Inclusion criteria: ages 18-40 years Cases: diagnosis of PCOS Controls: without PCOS	Serum measurements Values below the LOD were calculated as LOD÷√2 LOD: 0.022 % < LOD: 5 Geometric mean: 0.14 for cases, 0.08 for controls Tertiles: Lowest < 0.09 Middle 0.09-0.21 Highest >0.21 Timing of samples: at beginning of study	PCOS diagnosed prior to study	For continuous, per unit increase in ln BPS, stratified by tertile of BPS **In T1 (< 0.09 ng/mL), OR: 1.12 (95% CI:1.03, 3.71) (p = 0.029).** In T2 (0.09-0.21 ng/mL), OR: 1.29 (95% CI: 0.79, 6.89) (p = 0.163) In T3 (> 0.21 ng/mL), OR: 1.33 (95% CI: 0.58, 4.88) (p = 0.548) BPS serum levels were higher in cases than controls (geometric mean 0.14 ng/mL vs. 0.08 ng/mL, p = 0.023); cases and controls differed in other characteristics, e.g., age, marital status, education, smoking status.	Models adjusted for: age, education, BMI, income, smoking. Other Bisphenols: BPA, BPF	BPA associated with decreased HOMA-IR (Homeostasis Model of Assessment-Insulin Resistance) and testosterone. The comparison group used for the effect estimate was not clearly reported, assumed to be per 1-unit increase in BPS. No mention of matching controls to cases, including significant differences between the groups based on age, marital status, education, and smoking status. Correlations between BPS and other bisphenols not reported.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Zhan et al. 2023 China 2014-2016 Case-control study, hospital-based Cases = 321, controls = 412 Mean age 29.0 years (14% 35+) Inclusion criteria cases: women diagnosed with infertility from PCOS; controls as stated by author: “The controls consisted of healthy women with no endocrine disorders and had AID”	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.003 % < LOD: 6.8 Percentiles: 25th: 0.02 50th: 0.12 75th: 0.49	PCOS identified prior to BPS sample collection Hormones: luteinizing hormone (LH), follicle stimulating hormone (FSH), E2, TT, prolactin (PRL)	Odds of PCOS For continuous, per unit increase in ln BPS **OR: 1.18 (95% CI: 1.10, 1.25)** 4-level BPS variable (quartiles): **Q2 vs Q1, OR: 1.49 (95% CI: 1.12, 1.91)** **Q3 vs Q1, OR: 1.70 (95% CI: 1.28, 2.22)** **Q4 vs Q1, OR: 2.12 (95% CI: 1.58, 2.79)** ***P* trend: <0.001** Associations were stronger among overweight and obese women after stratification by BMI. Within mixture analyses, BPS had the highest weights in quantile-based g-computation models. Hormones (in no-PCOS group) For continuous BPS, per unit increase in ln BPS: **TT [β = 0.07 (95% CI: 0.02, 0.12)], ($p < 0.05$)** No associations with LH, FSH, E2, PRL	Models adjusted for: age, BMI, level of education, annual household income, employment status, study site, smoking status, alcohol consumption. Effect modification: BMI Other Bisphenols: BPA, Bisphenol P (BPP), BPB, Bisphenol Z (BPZ), bisphenol AP (BPAP), and BPAF Used quantile-based g-computation to assess chemical mixtures and extreme gradient boosting to assess interactions between bisphenol analogs. Secondary analyses of BPS and hormones in no-PCOS group	BPA, BPP, BPB, BPZ, and BPAF were associated with increased odds of PCOS. BMI was evaluated as an effect modifier and could be significant for other bisphenols, but not for BPS. Correlations between BPS and other bisphenols: positively correlated with BPA, BPZ, BPAF, BPB, negatively correlated with BPB (exact correlation coefficients not reported).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Other Female Reproductive Outcomes					
Ao et al. 2022 Shanghai and Zhejiang Providence, China 2014-2016 Case-control study, hospital-based n = 1,180 cases, 571 controls Mean ages (±SD) cases: 30.2 ± 4.1 years, controls: 30.3 ± 3.7 years Inclusion criteria Cases: ≥ 2 unexplained recurrent miscarriage (URM), lack of genetic and infectious disorders, normal hormones, lack of known fertility problems Controls: multiparous women, no history of miscarriage, preconception visit	Urine spot samples Adjusted with creatinine LOD: 0.003 % < LOD: 17.5 Values below the LOD were calculated as LOD÷√2 Percentiles (adjusted with creatinine expressed as μg/g): 25th: 0.01 50th: 0.11 75th: 0.48 Timing of samples: measured after miscarriage (before 20 weeks gestation)	Unexplained recurrent miscarriage (URM) identified prior to study start	BPS quartiles **Q2 vs Q1, OR: 1.27 (95% CI: 1.22, 2.22)** **Q3 vs Q1, OR: 1.37 (95% CI: 1.02,1.85)** Q4 vs Q1, OR: 0.92 (95% CI: 0.69,1.23) *P* trend: 0.99 Effect modification by women < 30 and ≥ 30 years of age with BPS modeled as tertiles: For women ≥ 30: **T2 vs T1, OR: 1.62 (95% CI: 1.13, 2.33)** T3 vs T1, OR: 1.19 (95% CI: 0.83, 1.71) For women < 30: T2 vs T1, OR: 1.01 (95% CI: 0.68, 1.50) **T3 vs T1, OR: 0.65 (95% CI: 0.44, 0.97)** Restricted cubic splines showed an upside-down U shaped dose-response, **p-nonlinear: <0.001** BPS was least weighted chemical in mixture.	Models adjusted for: age, BMI, study site, education, study site. Effect modification: age (above or below age 30), study site Other Bisphenols: BPA, BPAF, BPAP, BPB, BPP BKMR models and quantile-based g-computation used to estimate effects of BPS as part of a mixture of chemicals.	BPA, BPAF, BPAP, BPB, BPP were associated with increased odds of URM, effect modified by those over 30 years for most BPs. Mixture analyses showed that BPAF/BPAP/BPA were the highest contributors to the effects of BPs on URM. Ranges of BPS values included in each tertile were not reported. Correlations between BPS and other bisphenols: BPA (r = 0.23), BPAF (r = 0.46), BPAP (r = 0.44), BPB (r = 0.36), BPP (r = 0.45).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Blaauwendraad et al. 2022 Rotterdam, Netherlands 2004-2005 Prospective cohort study Generation R Study n = 673 boys and n = 524 girls Mean (±SD) maternal age in years for: 30.7 (±4.8) boys and 30.9 (±4.6) girls Inclusion criteria: mothers were ≤18 weeks gestation, singleton	Urine spot samples Adjusted with creatinine LOD: 0.15 % < LOD (per trimester sampling time): 31.5, 70.3, 80.0 Values below the LOD were calculated as LOD÷√2 <u>Percentiles (per trimester sampling time)</u>: 25th: <LOD, < LOD, < LOD 50th: 0.2, <LOD, <LOD, 75th: 0.6, 0.08, <LOD, <u>Timing of samples</u>: Three time points as median (IQR): 12.9 (12.1-14.1), 20.4 (19.9-20.9), and 30.2 (29.9-30.8) weeks gestation	Reproductive development of infants and children: infant reproductive tract abnormalities, ovarian and testicular volume at 10 years using magnetic resonance imaging and pubertal development and onset of menstruation at 13 years	<u>Per IQR increase in BPS</u> age at first menstruation (years) for girls **[β = 0.17 (95% CI: 0.02, 0.31)] (p = 0.025)** Non-significant decrease in ovarian volume at age 10; Non-significant differences in pubic hair and breast development	Models adjusted for child's maternal age, ethnicity, pre-pregnancy BMI, education level, parity, intake, smoking and alcohol use, breastfeeding, child's gestational age adjusted birthweight, age and age adjusted BMI at time of measurement. Used quantile-based g-computation to assess chemical mixtures. Other Bisphenols: BPA and BPF	BPF was not included in the study with low detection levels in the second trimester. Exposure concentrations converted from nmol/L to ng/mL. Correlations between BPS and other bisphenols: BPA range 0.03 – 0.06 by trimester, BPF range 0.04 – 0.28 by trimester. Only analyses for female offspring presented here.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Peinado et al. 2020 Granada, Spain 2018-2019 Case-control study, hospital-based Endometriosis y Exposición Ambiental (EndEA) study n = 124, n = 35 cases and n = 89 controls Mean ages (±SD): cases: 38.3 ± 9.3 years, controls: 35.8 ± 10.4 years Inclusion criteria: premenopausal women ages 20-54 years, undergoing abdominal surgery, with BMI below 35 kg/m^2 Cases: diagnosed with endometriosis Controls: without endometriosis	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷2 LOD: 0.2 % < LOD: 88.6 for cases, 83.9 for controls Geometric mean: below LOD 25th: <LOD 75th: < LOD <u>Timing of samples</u>: First morning urine samples at start of study, prior to surgery	Endometriosis diagnosis following abdominal surgery at time of enrollment	No significant association between BPS and endometriosis Above vs below LOD, OR: 2.0 (95% CI: 0.4, 10.2)	Models adjusted for: creatinine, age, BMI, parity and residence. Other Bisphenols: BPA, BPF	BPA associated with increase in odds of endometriosis [OR 1.5 (95% CI: 1.0, 2.3)] per log unit increase, and with the second tertile of exposure [OR 3.7 (95% CI: 1.3-10.3)]. Thiobarbituric acid reactive substances (TBARS), a marker of lipid peroxidation, were also analyzed in urine. Correlation between BPS and other bisphenols not reported. LOD units given as mg/mL in text (assumed to be a typo for ng/mL based on expected range of values).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Philips et al. 2018 Rotterdam, Netherlands 2002-2006 Prospective cohort study Generation R Study n = 877 Mean ages (±SD): 31.2 ± 4.4 Inclusion criteria: singleton pregnancy and live birth, no prior use of infertility treatment	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.05 % < LOD: 29.5 Percentiles: 25th: 0.17 50th: 0.35 75th: 1.03 Timing of samples: First trimester (median 12.9 weeks, IQR 12.1-14.4 weeks)	Questionnaire answers for time to pregnancy (fecundability) measured in months and use of artificial fertility treatments	No significant association of BPS with fecundability For continuous, per unit increase in ln BPS: First trimester BPS fecundability ratio (FR) = 0.98 (95% CI: 0.94, 1.02) By folic acid supplement use: Inadequate folic acid FR = 0.94 (95% CI: 0.87, 1.01) (p <0.1) Adequate folic acid FR = 1.02 (95% CI: 0.96, 1.09)	Models adjusted for: maternal age, education, parity, folic acid supplement use, and urinary creatinine concentration. Other Bisphenols: BPA, BPF, BPZ, BPB, BPAP, BPP, BPAF Effect modification: folic acid Sensitivity analyses examined BPS and BPA in same model (no change in estimates)	Correlation between BPS and other bisphenols not reported, authors stated “correlations between first trimester BPA and BPS are weak”. Fecundability ratio interpreted as relative probability of becoming pregnant in a single menstrual cycle per log unit increase in bisphenol (FR < 1 indicates longer time to pregnancy). Stratification by folic acid was motivated by prior studies showing folic acid buffers adverse effects of BPA.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Philips et al. 2019 Rotterdam, Netherlands 2004-2005 Prospective birth cohort Generation R Study n = 1,233 Mean age (±SD): 30.5 ± 4.8 years Inclusion criteria: <18 weeks gestation, singleton pregnancy, no pre-existing hypertension	Urine spot samples Adjusted with creatinine LOD: 0.05 % < LOD: 31.5 Values below the LOD were calculated as LOD÷√2 Percentiles: 25th: 0.17 50th: 0.35 75th: 1.03 Timing of samples: Early pregnancy (median gestational age 13.1 weeks, IQR 12.1-14.5 weeks)	Blood measurements: Placental angiogenic markers measured in early and mid-pregnancy: placental growth factor, tyrosine kinase ratios, hemodynamic function Umbilical blood: umbilical artery pulsatility index (PI), uterine artery resistance index (RI), notching and placental weight, Physical measures: blood pressure measured at each visit, gestational hypertensive disorders via medical records	No significant findings for BPS with placental angiogenic markers, umbilical cord measures, blood pressure or gestational hypertensive disorders	Models adjusted for: maternal age at enrollment, education level, ethnicity, parity, folic acid supplementation, gestational age, pre-pregnancy BMI (kg/m^2), smoking, and alcohol consumption. Other Bisphenols: BPA	BPA associated with decreasing slope of the umbilical artery PI Z-score, increasing slope of the uterine artery RI Z-score, but nothing else. This was over time and was not seen in the individual models. Correlation between BPS and BPA not reported.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Philips et al. 2020 Rotterdam, Netherlands 2002-2006 Prospective cohort study Generation R Study n = 1,213 Mean ages (±SD): 30.6 ± 4.8 years Inclusion criteria: singleton pregnancy and live birth	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.05 % < LOD: 31.9 early and 70.9 mid-pregnancy Median (IQR) early pregnancy: 0.35 (0.17,1.09) Median (IQR) mid-pregnancy: 0.24 (0.12, 0.49) <u>Timing of samples</u>: Early (median 13.1 weeks [IQR 12.1-14.5]) and mid-pregnancy (median 20.4 weeks [IQR 19.9-20.9])	Gestational weight gain measured during study (grams)	For continuous, per unit increase in ln BPS measured in early pregnancy: gestational weight gain **[*β*: –261 grams (95% CI: –466, –56)]** over total pregnancy gestational weight gain [*β*: –142 (95% CI: –282, –2)] until late pregnancy (~ 30 weeks), not significant after correction for multiple comparisons as stated by authors. No associations with binary outcomes of insufficient weight gain and excessive weight gain	Models adjusted for: maternal age, daily dietary caloric intake, parity, ethnicity, education, maternal smoking, maternal alcohol, mid pregnancy total bisphenols, and folic acid supplementation. Other Bisphenols: BPA, BPF	BPA showed a 132 gram (95% CI: –231, –34) decrease in gestational weight gain during mid to late pregnancy. Correlations between BPS and other bisphenols: Early pregnancy BPS positively associated with mid-pregnancy BPA (r = 0.192); cross-sectional BPS-BPA correlations not reported. BPS-BPF correlation coefficient not reported. Mid pregnancy BPS not analyzed due to low detection.

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Wesselink et al. 2021 Detroit, Michigan 2010-2012 Case-cohort study Study of the Environment, Lifestyle, and Fibroids prospective cohort study n = 754 Mean age: 28.6 ± 3.5 years Inclusion criteria: Black women, no previous uterine leiomyomata (UL, fibroids), no history of cancer, and had an intact uterus	Urine spot samples Adjustment: creatinine For values below the LOD, authors used instrument values. LOD: 0.1 % < LOD: 4.6 (Baseline), 5.7 (20 months), 4.1 (40 months) <u>Percentiles (baseline, 20 months, and 40 months)</u>: 50th: 0.5, 0.6, 0.8 90th: 2.6, 2.5, 3.5 <u>Timing of samples</u>: Baseline measurement followed by 20 and 40 months	UL incidence, developed over the course of 60 months. Fibroid growth, measured via transvaginal ultrasound on a subset of fibroids, with measurements on up to 6 fibroids per participant. Fibroid growth measured from 20-40 months (n = 67 fibroids), and from 40-60 months (n = 145 fibroids).	Per 50% increase in BPS: Hazard ratios (HRs) of UL: **0.93 (95% CI: 0.87, 0.99)** %Δ in UL volume over 18 months: **4.1% (95% CI: 0.3, 8.0)** Stratification by age: Below age 30, HR of UL: **0.90 (95% CI: 0.82, 0.99)** 30+ years: HR of UL: 0.95 (95% CI: 0.87, 1.04) No evidence of effect modification by parity or BMI BPS showed a nonlinear U-shaped dose-response with restricted cubic splines	Models adjusted for: age, education, parity, cigarette smoking, alcohol intake, BMI, age at menarche, depot medroxyprogesterone acetate use, time since last birth, and creatinine. Effect modification: maternal age, parity, BMI Other Bisphenols: BPA, BPF	Multiple imputation approaches were used in the study: (i) for missing urinary phenol concentrations (10.1 – 18.1% of study participants based on timepoint of collection), (ii) for missing outcome status for those who did not complete any follow-up (3.1% of study participants), and (iii) for missing confounder data (<1% of study participants). Complete case estimates presented in supplement are similar, but less precise. Correlations between BPS and other bisphenols: BPS positively correlated with BPA and BPS (exact coefficients not reported).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Yue et al. 2023a Beijing, China 2017 Cross-sectional Study n = 86 Mean (±SD) age: 40 (±10) years Inclusion criteria: adults having health physical examination at Beijing Hospital, China without major diseases/disorders of the thyroid or other severe diseases	Urine spot samples Adjusted with creatinine LOD: 0.002 % < LOD: 2.3 Values below LOD were given the LOD Mean ± SD: 0.23 ± 0.78 Percentiles: 10th: 0.01 25th: 0.03 50th: 0.07 75th: 0.15 90th: 0.41	Thyroid hormones: TT4, TT3, FT4, FT3, and TSH	No significant results with BPS and thyroid hormones Non-significant increase for TT3, FT3, TSH, TT3/TT4 ratio, FT3/FT4 ratio Non-significant decrease for TT4, FT4	Models adjusted for: gender, age, BMI, smoking status, education levels, and urinary iodine, and co-exposure to other endocrine disrupting chemicals with p -value < 0.15 in simple linear regression models. BKMR models used to estimate effects of BPS as part of a mixture of chemicals. Other Bisphenols: BPA and BPF	BPF associated with decreases in TT3, TT4, fT3, and FT4. BPF associated with increases in TT3/TT4 ratio and BPA associated with increases in FT3/fT4 ratio. Correlations between BPS and other bisphenols: BPA (r = 0.29) and BPF (r = 0.18). Only results for females are presented here (sex-stratified analyses were displayed graphically in Figure 2).
Zhan et al. 2022 United States 2013-2016 Cross-sectional study NHANES n = 857 Median ages (IQR): 31.0 (23.0 – 39.0) years Inclusion criteria: systematic sample of US population, over 6 years of age, those who did not have hysterectomy or oophorectomy	Urine spot samples Adjusted with creatinine Values below the LOD were calculated as LOD÷√2 LOD: 0.1 % < LOD: 9.6 Percentiles: 25th: 0.20 50th: 0.60 75th: 1.30	History of infertility either not conceiving after one year of trying or seeing a reproductive specialist based on questionnaire	For continuous, per unit increase in ln BPS: No significant associations of BPS with infertility OR: 1.08 (95% CI: 0. 92, 1.28) (p = 0.35) Exposure-response curve for BPS with risk of infertility by BKMR analysis showed a slight inverse U-shape. No evidence of interactions between various pollutants	Models adjusted for: age, race/ethnicity, education level, BMI, marital status, serum cotinine, drinking status, poverty-income ratio, physical activity, age at menarche, pregnancy history. BKMR models used to estimate effects of BPS as part of a mixture of chemicals. Other Bisphenols: BPA	BPA associated with an increased odd of infertility. Correlations between BPS and other bisphenols: BPA (r = 0.11).

Reference; study location, period, design, sample	Exposure matrix and concentration (ng/mL)	Outcomes	Results	Covariates, other bisphenols analyzed	Comments
Zhang et al. 2023 Shenyang, China 2020-2021 Cross-sectional study Shenyang Reproductive and Environmental (SYRAE) study n = 111 Median (IQR) age in years 32.0 (4.1) Inclusion criteria: over 18 years old, seeking assisted reproductive technology at study center, available ovarian assessment data, did not have PCOS	Urine spot samples Adjusted with creatinine LOQ: 0.1 % < LOQ: 40 Values below the LOQ were calculated as LOQ÷2 Geometric mean (creatinine adjusted, expressed as µg/g): 0.37 Percentiles (creatinine adjusted, expressed as µg/g): 25th: 0.19 50th: 0.32 75th: 0.55 Timing of samples: Provided prior to study	Diminished ovarian reserve (DOR) and indicators of ovarian reserve: anti-müllerian hormone (AMH, ng/mL), FSH milli-international units (mIU/mL), and E2 (pg/mL)	BPS modeled as categorical variable of higher (above the median) or lower (below the median): odds of DOR **OR: 6.85 (95% CI: 1.24, 37.82) (p = 0.027)** Non-significant lower AMH, higher FSH, higher E2 For continuous, per unit increase in ln BPS: AMH for all women: **[*β* = –0.287 (95% CI: –0.505, –0.070)] (p = 0.01)** Non-significant higher FSH and lower E2 AMH excluding women with ovulation disorders: **[*β* = –0.296 (95% CI: –0.518, –0.075)] (p <0.01)** BPS exposure showed subtle inverse U-shape dose response with AMH levels (non-linear term not significant, p = 0.37)	DOR models adjusted for: age, BMI, and household income. AMH, FSH, E2 models adjusted for: age, BMI, smoking, passive smoking, and infertility diagnosis. Other Bisphenols: BPA and BPF	BPA associated with increased odds of DOR and decreased E2 levels. BPA showed an inverse U-shape dose response with AMH levels. Imputation for continuous values at 40% of BPS samples. Correlations between BPS and other bisphenols: BPA (r = 0.585) and BPF (r = 0.237).

4.2 Studies in animals

OEHHA identified **43** studies that examined the effects of BPS on the female reproductive system in mammalian and non-mammalian animal models. These included **18** studies in mice, **13** in rats, **three** in ewes, **one** in gerbils, **one** in hamsters, **five** in zebrafish, **one** in chickens, and **one** in *C. elegans*. A summary of each study is presented in Table 4.2.1, and the evidence from these studies is discussed below.

4.2.1 Overview

The BPS related reproductive toxicity outcomes reported in the reviewed studies included effects on weight and histology of the ovary, uterus, placenta, and – one accessory reproductive gland – the female prostate. Other reproductive toxicity outcomes included effects on the endocrine system, puberty onset, estrous cycle, mammary gland development, reproductive performance, and sex ratio. 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).

Differences in experimental design should be considered when comparing findings across studies. 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, perinatal, pubertal, adult), and timing of outcome examination. 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 five studies in rats, three in mice, and one in ewes used subcutaneous (sc) injections, and one study in rats and two in mice used intraperitoneal (ip) injections. The duration of exposure in mammalian studies ranged from a few hours (in studies assessing the effects of exposure on hormone levels) to 90 days (in a repeated-dose oral toxicity study). Chickens were exposed by gavage, zebrafish were exposed in tank water, and *C. elegans* were exposed directly via the nematode growth medium. Daily exposure ranged from 0.001 µg/kg to 1,000 mg/kg in mammals and chickens, 0.5 µg/L to 200 µg/mL in zebrafish, and 31.2 to 125 µg/mL [125 to 500 µM] in *C. elegans*.

4.2.2 Organ weight and histology

Effects on the ovary

Alterations in ovarian weight may reflect changes in the physiology of the organ. For example, faster or slower follicular development, inhibition of corpus luteum (CL)

formation after ovulation, and ovarian cyst development are a few factors that can affect ovarian weight. The following paragraphs focus specifically on the effects of BPS on oocyte and follicle development.

A change in ovarian weight after exposure to BPS was assessed in several studies and results were mixed. Decreased ovarian weight on postnatal day (PND) 75 was reported in rats (strain not specified) subcutaneously injected with 5 and 50 mg/kg-day BPS on PND 1 to 10 (Ahsan et al. 2018), in adult Sprague-Dawley (SD) rats intraperitoneally injected with 50 mg/kg-day BPS for 28 days (Ijaz et al. 2020), and in pubertal ICR mice exposed to 0.001 to 100 µg/kg-day BPS in drinking water for 28 days (Nevoral et al. 2018). Two studies reported increased ovarian weight, one in Wistar rats dosed by gavage at 1,000 mg/kg-day BPS for 90 days (BASF 2014a) and in adult female Hy-Line W-36 laying chickens dosed by gavage at 50 µg/kg-day for 90 days (Eldefrawy et al. 2021). Other studies did not detect significant changes in ovarian weight with daily BPS doses ranging from 0.001 mg/kg-day to 1,000 mg/kg-day in SD and Wistar rats, gerbils, and ewes (BASF 2020; Demacopulo and Kreimann 2019; Kaimal et al. 2021; Silva et al. 2019; Téteau et al. 2022), Anonymous Studies 12, 14, and 16 as described in (ECHA 2019).

There are several studies that assessed the effects of BPS on oocyte and follicle development at very early stages in fetal life. During gestation, fetal germ cells undergo incomplete mitosis (lacking cytokinesis) and form syncytia, multinucleated structures known as germ cell nests or cysts. Germ cell nests ultimately break down and granulosa cells surround individual oocytes to form primordial follicles. At this point, oocytes have started miotic division but are arrested in meiosis prophase I (diplotene stage). Altered follicular or oocyte development can have downstream consequences on ovulation and the formation of CL.

Prenatal exposure of ICR mice via treatment of pregnant dams with BPS at 2, 10, 50, 100, or 200 µg/kg-day from gestational day (GD) 12.5 to 15.5, targeting the period of meiosis, resulted in accelerated meiotic progression in fetal ovaries at GD 15.5 at all doses, compared to controls. Accelerated meiotic progression was defined by a smaller percentage of oocytes at earlier stages of meiosis prophase I (zygotene) and a larger percentage of oocytes at later stages (pachytene and diplotene), and increased rate of aberrant spindles and misaligned chromosomes at 2 and 10 µg/kg-day (Zhang et al. 2020). Because the effects on meiotic progression were observed at all BPS doses, the researchers continued studying the effects of prenatal exposure to BPS on ovarian development with only the two lower doses, 2 and 10 µg/kg-day. Both doses accelerated germ cell nest (cyst) breakdown in F1 mice at PND 3, as evidenced by a decreased proportion of oocytes in nests and a relative increased proportion of oocytes in primordial follicles, with no effect on the total number of oocytes, compared to

controls. To test whether this accelerated nest breakdown affected subsequent follicular development, the researchers examined outcomes at PND 21, where they observed a higher proportion of oocytes in primordial follicles and a lower proportion in secondary and antral follicles in the prenatally exposed mice (Zhang et al. 2020). Acceleration of cyst breakdown was similarly observed in the F2 generation[3] at PND 3 (Zhang et al. 2020).

In a study with a different strain of mice (CD-1) and longer duration of treatment (GD 11 to birth), BPS at 0.5 and 20 µg/kg-day inhibited germ cell nest breakdown, resulting in more germ cells retained in nests at PND 4. The authors also reported decreases in the ratio of primary follicles to total oocytes at doses of 0.5, 20, or 50 µg/kg-day, non-significant decreases for the ratio of secondary follicles to total oocytes, and no effects on the ratio of primordial follicles to total oocytes (Shi et al. 2019).

Two studies investigated the effects of gestational BPS exposure on the number of CL. One study reported a reduced number of CL in SD rats exposed orally to 5 µg/kg-day BPS from GD 6 to 21 (Kaimal et al. 2021). The second study reported no changes in the number of CL in a different strain of rat (Wistar) exposed orally with up to 300 mg/kg-day BPS from GD 6 to GD 19 (BASF 2014b).

In rodents, germ cell breakdown and formation of primordial follicles continues until shortly after birth. Treatment of newborn CD-1 mice with 10 µg/kg-day by ip injection for three days decreased the percentage of oocytes in cysts and increased the numbers of oocytes in primordial follicles (Liu et al. 2021), a finding that is consistent with the accelerated nest breakdown identified by Zhang et al. (2020). Similar to Zhang et al. (2020), after following the animals to PND 21 to measure effects on downstream folliculogenesis, the authors observed decreased numbers of secondary and antral follicles in treated mice (Liu et al. 2021). The finding that BPS exposure decreased numbers of antral follicles was observed, again, in a separate study that treated female rats with 0.5, 5 or 50 mg/kg-day BPS by sc injections for 10 days postnatally (PND 1 to 10). This treatment regimen also resulted in consequent decreases in the number of CL at 5 or 50 mg/kg-day BPS and increases in the number of atretic cystic follicles at 50 mg/kg-day BPS (Ahsan et al. 2018). A final perinatal exposure study examined the interaction between perinatal exposure to 200 µg/kg-day BPS in CD-1 mice from GD 8 to PND 19 followed by 1 µg/kg-day ethinyl estradiol (EE) from PND 19 to PND 21. BPS treatment alone resulted in a higher number of secondary follicles with no effect on other follicle types. BPS + EE, however, showed that BPS inhibited the EE-induced

[3] BPS was administered at doses of 2 or 10 µg/kg-day to F0 pregnant dams on GD 12.5 to 15.5 (Zhang et al. 2020).

increases in total follicles. This finding suggests an endocrine mechanism of action for BPS (Hill et al. 2017).

Two studies examined the effects of pubertal BPS exposure on ovarian histopathology. At this developmental stage, the processes of resuming meiosis of oocytes, and follicle recruitment and growth are tightly regulated by hormones. BPS treatment for 28 days in pubertal ICR mice decreased relative ovary weight at all tested doses (0.001, 0.1, 10, 100 µg/kg-day) and decreased total ovarian volume at 10 and 100 µg/kg-day. These changes in ovary morphology paralleled reductions of CL volume and dose-dependent decreases in primary, preantral, and antral follicles. The authors also observed increased mean volume of antral follicles at 0.001 and 0.1 µg/kg-day, decreased atretic follicle volume at 0.1 and 10 µg/kg-day, decreased medulla volume at all doses, and decreased numbers of granulosa cell layers at 10 and 100 µg/kg-day (Nevoral et al. 2018). Abnormalities in oogenesis were also evident. The study reported increased incidence of spindle malformation at 1 and 100 µg/kg-day, increased numbers of abnormal oocytes at 0.1 and 10 µg/kg-day, and increased methylation of H3K27 (a specific lysine residue in histone H3) at 10 and 100 µg/kg-day without abnormal chromosome alignment (Nevoral et al. 2018). The second pubertal exposure study used Wistar rats treated with 60 mg/kg-day dehydroepiandrosterone (DHEA) to induce polycystic ovary-like syndrome (PCOS). In this study, co-treatment with sc injections of BPS at 1 µg/kg-day for 20 days decreased the average number of cysts per ovary compared to treatment with DHEA alone, providing more evidence of an endocrine mechanism of action for BPS. BPS treatment alone induced a non-significant increase in follicular cysts (absent in controls) (Demacopulo and Kreimann 2019).

The last set of mammalian studies examined effects of BPS exposure on the ovary in adult mammals. Similar to the studies described above, there was evidence to suggest that BPS exposure disrupts folliculogenesis and oocyte maturation. For example, adult (8-9 weeks old) CD-1 mice treated with BPS at 300 µg/kg-day for 28 days by oral gavage had atrophy in immature follicles, an increased number of atretic follicles, damaged oocyte structure, and more wrinkled and depressed zona pellucida compared to controls (Yue et al. 2023b). In a separate study, seven days of oral BPS treatment in adult ICR mice increased the proportion of abnormal mature oocytes at all doses (0.001 to 100 µg/kg-day) and increased abnormal spindles at 0.01, 10, and 100 µg/kg-day. This study also reported non-significant increases in abnormal chromatin in in mature metaphase II (MII) oocytes, increased apoptosis at 0.01, 10, and 100 µg/kg-day in, but no effects on oocyte quantity or quality (Prokešová et al. 2020). A third study reported that adult SD rats treated with BPS by ip injections at 50 µg/kg-day to 50 mg/kg-day for 28 days had changes in ovarian structure, reporting a reduced number of CL at ≥ 500 µg/kg-day and reduced gonadosomatic index (GSI) and increased CL diameter at 50 mg/kg-day [50,000 µg/kg-day]. Also, antral and atretic follicles were decreased in rats

treated with 5 and 50 mg/kg-day [5,000 and 50,000 μg/kg-day] and antral follicle diameter was increased at all doses. Granulosa cell height was decreased at 5 mg/kg-day but increased at 50 mg/kg-day with no effect on theca cell height (Ijaz et al. 2020). In one study in adult female golden hamsters treated with BPS at a daily oral dose of 150 mg/kg-day for 28 days, a decrease in the number of growing follicles and CL (lack of ovulation) with an increase in the number of atretic follicles was reported (Pal et al. 2023). Lastly, one non-rodent study treated mature ewes with BPS at 4 or 50 μg/kg-day and reported no effect on the total number of follicles available for aspiration (Desmarchais et al. 2022).

In wild-type zebrafish, five studies examined changes in ovarian maturation and ovarian structures, including changes in oocyte morphology. In most studies, zebrafish (AB strain) were exposed to BPS concentrations between 0.1 μg/L and 200 μg/L (with one study using BPS concentration up to 200,000 μg/L) for a wide range of durations (7 to 240 days). Two studies reported that BPS treatment reduced GSI in adult zebrafish exposed for 21 days at 0.5, 5, and 50 μg/L (Ji et al. 2013) and in zebrafish embryos exposed for 75 days at 100 μg/L (Naderi et al. 2014). Delays in oocyte and follicle maturation were evident in other studies. For example, BPS treatment at 1, 10 and 100 μg/L for 14 days increased the number of perinucleolar oocytes, decreased the number of cortical alveolar oocytes, and decreased the percentage of late vitellogenic and spawning oocytes at all doses (Wang et al. 2020). Treatment at different days post fertilization (dpf) from days 30 to 240 increased the number of cortical follicles at 1 μg/L, increased the number of vitellogenic-stage oocytes at 100 μg/L, and increased egg production at 1 and 100 μg/L. This study also reported fewer oocytes at the primary growth stage and increased numbers of oocytes at the full-grown stage at 1 and 100 μg/L (Qin et al. 2021). A final study treated zebrafish at substantially higher concentrations (8, 40, and 200 μg/mL) for 21 days and observed increased GSI and hepatic somatic index (HSI) at 40 μg/mL and oocyte degeneration at 40 and 200 μg/mL compared to controls, as evidenced by regressed oocytes, perinucleolar oocytes, and yolk vesicle oocytes (Park et al. 2022).

Two final studies used chicken and *C. elegans* models. Chickens are a useful experimental model in which to study folliculogenesis considering the relative short period of follicle development and maturation and daily ovulation in these animals, while *C. elegans,* a hermaphroditic species, has been a useful animal model to study meiosis. In adult Hy-Line W-36 chickens orally exposed to BPS at 50 μg/kg-day for three months there was an increase in weight of the left ovaries (the active one in chickens), a decrease in the number of preovulatory follicles, an increase in lymphocyte infiltration (presence of lymphocytes around the growing follicle and ovarian medulla), and an increase in ovarian inflammation (Eldefrawy et al. 2021). In *C. elegans* exposed to BPS at 125, 250 and 500 micromolar (μM) (internal dose = not detected, 0.21, and 0.39 μg/g,

respectively) there were dose-dependent effects in germline cells such as irregular distribution of nuclei along the distal to the proximal axis, nuclear loss, and cell death by apoptosis (Chen et al. 2016b).

Effects on the uterus

Changes in uterine weight following exposure to BPS have been assessed in several studies. SD rats treated with BPS (from 50 µg/kg-day to 50 mg/kg-day) by ip injection for 28 days had reduced relative uterine weights at 5 and 50 mg/kg-day, compared to controls (Ijaz et al. 2020). The study by Ahsan et al. (2018) also reported decreases in relative weight of paired uteri in rats treated from PND 1 to 10 with 50 mg/kg-day by sc injection and assessed at PND 75. In two other rat studies, BPS treatment resulted in increased uterine weight. One study treated SD rats with 30, 100, or 300 mg/kg-day BPS by oral gavage for about 14 weeks from 6 weeks premating to weaning and observed effects at 300 mg/kg-day Anonymous Study 14 as described in (ECHA 2019), while the other treated SD rats with sc injections for three consecutive days and reported increased uterine weight at 20 and 500 mg/kg-day (Yamasaki et al. 2004).

Other studies reported no effects of BPS treatment on uterine weight. These included a study that treated Wistar rats from GD 6 to GD 19 and evaluated dam uterine weight on GD 20 (BASF 2014b), a study that treated SD rats with BPS for a total of 40-46 days from premating to postpartum day 3 Anonymous Study 12 as described in (ECHA 2019), and a study that treated SD rats with BPS by daily oral gavage at doses of 0, 100, 300 or 600 mg/kg-day for 28 days (BASF 2020). A final study reported no changes in uterine weight among adult ICR mice treated with BPS by oral gavage at 300 µg/kg-day for 28 days (Yue et al. 2023b).

Other reported effects of BPS exposure on the uterus include histological and structural alterations. The study by Yue et al. (2023) in adult ICR mice reported that BPS treatment resulted in multiple changes in the shape and structure of the uterus in the absence of an effect on uterine weight. These histopathological changes included a narrowed uterine cavity, increased number of uterine glands, reduced endometrial area, and atrophied lamina propria, myometrium, and plasma layer (Yue et al. 2023b). Adult female C57BL/6J mice treated with BPS by gavage at a dose of 30 mg/kg-day for 21 days developed “non-atypical” endometrial hyperplasia (increased number of normal endometrial cells), increased number of glands in the endometrium, and increased thickness of the myometrium, compared to controls. Migration of endometrial cells into the myometrium (adenomyosis) was also observed in 2 out of 5 treated mice, compared to 0 out of 5 controls (Benjamin et al. 2023).

In immature Wistar rats treated with 60 mg/kg-day DHEA for 20 days to induce PCOS, cotreatment with BPS at 1 µg/kg-day resulted in the following alterations in the uterus:

an increased intraepithelial lumen, increased cell proliferation in the endometrial perimeter, and increased apicobasal polarity of the epithelium when compared to the DHEA alone group. BPS treatment alone (no DHEA) increased cell vacuolization in the endometrium compared to controls (Demacopulo and Kreimann 2019). These findings are similar to those presented by (Yamasaki et al. 2004) in rats and (Hill et al. 2017) in mice which showed that BPS treatment altered the effects of EE treatment. For example, BPS at 20 mg/kg-day further increased the uterine weight of an EE treated group of SD rats and at 500 mg/kg-day diminished the EE effect on increased uterine weight (Yamasaki et al. 2004). In female CD-1 mice exposed to BPS from GD 8 until PND 19 (via oral administration to dams during gestation at 200 µg/kg-day and direct oral administration after birth at 200 µg/kg-day) there were no observed effects on uterine tissue organization on PND 22, however, increased uterine epithelial height was apparent at PND 22 in mice that received additional treatment with EE at 1 µg/kg-day from PND 19 to PND 21 (Hill et al. 2017). Together, these findings suggest that BPS alters uterine responsiveness to hormonal changes normally experienced during puberty.

The last histopathological uterine outcome reported was increased squamous metaplasia of the uterus at a BPS dose of 1,000 mg/kg-day in 42-day-old female Wistar rats treated for 90 days (BASF 2014a).

Effects on the female prostate

One study presented data on the effects of BPS on an accessory gland, the female prostate. In this study adult female gerbils were orally treated with BPS at 40 µg/kg-day for 28 days, which affected the three-dimensional structure of female prostatic tissue. Compared to controls, BPS treated gerbils had increased relative frequency of epithelium and muscular stroma and decreased ductal lumen. Prostatic histology showed decreased percentage of normal tissue, increased hyperplasia, and increased intraepithelial neoplasia without affecting prostate weight. There were no alterations in body or gonadal weight (Silva et al. 2019).

Effects on placenta

Two studies, in different species, examined effects of BPS on placenta histopathology. One study treated mice with BPS through oral ingestion of wafers at 200 µg/kg-day for 2 weeks prior to mating and through GD 12.5 and observed in placental cross-sections (through three placental layers: labyrinth, spongiotrophoblast, and giant cell) a decreased ratio of spongiotrophoblast to giant cell area, which the authors propose may be due to changes in gene expression (Mao et al. 2020). The second study treated pregnant sheep with a sc injection of BPS at 0.5 mg/kg-day from GD 30 to 100. BPS treatment resulted in a non-significant increase in placental weight at GD 120, and no

effects on placental stereology, or number of placentomes per placenta (Gingrich et al. 2018).

4.2.3 Endocrine effects

Effects of BPS on the endocrine system involved variations in hormone levels, including synthesis and/or metabolism, and alterations in hormone receptor activity and/or expression. BPS effects on hormone levels were observed in various body fluids and tissues and included alterations in gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH); steroid hormones: progesterone (P), testosterone (T), dihydrotestosterone (DHT), estradiol (E2) and estrone (E1); thyroid hormones; and prolactin.

In the female reproductive cycle, there are two well characterized ovarian phases: follicular and luteal. During the follicular phase, the ovarian follicles are maturing, and the surrounding granulosa cells are producing increasing amount of E2. In the middle of the cycle, levels of gonadotropins secreted by the pituitary gland surge in response to positive feedback from E2 and trigger ovulation of the fully mature antral follicle. The remaining follicular cells (granulosa and theca) now form the CL that increases the production of P in the luteal phase. Disruption of this hormone balance may affect, or may be a consequence of, alterations in follicle development (e.g., FSH), ovulation (e.g., LH, E2), implantation (e.g., P) or other processes involved in female reproduction.

A small number of studies have reported effects of BPS on some steroidogenic enzyme mRNA and protein levels, and other studies have reported effects on gene and/or protein expression of the progesterone receptor (PR), both estrogen receptor alpha and beta (ERα, ERβ) and related growth factors, the androgen receptor (AR), and the prolactin receptor (PrlR) and related factors.

Gonadotropins

Two studies in rats and one in mice reported the effects of BPS on gonadotropin levels. In rats (unspecified strain), a decrease in plasma levels of LH and FSH was reported in females treated with 50 mg/kg-day BPS by sc injection from PND 1 to 10 and assessed on the first day of estrus starting on PND 75 (Ahsan et al. 2018) and in adult female SD rats treated with BPS at doses between 5 and 50 mg/kg-day by ip injection for 28 days (Ijaz et al. 2020). In adult female NMRI mice, a dose-dependent decrease in serum LH and FSH concentrations was observed following sc injection with BPS for 21 consecutive days at doses ranging from 1 to 100 µg/kg-day (Nourian et al. 2020).

Steroid hormones

Progesterone

Five studies reported that exposure to BPS decreased levels of P in serum, plasma, or follicular fluid in rats, mice, and ewes. Two studies reported increased levels of P in mice and zebrafish while one study in ewes reported no changes in P levels in plasma after BPS treatment.

Decreases in plasma P levels at 50 mg/kg-day were found in neonatal female rats (unknown strain) exposed to BPS (0.5, 5, or 50 mg/kg-day) by sc injection in the first 10 days of life (Ahsan et al. 2018). One study in CD-1 mice with BPS exposure during gestation (GD 10.5 to GD 17.5) reported decreased serum P levels at 3 months of age in the 5 mg/kg twice a day dose group, with no effects at PND 28 or at 14 months of age (Tucker et al. 2018). Decreases in plasma P levels were also observed in adult female SD rats treated with BPS doses of 5 and 50 mg/kg-day via ip injection for 28 days (Ijaz et al. 2020). In one study in adult female ewes, an interaction between BPS and diet was observed on P levels. Two groups of animals – restricted feed and well fed – were exposed to BPS to assess the effects on steroid hormones. Most of the BPS effects were observed in the restricted feed group. In this study, BPS exposure at 50 µg/kg-day for at least three months decreased P and 5α-dihydroprogesterone (P metabolite) levels in serum, and 17α-hydroxyprogesterone levels in preovulatory follicular fluid in the restricted feed group (Téteau et al. 2022). In pregnant ewes exposed to daily sc injections of BPS at 0.5 mg/kg-day from GD 30 to 100, a non-significant decrease in serum P levels was observed between GD 75 -105 (Gingrich et al. 2018).

Increases in P levels following BPS exposure have also been reported in some studies. In the mouse gestational exposure study discussed above by Tucker et al. (2018), an increase in serum P levels was observed on PND 20 in the 0.05 mg/kg twice a day dose group; this dose is a notably smaller than the dose that resulted in decreased P at three months of age in the same study (5 mg/kg twice a day). In a zebrafish study, there were increased whole-body P levels observed after BPS exposure to 40 and 200 µg/mL for 21 days (Park et al. 2022). In the last study examining P, treating adult ewes orally with BPS at 4 or 50 µg/kg-day had no effect on plasma or follicular fluid P levels (Desmarchais et al. 2022).

Androgens: T, DHEA, DHT

BPS effects on androgen levels were investigated in three studies in mice, two studies in rats and one study in ewes. Increased serum T levels were reported after BPS exposure during pregnancy or early in life in mice and rats, whereas decreased T levels were observed following exposure later in life (PND 35 or at eight months of age).

Increased serum T levels were observed in mice on PND 28 after gestational exposure to BPS in the 0.05 mg/kg twice a day dose group (Tucker et al. 2018), at 9 months of age after BPS treatment with 50 µg/kg-day [0.05 mg/kg-d] during gestation (Shi et al. 2019), and in newborn mice treated with BPS at 10 mg/kg (every three days) from PND 0 to PND 60 (Shi et al. 2017). Similarly, an increase in plasma T levels was observed in neonatal female rats exposed to BPS at 50 mg/kg-day by sc injection in the first 10 days of life and evaluated on the first day of estrus starting on PND 75 (Ahsan et al. 2018). Increased plasma T levels were also reported in adult female SD rats treated with BPS doses of 5 and 50 mg/kg-day by ip injection for 28 days (Ijaz et al. 2020). In the BPS gestational exposure study by Tucker et al. (2018) in mice, there were increased serum DHEA (T precursor) levels on PND 20, increased serum T levels on PND 28, in the 0.05 mg/kg twice a day dose group, and no effects on serum DHEA on PND 28 or later. However, there were decreased serum T levels on PND 35 (no effects on PND 56) in the 0.5 and 5 mg/kg twice a day dose groups, and decreased serum T and DHEA levels at eight months of age in the 0.5 mg/kg twice a day dose group, with no effects at 14 months of age (Tucker et al. 2018). Finally in the study by Téteau et al. (2022) in adult ewes, exposure to 50 µg/kg-day BPS for at least three months resulted in decreased levels of 11-dehydrocorticosterone and DHT and increased levels of 5β-dihydrotestosterone (T metabolite) in follicular fluid in the restricted feed group.

Estrogens: E2 and E1

Three studies in mice, one in rats and one study in ewes reported decreased E2 levels in different media. One study reported a decrease in serum E2 on lactation day (LD) 21 after BPS treatment at 2 or 200 µg BPS/kg-day during gestation and lactation in CD-1 mice (LaPlante et al. 2017). Mice treated during puberty for 4 weeks had decreased plasma E2 levels and, as reported in Section 4.2.2, decreased number of granulosa cell layers – the cells producing E2 – at BPS doses of 10 and 100 µg/kg-day and increased volume of antral follicles at 0.001 and 0.1 µg/kg-day (Nevoral et al. 2018). After a single sc injection to adult CF-1 mice, a decrease in urinary E2 levels was observed 10 hours after BPS treatment at doses of 25.2 or 79.8 mg/kg, and a dose-dependent decrease in serum ^{3}H-E2 levels was observed 90 minutes after treatment at doses of 78.5 or 252.7 mg/kg (Pollock et al. 2019). In adult female SD rats treated with BPS by ip injection for 28 days there was a decrease in plasma E2 levels at doses of 5 and 50 mg/kg-day and, as reported in Section 4.2.2, a decrease in the number of antral and atretic follicles was also observed at these doses (Ijaz et al. 2020). In the study by Téteau et al. (2022), in adult ewes treated with BPS at 50 µg/kg-day the plasma had decreased E2 and increased E1 levels in the restricted feed group, the oviduct fluid had decreased E2 and E1 in the well fed and restricted feed group, respectively, and the follicular fluid had increased E2 levels in the well fed group.

Increased E2 levels after treatment with BPS were reported in two studies in mice and three studies in zebrafish. Mice treated with BPS by sc injection (every three days) with 50 µg/kg or 10 mg/kg starting at birth for 60 days had increased serum E2 levels at PND 60 at both doses (Shi et al. 2017). In the gestational exposure study in mice by Tucker et al. 2018, serum E2 levels were increased on PND 20 in the 0.5 and 5 mg/kg twice a day dose groups. In zebrafish exposed to BPS at 10 or 100 µg/L for 75 days, increases in plasma E2 levels and vitellogenin (VTG, marker for estrogenic activity) production were observed (Naderi et al. 2014). In another study, an increase in plasma E2 levels and the E2/T ratio was reported in female zebrafish exposed to BPS at 50 µg/L, with no effect on plasma T levels (Ji et al. 2013). The final zebrafish study reported that after 21 days of BPS exposure there was an increase in whole-body E2 levels at all concentrations tested (8, 40, and 200 µg/mL) (Park et al. 2022).

No effects of BPS on serum or plasma E2 levels were reported in several studies. These included studies in mice that were exposed from GD 11 to birth at doses of up to 50 µg/kg-day and evaluated at three, six, or nine months of age (Shi et al. 2019), in mice that were exposed from GD 10.5 to GD 17.5 at doses of up to 5 mg/kg twice a day) and evaluated on PND 28, 35, or 56 (Tucker et al. 2018), and in adult mice orally treated with BPS at 300 µg/kg-day for 28 consecutive days (Yue et al. 2023b). Other studies observed no effects on plasma E2 levels in rats (unknown strain) treated with daily BPS sc injections of up to 50 mg/kg-day from PND 1 to PND 10 (Ahsan et al. 2018) and no effects on plasma or follicular fluid E2 levels in ewes treated orally with BPS at 4 or 50 µg/kg-day (Desmarchais et al. 2022). Finally, there was no effect on placental E2, or E1 levels in mice treated orally with BPS at 200 µg/kg-day for two weeks before mating (Mao et al. 2020).

Other hormones

Several studies assessed the effect of BPS exposure on levels of other hormones. In adult female CD-1 (ICR) mice, no effects on serum levels of free triiodothyronine or free thyroxine were observed after treatment with 300 µg/kg-day BPS for 28 days (Yue et al. 2023b).

In zebrafish, one study reported decreased plasma T3 and T4 levels after exposure to 100 µg/L BPS for 75 days (Naderi et al. 2014), while another study found a concentration-dependent increase in whole body T3 and T4 levels after BPS treatment of adult zebrafish for 21 days at concentrations of 8, 40, and 200 µg/mL (Park et al. 2022).

In mice, BPS treatment at 200 µg/kg-day for 2 weeks prior to mating and through GD 12.5 resulted in an increase in placental dopamine concentrations and in the percentage of dopamine-positive trophoblast giant cells, a decrease in placental

serotonin (5-HT) concentrations and in the proportion of serotonin-positive giant cells, and a substantial increase in the ratio of 5-hydroxyindoleacetic acid (primary 5-HT metabolite) to 5-HT (Mao et al. 2020).

In ewes orally treated with BPS at 4 or 50 µg/kg-day, there were no reported effects on plasma anti-müllerian hormone (AMH) levels (Desmarchais et al. 2022).

Steroidogenic enzymes

Steroidogenesis, i.e., the synthesis of steroid hormones from cholesterol, occurs through metabolic processes involving cytochromes P450 and hydroxysteroid dehydrogenases (HSD). Steroidogenesis can be modulated by many factors, including changes in gene expression, protein production and enzyme activity. Three studies were identified that assessed the effects of BPS on steroidogenic enzyme gene and/or protein expression.

In mice exposed to BPS during gestation from GD 11 to birth there was an increase in ovarian mRNA for *Cyp11a1* (P450scc) – the enzyme that catalyzes the initial step in steroidogenesis – at 3 months of age in the 0.5 µg/kg-day dose group (Shi et al. 2019), while no effect on steroidogenic enzyme mRNA expression was observed in mice exposed from birth to PND 60 (once every three days) at doses of up to 10 mg/kg (Shi et al. 2017).

In Wistar rats exposed to BPS during gestation and postnatally from birth through PND 21, there was a decrease in brain mRNA levels of 5α-reductase-3 – one of three isozymes involved in the conversion of T into the non-aromatizable androgen, DHT– with no changes observed in brain 5α-reductase protein levels for this isozyme (Castro et al. 2015).

Hormone receptors

Effects of BPS exposure on hormone receptors were analyzed in several studies. Results included effects on gene and/or protein expression of the P receptor (PR), estrogen receptors (ERs) and related growth factors, the androgen receptor (AR), and the PrlR and related factors.

Progesterone receptor

Increases in PR protein levels were observed in mammary glands from pubertal (PND 32-35) female CD-1 mice exposed to BPS during gestation and lactation via maternal oral administration (2 or 200 µg BPS/kg-day) from GD 9 to LD 20, but not from newly weaned (PND 24) or adult (9-week-old) mice (Kolla et al. 2018). Another study reported a decrease in mammary gland PR mRNA levels at 8 months of age in mice exposed to

5 mg BPS/kg twice a day during gestation (via maternal oral administration from GD 10.5 – GD 17.5), with no changes observed at the two lower doses (Tucker et al. 2018). No effects on mammary gland PR mRNA levels were found on PND 31 in CD-1 mice exposed to BPS during gestation and lactation (GD 8 to PND 2) via maternal oral administration (2, 200, or 2,000 µg/kg-day) (Kolla and Vandenberg 2019).

Estrogen receptor

In the gestational and lactational study in female CD-1 mice by Kolla et al. (2018), there was a NS increase in estrogen receptor alpha (ERα) protein in the mammary gland at puberty (PND 32-35), no effect at PND 24, and a decrease (200 µg/kg-day dose group only) at nine weeks of age. The gestational and lactational study in female CD-1 mice by Kolla and Vandenberg (2019) reported an increase in *Esr1* (*Erα* gene) mRNA levels in the mammary gland on PND 31 in the 200 µg/kg-day dose group, with no effect at other doses. This study also reported that the effect of BPS on mammary gland *Esr1* mRNA levels was not modified by a peripubertal estrogen (ethinyl estradiol, EE) challenge (Kolla and Vandenberg 2019).

In the mammary glands of adult female CD-1 mice orally treated with BPS at 2 or 200 µg/kg-day during pregnancy and lactation (GD 8 to LD 21) and assessed on LD 21, an increase in the percentage of Erα protein positive cells was observed at both doses. *Esr1* gene expression in the mammary gland was not significantly different from controls on LD 21 at 2 µg/kg-day; however, mRNA levels were lower in the 200 µg/kg-day dose group compared to the 2 µg BPS/kg-day dose group (LaPlante et al. 2017). In addition, BPS exposure had no effect on the number of ERα positive cells in the arcuate nucleus of the hypothalamus (LaPlante et al. 2017).

A study in female CD-1 mice exposed to BPS during gestation and lactation via maternal oral administration (200 µg BPS/kg-day from GD 8 to PND 19) reported non-significant increases in *Esr1* (Erα) and *Esr2* (Erβ) mRNA in the ovary and a non-significant decrease in Erα mRNA in the uterus on PND 22 (Hill et al. 2017).

Ovarian and uterine gene expression of epidermal growth factor-1 receptor (EGF-R) and insulin growth factor-1 (IGF-1) can be regulated by estrogens and were also assessed on PND 22. Ovarian EGF-R and IGF-1 mRNA levels and uterine IGF-1 mRNA levels were increased in the BPS exposed mice, compared to controls (Hill et al. 2017). The authors also investigated the effect of a peripubertal EE challenge on ovarian and uterine gene expression in BPS exposed mice: ovarian *Esr2* (ERβ) and EGF-R mRNA levels were decreased and *Esr1* (ERα) mRNA was non-significantly decreased in EE-challenged mice exposed to BPS, compared to EE-challenged controls. Uterine IGF-1 mRNA levels were increased in EE-challenged mice exposed to

BPS compared to EE-challenged controls, while no differences were observed in uterine *Esr1* (ERα) and EGF-R mRNA levels (Hill et al. 2017).

In adult female gerbils orally treated with BPS at 40 µg/kg-day per day for 28 days, an increase in the percentage of ERα positive cells was observed in the stroma of the female prostate, compared to controls (Silva et al. 2019).

In adult female zebrafish exposed to BPS for 21 days (8, 40, 200 µg/mL) a non-significant increase in hepatic ERα mRNA levels was reported at 8 µg/mL, with non-significant decreases reported by the authors at the two higher concentrations. [Note that the decrease at 200 µg/mL appears to be significant, based on visual inspection of Figure 2B in the publication.] BPS exposure had no effect on hepatic ERβ mRNA levels, while mRNA levels of the estrogen-responsive gene VTG were decreased at 200 µg/mL, with non-significant decreases in VTG mRNA observed at the two lower concentrations (Park et al. 2022).

Androgen receptor

In the adult female gerbil study by Silva et al. (2019) discussed above, an increase in the percentage of AR positive cells was observed in the epithelium and stroma of the female prostate.

Prolactin receptor and related factors

In the study by LaPlante et al. (2017) of adult female CD-1 mice treated with BPS during pregnancy and lactation and assessed on LD 21, mammary gland PrlR mRNA levels were increased at 2 µg/kg-day and non-significantly decreased at 200 µg/kg-day, and a non-significant decrease was observed in the number of mammary epithelial cells staining positive for signal transducer and activator of transcription 5 (stat5) protein, a marker of prolactin signaling and cell differentiation. BPS exposure had no effect on PrlR mRNA levels in the pituitary, or on the number of stat5 positive cells in the arcuate nucleus of the hypothalamus (LaPlante et al. 2017).

4.2.4 Puberty onset

Vaginal opening happens in concert with hormonal fluctuations in the hypothalamic-pituitary-gonadal (HPG) axis and occurs as early as PND 35 (five weeks) in mice and PND 32 in rats, and as late as PND 42 to PND 56 (six to eight weeks) in both species. This wide variation in pubertal timing underscores the need for concurrent controls in experimental designs. Accelerations or delays in pubertal timing, or deviations in HPG hormonal fluctuations when comparing treated versus untreated rodents may indicate

reproductive toxicity. Seven studies in rodents examined effects of BPS on puberty onset measured by vaginal opening.

Two studies in rats and one in mice observed delays in puberty onset after early life BPS exposure. One study treated newborn female rats with BPS by sc injections (0.5, 5, or 50 mg/kg-day) on the first 10 postnatal days and observed delays in vaginal opening in the 50 mg/kg-day dose group. These delays were accompanied by changes in plasma hormone levels, including decreases in gonadotropins and progesterone (Ahsan et al. 2018). Delays in vaginal opening (PND 33.2), relative to the control group, were also observed in female SD rats exposed during gestation and lactation via maternal gavage administration of 60 mg/kg-day BPS (from 10 weeks premating through mating, gestation, and lactation to PND 21), but not in rats exposed to lower or higher doses (i.e., 20 and 180 mg/kg-day) (BASF 2019a). Puberty onset in this study ranged from PND 28 to PND 40 (across all control and exposed animals) (BASF 2019a). A study in CD-1 mice reported a 2.5-day delay in vaginal opening following exposure during gestation (GD 11 to birth) via maternal oral administration of 20 µg/kg-day BPS, while puberty onset was 1-2 days earlier in the 0.5 µg/kg-day dose group, and no effects observed in the 50 µg/kg-day dose group (Shi et al. 2019).

Four studies reported no significant effects of BPS on puberty onset. These included a gestational exposure study in CD-1 mice (maternal gavage administration of 0.05, 0.5, or 5 mg/kg twice a day from GD 10.5 to GD 17.5) (Tucker et al. 2018), a gestational and early postnatal exposure study in CD-1 mice (maternal oral administration of 2, 200, or 2,000 µg/kg-day from GD 8 to PND 2) (Kolla and Vandenberg 2019), a study that treated CD-1 mice, starting as newborns, with sc injections of 50 µg/kg-day or 10 mg/kg-day BPS once every three days from PND 0 to 60 (Shi et al. 2017), and a lactational exposure study in ICR mice (maternal administration of 0.375 or 37.5 ng/mL BPS in drinking water from PND 0 to PND 15) (Nevoral et al. 2021).

4.2.5 Effects on estrous cycle

The rodent estrous cycle is a well-defined four-to-five-day cycle that includes four phases (proestrus, estrus, metestrus, and diestrus). The preovulatory gonadotropin surge occurs on the proestrus day followed by ovulation which occurs during estrus, the day that the female is receptive to mating. Altering the length of the estrous cycle, especially more days in diestrus, may impair overall female reproductive success. One study in mice and three in rats observed changes in estrous cyclicity after BPS exposure.

In mice, gestational BPS exposure (maternal oral administration of 0.5, 20, or 50 µg/kg-day from GD 11 to birth) resulted in irregular estrous cyclicity (several days in estrus

and diestrus) at all doses tested (Shi et al. 2019). In adult SD rats, there were increases in estrous cycle length observed after gestational and lactational exposure to BPS (treatment of dams with 180 mg/kg-day BPS by gavage for about 90 days from premating to PND 21) (BASF 2019a), increases in the number of days in diestrus after gestational and early postnatal lactational exposure to BPS (treatment of dams by gavage with 300 mg/kg-day for 40-46 days from premating to PND 3) Anonymous Study 12 as described in (ECHA 2019), and increases in estrous cycle length after gestational and lactational exposure to BPS (treatment of dams by gavage with 300 mg/kg-day for about 14 weeks from 6 weeks premating to weaning) Anonymous Study 14 as described in (ECHA 2019). No effect on the day of first estrus or on estrous cycle length was observed in mice (Tucker et al. 2018).

4.2.6 Mammary gland development

Gestation is a sensitive period for exposure because mammary gland development begins during this time. Development continues in puberty when production of ovarian E2 and P stimulate the growth of terminal end buds (TEB). In adulthood, TEBs regress and alveolar buds are formed, and in pregnancy, prolactin secreted from the pituitary gland stimulates development of lobuloalveolar units in preparation for lactation. In the reviewed studies, effects of BPS exposure included alterations in the development and retention of TEBs, development of alveolar buds, and mammary gland proliferation. In general, the reported effects of early life BPS exposure varied by timing of assessment of the mammary gland (e.g., pre-pubertal, pubertal, adult). Four studies investigated changes in mammary gland development in mice exposed to BPS during gestation (Tucker et al. 2018) or during gestation and lactation (Kolla et al. 2018; Kolla and Vandenberg 2019; LaPlante et al. 2017).

There were data to suggest that gestational and lactational exposure to BPS results in accelerated development of TEBs in pre-pubertal mice, while no effect of exposure was apparent in pubertal mice. One study observed dose-dependent increases in TEB counts on PND 20 in (pre-pubertal) CD-1 mice exposed during gestation (maternal administration of 0.05, 0.5, or 5 mg/kg twice a day from GD 10.5 to GD 17.5) (Tucker et al. 2018). A second study observed the following changes on PND 24 in (pre-pubertal) CD-1 mice exposed during gestation and lactation (maternal oral administration of 2 or 200 µg/kg-day from GD 9 to LD 20): larger TEB area in the low dose group; increased average TEB size in both dose groups; and non-significant increases in TEB count in both dose groups (Kolla et al. 2018). In this same study, no effects of early life BPS exposure were apparent on TEBs when mice were assessed during puberty (PND 32-35) (Kolla et al. 2018). A third study also reported no effects of early life BPS exposure (maternal oral administration of 2, 200, or 2,000 µg/kg-day from GD 8 to PND 2) on

number or total area of TEBs in mice assessed on PND 31 (Kolla and Vandenberg 2019).

Two of these studies examined TEB counts in adult mice, both showing increased retention of TEBs in adult mammary glands of mice with early life BPS exposure. Increased TEBs were observed at 3 months of age in mice exposed during gestation in the 0.5 mg/kg twice a day dose group (Tucker et al. 2018), and increased TEB-like structures were observed at 9 weeks of age in mice exposed during gestation and via lactation in both the 2 and 200 µg/kg-day dose groups (Kolla et al. 2018). The latter study also reported increased numbers of mammary gland alveolar buds in the high dose group and increased incidence of intraductal hyperplasia in both dose groups at 9 weeks (Kolla et al. 2018) while the former study observed increased incidence of mixed cell inflammation at 3 months of age (Tucker et al. 2018). At 14 months, there was increased incidence of all types of mammary gland inflammation, non-neoplastic lesions, and lobuloalveolar hyperplasia in the 0.5 mg/kg twice a day dose group (Tucker et al. 2018).

Additional effects on mammary gland development were reported in each of these studies. In the Kolla et al. (2018) study, Ki67 expression indicated a decrease in mammary gland cell proliferation at 2 µg/kg-day BPS on PND 24, a non-significant increase in proliferation at puberty in both dose groups and a significant increase in proliferation in adults at 2 µg/kg-day (Kolla et al. 2018). In the Kolla and Vandenberg (2019) study, a decrease in ductal area was observed in the 2 µg/kg-day dose group on PND 31 (Kolla and Vandenberg 2019). And in the Tucker et al. (2018) study, increases in the mammary gland developmental score were reported on PND 20 in the 5 mg/kg twice a day dose group, on PND 35 in all dose groups, and on PND 56 in the 0.5 mg/kg twice a day dose group, without any effects observed on mammary epithelial area (Tucker et al. 2018).

A fourth study, in CD-1 mice, evaluated not only the anatomical effects on the mammary gland of dams treated with BPS during pregnancy and lactation (2 or 200 µg/kg-day from GD 9 to LD 20), but also effects on nursing behavior. The authors concluded from histological examination of whole-mount mammary glands collected on either LD 2 or LD 21 that there was the suggestion of an overall treatment-related trend of increased functional mammary gland tissue at LD 2 and decreased functional mammary gland tissue on LD 21. A significantly reduced volume of lobules, a non-significant decrease in lobule size, and a significantly increased volume of adipose tissue were also reported in the high dose group on LD 21. Regarding nursing behaviors, on LD 14, dams treated with 2 µg/kg-day BPS spent increased time in the “high-crouch” lactating position compared to controls, while dams treated with 200 µg/kg-day BPS spent increased time

nursing and a decreased percentage of pups initiated nursing (control: 91%; 200 µg/kg-day: 50%) (LaPlante et al. 2017).

4.2.7 Reproductive performance

Reproductive performance or efficiency is closely related to fertility. Among anatomical and physiological features in female reproduction, the ovary plays an important role mostly because of the fixed number of oocytes at birth. After gamete production the reproductive process continues through mating, conception, implantation, and delivery of live offspring, where all these processes are closely controlled by the nervous and endocrine systems.

Reproductive performance was assessed in female CD-1 mice gestationally exposed to BPS (maternal administration of BPS at 0.5, 20, or 50 µg/kg-day from GD 11 to birth) at 3, 6 and 9 months of age. No effects of BPS exposure were observed at 3 months, while at 6 months "time to vaginal plug" (i.e., successful mating) was increased at all doses, with further increases in "time to vaginal plug" observed for all doses at 9 months (Shi et al. 2019). A decrease in the percentage of successful mating was reported in the low and mid dose groups at 6 months and in all dose groups at 9 months, with a greater decrease at 9 (60%) compared to 6 months (25%). In addition, a decrease in the number of pups born per litter was observed at 9 months in the mid dose group (Shi et al. 2019).

Reproductive performance was assessed in female rats exposed as newborns to BPS by sc injections (0.5, 5, 50 mg/kg-day) on the first 10 post-natal days. A decrease in the percentage of females that conceived and a decrease in the number of pups born per litter was observed in the high dose group (Ahsan et al. 2018). A non-significant decreased fertility index (58.3 % vs 91.7 %) was reported in adult SD rats treated with BPS at 300 mg/kg-day for a total of 40-46 days from premating to PND 3 Anonymous Study 12 as described in (ECHA 2019).

Three studies in mice reported no effects of BPS on reproductive performance. The first study treated adult female mice orally with BPS daily at 200 µg/kg-day (from before mating to GD 12.5) (Mao et al. 2020), and the second treated female mice with sc injections of BPS early in life from birth to PND 60 (every three days at 50 µg/kg-day or 10 mg/kg-day) (Shi et al. 2017). These studies assessed time to conception (Shi et al. 2017), pregnancy success (Mao et al. 2020), gestational length (Shi et al. 2017), maternal gestational weight (Mao et al. 2020; Shi et al. 2017), uterine and pup weights (Shi et al. 2017), implantation sites (Mao et al. 2020), and litter size (Mao et al. 2020; Shi et al. 2017). No effect on litter size or body weight was observed in the F2

generation of ICR mice in a study where BPS was administered orally to F0 dams on GD 12.5-15.5 at doses of 2, 10, 50, 100, or 200 µg/kg-day (Zhang et al. 2020).

One study in SD rats reported no effects of BPS on reproductive performance parameters. In rats treated orally during pregnancy on GD 6 to GD 21 with BPS at 5 µg/kg-day, no statistically significant effects were reported on dam body weight gain, proportion of dams with normal pregnancies or abortions, litter size, or offspring body weights and morphometric measurements. However, there were two stillborn pups (one each in two out of 13 litters) in the BPS group versus none in the control (Kaimal et al. 2021).

Female zebrafish exposed to BPS at 10 and 100 µg/L, starting as embryos, for 75 days produced fewer numbers of eggs during a 7-day spawning period and the eggs had a lower hatching rate with an increased time to hatching (Naderi et al. 2014).

BPS altered shoaling behavior, which is important during mating, of adult female zebrafish exposed to BPS at 100 µg/L for 14 days, such that females spent less time in the zone close to other fish and increased time far from other fish (Wang et al. 2020).

In *C. elegans* exposed to BPS at 125, 250 and 500 µM there was a concentration-dependent increase in embryonic lethality and a decrease in brood size at 125 and 500 µM (Chen et al. 2016b).

Effects on fertilization

A decrease in fertilization rate was reported in ICR mice treated with BPS in drinking water during puberty for four weeks at a dose of 10 µg/kg-day, with non-significant decreases observed at lower doses (0.001 and 0.1 µg/kg-day), consistent with a dose-related effect. However, a significant increase in fertilization rate was reported at 100 µg/kg-day (Nevoral et al. 2018). F1 oocytes from female mice that were treated with BPS at 2 or 10 µg/kg-day for 4 days from GD 12.5 to 15.5 were used for *in vitro* fertilization with sperm from untreated animals. Reproductive outcomes observed in this study included a dose-dependent decrease in the proportion of two- and four- cells embryos, and lower proportion of blastocysts (Zhang et al. 2020).

BPS also decreased the *in vitro* fertilization rate in mouse (strain not specified) oocytes harvested from females treated by ip injection for 21 days during adulthood with doses of 10, 50 and 100 µg/kg-day BPS (Nourian et al. 2017). Dose-dependent decreases in the percentages of two-cell embryos and blastocysts were observed. In addition, type I embryo arrests were increased at 50 and 100 µg/kg-day (Nourian et al. 2017). In adult ewes on either a restricted or well-fed diet, BPS treatment at doses of 4 or 50 µg/kg-day

for three months had no effect on early embryo development (e.g., 2-cells, 4-cells, or blastocyst), following *in vitro* fertilization (Desmarchais et al. 2022).

Effects on implantation

A decreased number of implantation sites was reported in two studies in adult female SD rats treated with BPS at 300 mg/kg-day by gavage, either from premating to PND 3 in Anonymous Study 12 as described in ECHA (2019) or for a total of approximately 14 weeks, from 6 weeks premating to weaning in Anonymous Study 14 as described in ECHA (2019). In the latter study, an increased rate of post implantation loss was also observed in the 300 mg/kg-day dose group in the Anonymous Study 14 as described in ECHA (2019). A dose-related decreased trend in the number of implantation sites, with no effect on fertility index, was reported in adult female SD rats treated by gavage with BPS at doses of 20, 60 or 180 mg/kg-day for about 90 days from premating through pregnancy (and weaning on PND 21) (BASF 2019a). No effects on mean number of implantation sites or percentage of post implantation loss was reported in adult female Wistar rats treated with BPS by gavage at doses of 30, 100, or 300 mg/kg-day during pregnancy (GD 6 to GD 19) and evaluated on GD 20 (BASF 2014b), or in mice treated orally with BPS at 200 µg/kg-day for 2 weeks prior to mating and through GD 12.5 (Mao et al. 2020).

4.2.8 Sex ratio

No effect on sex ratio was seen in the F2 generation of CD-1 mice in a study where BPS was administered orally to F0 dams on GD 11 to birth at doses of 0.5, 20, or 50 µg/kg-day (Shi et al. 2019) or in the F2 generation of ICR mice in a study where BPS was administered orally to F0 dams on GD 12.5-15.5 at doses of 2, 10, 50, 100, or 200 µg/kg-day (Zhang et al. 2020). In zebrafish exposed to BPS from 2 to 75 dpf at concentrations of 0.1, 1, 10, or 100 µg/L, sex ratio was skewed towards females at 10 and 100 µg/L BPS (Naderi et al. 2014).

Summary Table

Data discussed above on female reproductive toxicity from whole animal studies are summarized in Table 4.2.1 below. The results presented are statistically significant ($p<0.05$), unless otherwise stated (i.e., non-significant [NS] change). The studies are organized alphabetically by first author.

Table 4.2.1 BPS: Evidence on the female reproductive toxicity in animal studies

Study Design	Outcomes Assessed	Major Findings
Ahsan et al. 2018 Female rats (no strain provided), fifteen rats per group. Treatment: BPS (purity not reported) in castor oil, daily subcutaneous (sc) injections (50 µL total volume) at doses of 0, 0.5, 5. or 50 mg/kg-day for 10 days (postnatal day (PND) 1 to 10). Eight females/group were sacrificed on morning of the first estrus (starting on PND 75). Organs and blood samples collected for further analysis. Methods not very well described	Day of vaginal opening Start of estrous cyclicity (assessed from PND 60 to 70) Recorded weights of ovary and uterus on PND 75 Gonadosomatic index (GSI) = (100 × [gonad weight (g)/body weight (g)]) Starting on PND 75, blood was collected for plasma hormone levels of luteinizing hormone (LH), follicular stimulating hormone (FSH), testosterone (T), estradiol (E2), and progesterone (P) on the first estrus day. Histologic analysis of ovaries. Five adult females/group were assessed for fertility. Each female was mated with one fertile adult male: Litter size, litter weight and sex ratio were registered. General toxicity: Body weight	Puberty: Delayed vaginal opening at 50 mg/kg-day Decreased mean GSI at 50 mg/kg-day Decreased paired ovarian weight at 5 and 50 mg/kg-day Decreased absolute and relative weight of paired uteri at 50 mg/kg-day Hormonal analysis (at first estrus from PND 75): Decreased plasma levels of LH, FSH and P at 50 mg/kg-day Increased T levels at 50 mg/kg-day. No effect on E2 Histology: Decreased number of corpora lutea (CL) and antral follicles at 5 and 50 mg/kg-day Increased number of atretic follicles at 50 mg/kg-day, non-significant (NS) difference in the number of preovulatory follicles Estrus cycle: Altered estrus cyclicity at 50 mg/kg-day. Fertility: Percentage of females that conceived: 60% BPS and vs 100% in controls) Reduced number of pups born/female (5.33 ± 0.67) at 50 mg/kg-day (8.80 ± 0.58 in controls) General toxicity Increased body weight on PND 30, 45, and 60 at 50 mg/kg-day
BASF 2014a Female Wistar rats, 42 days old at the start of treatment, 10 rats per group. Treatment: BPS (99.4% purity) in water containing 1% carboxymethylcellulose (CMC) at a final volume of 10 mL/kg, daily oral gavage at doses of 0, 100, 300 or 1,000 mg/kg-day for a total of 90 days. Animals were sacrificed after 16 hours of fasting.	Reproductive organ weights Histopathology in ovary and uterus General toxicity: Body weight, organ weight, red blood cell (RBC) count	Increased ovarian weight at 1,000 mg/kg-day Squamous metaplasia of uterus at 1,000 mg/kg-day. General toxicity: No effect on body weight, increased adrenal and liver weight (absolute and relative), decreased RBC count, lower hematocrit at 1,000 mg/kg-day.
BASF 2014b Pregnant Wistar rats, 42 days old at the start of treatment, 25 rats per group. Treatment: BPS (99.3% purity) in water containing 1% CMC at a final volume of 10 mL/kg, daily oral gavage at doses of 0, 30, 100, or 300 mg/kg-day from gestational day (GD) 6 to GD 19. Animals were sacrificed on GD 20.	Number of corpora lutea Uterus weight Reproductive parameters: implantation sites, post implantation loss General toxicity: body weight	No effects on number of corpora lutea, gravid uterus weight, mean number of implantation sites or in % of post implantation loss General toxicity: Decreased net body weight gain at 300 mg/kg-day.
BASF 2019a)	F0: Estrous cycle duration, fertility index F1: Vaginal opening from PND 27	F0: Increased estrous cycle duration at 180 mg/kg-day F0: No effect on fertility index; decreased trend in number of implantation sites.

Study Design	Outcomes Assessed	Major Findings
Adult female Sprague-Dawley (SD) (Crl:CD) rats, 24 rats per group (F0 rats). Treatment: BPS (99.9% purity) in 0.5% CMC suspension, daily oral gavage (10 mL/kg-day) at doses of 0, 20, 60 and 180 mg/kg-day for about 90 days (from 10 weeks premating through mating, gestation, and lactation until weaning, PND 21). Mating of F0 females with treated males (with the same dose as females) after 10 weeks of treatment F0 females were sacrificed after weaning their offspring (PND 21).	General toxicity: Body weight	F1: Delay in vaginal opening (PND 33.2) at 60 mg/kg-day (range PND 28 – PND 40 for all doses) General toxicity: F0: Increased body weight at 60 and 180 mg/kg-day on days 14 and 28 of the premating period. No effect on the final body weight.
BASF 2020 SD female rats, five rats per group. Treatment: BPS (purity not reported) in 0.5 % methylcellulose in water, daily oral gavage (10 mL/kg) at dose of 0, 100, 300 or 600 mg/kg-day for 28 days. Animals were sacrificed at the end of exposure period.	Ovary and uterus weight General toxicity: Body weight	No effect on ovarian or uterus weight General toxicity: Reduced body weight at 600 mg/kg-day
Benjamin et al. 2023 7-month-old C57BL/6J female mice, five animals per group. Treatment: BPS (purity not reported) diluted in distilled water (vehicle). BPS at doses of 0 (control) or 30 mg/kg-day, daily by oral gavage for 21 days Estrous cycle monitored by daily vaginal smears and animals were sacrificed at the estrus after the 21-day treatment.	Mouse uteri were collected and fixed in 10% neutral buffered formalin for histopathological analysis (number of endometrial glands and uterine wall thickness)	Uterus histology: Developed non-atypical (increased number of normal cells) endometrial hyperplasia Increased thickness of the myometrium Increased number of glands in endometrium Adenomyosis (endometrial cells found in myometrium) in 40% of treated mice.
Castro et al. 2015 Female Wistar rats, ten rats per group. Treatment: BPS (98% purity) in 0.1% dimethyl sulfoxide (DMSO)/99.9% olive oil, daily sc injections at doses of 0 or 10 µg/kg-day from GD 12 to parturition. Female pups were treated similarly via sc injection from PND 1 to PND 21. Pups were sacrificed 30 min after last injection and brains saved for neuro-steroidogenesis analysis.	Effects on 5α-reductase (5α-R) isozymes (1, 2, and 3), dopamine, and serotonin (5-HT) related genes in the prefrontal cortex RT-PCR, western blot, and quantitative PCR array	Genes involved in steroidogenesis: No effect on 5α-R1 or 5α-R2 mRNA levels (no data on protein expression for these two isozymes) Decreased 5α-R3 mRNA levels, no effect on protein expression. Genes associated with the dopamine and serotonin systems: 5-HT: upregulation of 5 genes, including *Cyp2d4* (involved in 5-HT synthesis) and downregulation of 1 gene involved in metabolism and 2 genes involved in signal transduction pathways. Dopamine: NS downregulation of two dopamine receptor genes: *Drd1* and *Drd3*.
Chen et al. 2016b *C. elegans* hermaphrodite worms cultured at 20°C on nematode growth medium (NGM) plates without cholesterol, at least five worms per group.	Viable egg number and rate of embryonic lethality, number of adults (brood size), Germline effects	Female-like reproductive toxicity in hermaphrodites: No effect on the number of fertilized eggs Increased rate (5-fold) of embryonic lethality (dose-dependent) Decreased brood size (-25%) at 125 and 500 µM. Germline effects: Irregular distribution of germline nuclei

Study Design	Outcomes Assessed	Major Findings
Treatment: BPS (purity not reported) in 0.1% ethanol at 0, 125, 250, and 500 micromolar (µM) (internal concentrations for BPS were ND, 0.21 µg/g and 0.39 µg/g, respectively) Exposure from the L1 larval stage to adulthood: sodium hypochlorite-treated eggs plus BPS or control, followed by incubation at 20°C for 4 days; analysis at 20 and 24 hours post-L4 (first day of adulthood).		Dose-dependent germline nuclear loss Increased apoptosis and checkpoint activation at all doses Increased (about 3x) germline nuclear gap phenotype Alteration of SYP-1 disassembly (chromosome misalignment)
Demacopulo and Kreimann 2019 Immature Wistar rats (19–22 days old), five to six rats per group Exp 1. Treatment: BPS (purity not reported) in 20% ethanol / 80% sesame oil, daily sc injections (0.1 ml total volume) at 0 or 1 µg/kg-day for 20 days Exp 2. Polycystic ovary-like syndrome induction by dehydroepiandrosterone (DHEA). Treatment: daily sc injections of BPS at 1 µg/kg-day plus 60 mg/kg-day of DHEA for 20 days After treatment, estrous cycle day was determined for each animal and then euthanized for organ/tissue collection.	Estrous cycle stage Histologic analysis of ovaries and uterus Cell apoptosis, cell proliferation by measuring proliferation marker: proliferating cell nuclear antigen (PCNA), and EZRIN (marker of apicobasal polarity in endometrial cells) expression by immunohistochemistry	Exp 1. Ovary No difference in ovarian weight Ovaries had normal follicles and corpora lutea NS increase in ovarian cysts (no cysts in controls) Uterus NS effect on uterus morphology: decreased diameter and endometrial luminal height (increased variability in the data), increased cell vacuolization. Exp 2. BPS-DHEA interaction: Decreased average number of cysts in the BPS+DHEA group (compared to DHEA alone group) Increased intraepithelial lumens in the BPS+DHEA group (compared to DHEA alone group) Increased PCNA positive cells in endometrial perimeter in the BPS+DHEA group when compared to DHEA alone. Increased EZRIN expression in areas of the epithelium and increased apicobasal polarity of the epithelium in the BPS+DHEA group compared to DHEA alone.
Desmarchais et al. 2022 2.5 year old ewes (Ile-de-France) Study BPS and diet interaction. Two diet groups, restricted (RF) and well fed (WF), thirty ewes per group, further divided into ten animals per dose group. Treatment: BPS (purity not reported), daily in the diet (vehicle not reported) at doses of 0, 4 or 50 µg/kg-day for at least three months. Animals were estrous synchronized and stimulated for superovulation with FSH. Then, mature follicles were aspirated under anesthesia on all 60 ewes at 7-day intervals during the breeding season (September to December). The recovered cumulus–oocyte complexes (COCs) were used for *in vitro* maturation, *in vitro* fertilization (IVF), and embryo culture procedures. Oocytes were fertilized with sperm from untreated ram donors.	Plasma hormones measured: P and E2 (also in preovulatory follicular fluid), anti-müllerian hormone (AMH), and thyroid hormones, free triiodothyronine (FT3), free thyroxine (FT4) and total thyroxine (TT4) Embryo production data Metabolic effects: plasma glucose, and non-esterified fatty acid	Hormone levels: No effect of BPS on P or E2 levels in plasma or follicular fluid. No effect on plasma AMH, or any thyroid hormones. Ovary: No difference in the total number of follicles available for aspiration IVF: No significant effect on any of the parameters in terms of number of embryos and developmental rates, however, the following NS effects were observed: NS decreases (approximately 40%) at 4 µg/kg-day in the numbers of cleaved embryos, >4-cell embryos, blastocysts, and early blastocysts in the RF diet group. NS increases (approximately 200%) at 4 µg/kg-day in these parameters in the WF diet group. Significant diet and BPS dose interaction. There was a statistically significant interaction between dose and diet for the number and rate of cleaved embryos, the number of >4-cell embryos, the number of blastocysts and the number of early blastocysts.

Study Design	Outcomes Assessed	Major Findings
ECHA 2019 - Anonymous Study 12, 2000, (report following OECD TG 421 protocol) Adult female SD (F0) rats, 12 rats per group. Treatment: BPS (purity not stated) in 0.5 % aqueous sodium CMC solution with 0.1 % Tween 80, daily oral gavage at doses of 0, 10, 60 or 300 mg/kg-day for a total of 40-46 days (premating, gestation, and to lactation day (LD) 3).	Reproductive performance: Copulation, Implantation sites / index Reproductive tract weight Ovarian histology: number of corpora lutea General toxicity: Body weight	No effects on reproductive performance No effect on ovary or uterus weight No effect on number of corpora lutea Increased duration of estrous cycle (5.57 days vs 4.08 days); more days in diestrus (5 females vs 0 in control) at 300 mg/kg-day. Females in diestrus had a NS decreased fertility index (58.3 % vs 91.7 %) at 300 mg/kg-day Decreased implantation index at 300 mg/kg-day. General toxicity: Decreased final body weight at 60 mg/kg-day.
ECHA 2019 - Anonymous Study 14, 2017 Adult female SD rats, 10 rats per group. Treatment: BPS (purity not reported) in 0.5% CMC daily oral gavage (vehicle and route was according to other study in the report – Anonymous Study 16, 1999) at doses of 0, 30, 100 or 300 mg/kg-day for about 14 weeks (6 weeks premating, 2 weeks mating, gestation, and lactation)	Organ weights Estrus cyclicity Reproductive performance General toxicity: Mortality, body weight	(All effects at 300 mg/kg-day) No changes in ovarian weight Increased uterus weight Longer estrous cycles Decreased number of implantation sites Increased rate of post implantation loss General toxicity: No premature death, decreased body weight by 6% at 300 mg/kg-day
ECHA 2019 - Anonymous Study 16, 1999 Adult female SD rats, 6 rats per group. Treatment: BPS (purity not reported) in 0.5% CMC, daily oral gavage at doses of 0, 40, 200 or 1,000 mg/kg-day for a total of 28 days. Animals sacrificed at the end of dosing period	Ovary weight General toxicity: Body weight	No effect on ovary weight. No data reported on other reproductive organs General toxicity: Decreased body weight gain
Eldefrawy et al. 2021 Adult female Hy-Line W-36 laying chickens, six animals per group. Treatment: BPS (purity not reported) in corn oil, daily by gavage (from a 300 µg/mL solution) at doses of 0, or 50 µg/kg-day for 3 months.	Organ weight of ovary, oviduct, and liver after necropsy Histopathology: Numbers of preovulatory follicles and diameters of the five largest preovulatory follicles (F1, F2, F3, F4, and F5) were recorded. Oophoritis was scored using a 1–5 scale according to lymphocyte or plasma cell infiltration. RNA isolation and RNA-sequencing (RNA-seq) in theca and granulosa layers from the largest mature preovulatory follicle (F1) Bioinformatics analysis of RNA seq data and pathway analysis	Increased left ovary weight (the right ovary in chickens regresses and is not functional). Decreased numbers of preovulatory follicles. Lymphocytes present around the growing follicle, with ovarian inflammation (oophoritis). No oophoritis observed in controls. DEGs in largest mature preovulatory follicle: Examples of downregulated genes: STAR (Steroidogenic acute regulatory protein), CHRNA4 (Cholinergic receptor nicotinic alpha 4), ZAR1L (Zygote arrest 1 like), and LOC416931 (uncharacterized protein). Among the examined hens, only one gene was upregulated: AVD (avidin gene, can affect ovarian function)

Study Design	Outcomes Assessed	Major Findings
	RNA seq analysis was used to identify Differentially expressed genes (DEGs).	
Gingrich et al. 2018 Pregnant sheep (cross between Polypay and Dorset breeds), six to seven animals per group Treatment: BPS (purity not reported) in corn oil, daily sc injection (injection volume not reported), at doses of 0, or 0.5 mg/kg-day from GD 30 to 100 Biweekly serum samples from GD 30–120 for hormonal, protein, and biochemical analyses Animals were sacrificed, and placenta samples were collected on GD 120.	Placental endocrine function Pregnancy-associated glycoprotein 1 and pregnancy-specific protein B Serum P Placental morphology	Decreased serum maternal pregnancy-associated glycoprotein1 on GD 75 to GD 90 Decreased serum pregnancy-specific protein B on GD 60, 75, and 90 NS decrease in serum P at GD 75-105 (P=0.09) Placenta NS increase in placental weight (P=0.07) No effect on placental tissue distribution, or number of placentomes per placenta 50% lower e-cadherin protein expression in GD 120 placentomes 20% decrease in trophoblast derived binucleate cells Fusogenic genes: Decreased expression of enJSRV and HYAL2 Increased transcription factor glial cell missing factor 1.
Hill et al. 2017 Adult female CD-1 mice, five dams per group Treatment: BPS (99% purity) in tocopherol-stripped corn oil, orally in animals trained to drink the BPS containing solution (1 µL/g body weight) at a dose of 0 or 200 µg/kg-day from GD 8 until PND 19. On PND 19 (prepubertal), two female offspring from each litter were weaned from their mothers. One female was fed tocopherol-stripped corn oil (control) and the second was fed 1 µg/kg-day ethinyl estradiol (EE) in stripped corn oil from PND 19 to PND 21. Animals were sacrificed 24h after. Uterine responsiveness to EE was confirmed visually via change in uterine size on PND 22.	On PND 22, female reproductive tissues including the ovary, oviduct and uterus were collected. All organs were examined for gross malformations including ovarian cysts. Expression of several estrogenic activity related genes was measured in the uterus and ovary: Esr1 (estrogen receptor α (ERα)), Esr2 (ERβ), Igf-1 (insulin growth factor 1) and Egf-r (epidermal growth factor 1) BPS and EE interaction	No effect on tissue organization of oviduct or uterus including endometrial cell height. Uterus: NS decrease in Ki67 (marker for cell proliferation), or in cells undergoing apoptosis in the uterine stroma or epithelium Ovary: Increase in number of secondary follicles No effects on Ki67 or on apoptosis in granulosa or theca cells. Gene expression (mRNA levels): Uterus: increased Igf-1; NS decrease of Esr1; no effect on Egf-r Ovary: increased Igf-1 and Egf-r NS increase of Esr1 and Esr2 BPS and EE challenge: BPS + EE increased uterine epithelial height compared to unchallenged controls BPS inhibited the EE-induced increases in the number of total follicles. BPS + EE increased theca cells apoptosis compared to EE vehicle control BPS + EE increased uterine IGF-1 mRNA levels, compared to EE-challenged controls, BPS inhibited the effect of EE on expression of IGF-1 (NS decrease) and EGF-R (decrease), and on the Esr1 (NS decrease) and Esr2 (decrease) in the ovary.
Ijaz et al. 2020 Adult female SD rats, ten animals per group. Treatment: BPS (99% purity) in saline, daily intraperitoneal (ip) injection (1 mL/kg final volume) at doses of 0, 50, 500 µg/kg-day and 5, 50 mg/kg-day for 28 days On day 29, animals were sacrificed at the estrus day of the cycle	Ovaries and uteri weights; GSI Plasma concentration for T, E2, P, luteinizing hormone (LH) and follicle stimulating hormone (FSH) Biochemical and histological analysis of ovary and uterus Determination of oxidative stress in ovary: catalase (CAT), peroxidase, superoxide dismutase (SOD) activity, reactive oxygen species (ROS) assay, and thiobarbituric acid	Decreased GSI at the high dose (50 mg/kg-day) Hormones: Increased plasma T at 5 and 50 mg/kg-day Decreased plasma E2, P, LH, FSH at 5 and 50 mg/kg-day Ovaries: Decreased paired ovarian weight at 50 mg/kg-day Decreased number of CL at doses ≥ 500 µg/kg-day; increased CL diameter at 50 mg/kg-day Decreased number of antral and atretic follicles at 5 and 50 mg/kg-day Increased antral follicle diameter at all doses Decreased granulosa cell height at 5 mg/kg-day, and increased at 50 mg/kg-day No effect on theca cell height Uterus: decreased relative uterus weight at 5 and 50 mg/kg-day

Study Design	Outcomes Assessed	Major Findings
	reactive substance (TBARS)-lipid peroxidation assessment	Oxidative stress: No significant change in peroxidase activity Decrease in: CAT (U/mg protein) at 50 mg/kg-day SOD (U/mg protein) at 50 µg/kg-day and at 50 mg/kg-day Increase in: ROS (U/min tissue) at 500 µg/kg-day and at 5 and 50 mg/kg-day TBARS (nmol/mg tissue) at 50 mg/kg-day
Ji et al. 2013 Adult zebrafish (AB strain), four male and six female fish (and two replicates) per group. Treatment: BPS (purity not reported) in 0.1% methanol in 6 L exposure medium (filtered water) to a final concentration of 0.5, 5, and 50 µg/L for 21 days. After the exposure, fish were euthanized, and organs were collected for further analysis.	GSI, hepatic somatic index (HSI) = (100 × [liver weight (g)/body weight (g)]) Brain-somatic index (BSI) = (100 × [brain weight (g)/body weight (g)]) Plasma hormones: E2 and T Transcription of 21 genes related to functional processes of the hypothalamic-pituitary-gonadal axis (brain and ovary)	Decreased GSI at all doses, NS decreased BSI and NS increased HSI. Plasma hormone levels: Increased E2 at 50 µg/L, no effect on T and increase in E2/T ratio at 50 µg/L. Down-regulation of gene expression at 50 µg/L: Brain: *gnrh3* (gonadotropin releasing hormone) and *fshβ.* Ovary: *hmgr a* and *hmgr b* (Hydroxy methyl glutaryl CoA reductase). No effect on aromatase gene expression or on other genes analyzed.
Kaimal et al. 2021 Adult female Sprague-Dawley rats, at least nine animals per group Treatment: BPS (98% purity) in phosphate buffered saline (PBS), daily oral by micro pipetting the solution into the mouth (about 15 µL total volume) at doses of 0, or 5 µg/kg-day for 15 days (GD 6 to 21) F1 female rats were sacrificed at 16 to 24 weeks of age on the day of diestrus.	Number of pregnant dams, abortions per dam, stillbirths, and live births (gestational index In F1: Organ weight for pituitary, ovary, and uterus Morphometric analysis of ovaries: Follicle characterization	No effects on proportion of dams with normal pregnancies or abortions, litter size, or offspring body weight Two stillborn pups (one each in two out of 13 litters) and none in the control group F1: No effect on pituitary or ovary plus uterus weight. F1: Decreased number of CL in offspring ovaries; no effects on number of follicles at any stage (primordial, primary, secondary, antral, Graafian, or atretic).
Kolla et al. 2018 Pregnant CD-1 mice, at least eight animals per group Treatment: BPS (purity not reported) in 70% ethanol applied and allowed to dry on a small wafer, fed daily to dams at doses of 0, 2 or 200 µg/kg-day from GD 9 to LD 20 One female from each litter was selected for necropsy at each age: PND 24 (prepubertal), PND 32–35 (pubertal), and 9 weeks of age (adult)	Pregnancy loss Mammary gland analysis: Whole mount morphometric and histological analyses, and immunohistochemical analyses for Ki67 (marker of proliferation), and protein expression of ERα and progesterone receptor (PR)	Dams delivered naturally No effect on pregnancy loss Mammary gland morphology At PND 24: Larger TEB area in the low dose group, increased average TEB size in both dose groups At puberty: No significant effect at any dose At adulthood: Increased number of alveolar buds at 200 µg/kg-day; increased terminal ends at 2 µg/kg-day; increased incidence of TEB-like structures at both doses. Ki67 expression At PND 24: Decreased at 2 µg/kg-day (NS decrease at 200 µg/kg-day) At puberty: NS increase at both doses.

Study Design	Outcomes Assessed	Major Findings
		At adulthood: Increased at 2 μg/kg-day, no effect at 200 μg/kg-day. Increased incidence of intraductal hyperplasia at both doses (related to the K67 increase) Mammary gland steroid receptor protein expression At PND 24: No effects on ERα or PR At puberty: Increased expression of PR at both doses; NS increase in ERα at both doses. At adulthood: Decreased ERα at 200 μg/kg-day No effects on PR expression
Kolla and Vandenberg 2019 Pregnant CD-1 mice, at least seven animals per group Treatment: BPS (purity not reported) in tocopherol stripped corn oil, daily oral administration to dams with a pipette to the mouth (1 μg oil/g body weight) at doses of 0, 2, 200, or 2,000 μg/kg-day from GD 8 to PND 2 EE challenge: Two female pups per litter from each of the experimental groups described above were treated with EE at doses of 0, or 1 μg//kg-day, for 10 days (from PND 21 to PND 30) Animals were sacrificed on PND 31	Time to vaginal opening; anogenital index (AGI); uterus weight Mammary gland histology. Gene expression for ER and PR	NS effect on time to vaginal opening, AGI, or uterine weight. BPS effects on mammary gland: Decreased ductal area at 2 μg/kg-day No effect on the number or total area of TEBs Increased gene expression of *Esr1* (ERα) at 200 μg/kg-day No effect on *Pgr* (PR) gene expression. EE – BPS interaction: No effect of EE challenge on vaginal opening, AGI, uterine weight, mammary gland ductal area (BPS effects remained), number or total area of TEBs. No effect of EE challenge on gene expression of *Esr1* (BPS effects remained).
LaPlante et al. 2017 Timed pregnant CD-1 mice, at least 5 animals per group. Treatment: BPS (> 99% purity) in 70% ethanol applied and allowed to dry on a small wafer, fed daily at doses of 0, 2 or 200 μg/kg-day. Exp 1: Exposure from GD 8 until weaning on LD 21. Dams were euthanized on LD 21. Exp 2: BPS from GD 9 through LD 1. Dams euthanized on LD 2.	Histoarchitecture in dams whole-mount mammary gland on LD 2 and 21. On LD 21: Mammary gland receptor protein expression by immunohistochemistry: Prolactin receptor (PrlR), signal transducer and activator of transcription 5 (Stat5, a PrlR signaling marker), ERα, and Ki67 Mammary gland gene expression: Prlr and Esr1 Serum E2 Brain immunohistochemistry: ER, Stat5 and PrlR in the arcuate nucleus of the hypothalamus Nursing behaviors on LD 2, 7, and 14 General toxicity: Dam and pup body weights	Whole-mount mammary gland Trend to increased functional mammary tissue at LD 2, and decreased functional mammary gland tissue on LD 21 LD 2: NS increase in lobule size (23% at 2 μg/kg-day; 30% at 200 μg/kg-day). LD 21: Decreased volume fraction of lobules, and increase in the volume fraction of adipose tissue at 200 μg/kg-day NS decrease in lobule size (8% and 24% at 2 and 200 μg/kg-day, respectively) Prolactin signaling: NS decrease in Stat5 positive epithelial cells Increased *Prlr* gene expression at 2 μg/kg-day and NS decrease at 200 μg/kg-day. Gene and protein expression on LD 21: *Esr1*: NS increase in mRNA at 2 μg/kg-day (compared to control) and decrease at 200 μg/kg-day (compared to the 2 μg/kg-day dose group). No effect on number of ERα positive cells in the arcuate nucleus of the hypothalamus Ki67: No effect on protein expression Endocrine effects on LD 21: Decreased serum concentrations of E2 at both doses Increased ERα protein expression at both doses No effects on Prlr, or Stat5 positive cells in the brain. Nursing behavior: No effect on the time spent nursing at LD 2 and LD 7 Increased time nursing on LD 14 at 200 μg/kg-day Increased time in the high-crouch position on LD 14 at 2 μg/kg-day

Study Design	Outcomes Assessed	Major Findings
		Decreased percentage of pup-initiated nursing on PND 14 (91% in controls; 50% in 200 µg/kg-day dose group) General toxicity: Decreased pup wt on PND 14 at 2 µg/kg-day; NS decrease at 200 µg/kg-day.
Liu et al. 2021 Newborn CD-1 mice, number of animals not reported but the statistical analysis section states that each experiment was repeated at least three times. Treatment: BPS (purity not reported) in saline solution, daily ip injection (volume not specified) at doses of 0, 2 or 10 µg/kg-day for 3 days Animals were sacrificed after treatment (not clear how long after treatment) and ovaries saved for follicle analysis. Another set of animals (with the same treatments) were sacrificed at PND 21.	Ovaries were collected and oocytes in germ cell nests (or cysts) and primordial follicles were counted using mouse vasa homolog (MVH)—a germ cell marker. Immunofluorescence and immunohistochemistry for proliferation markers Folliculogenesis at PND 21 and measurement gene and protein expression of GDF9 and BMP15 (oocyte specific markers)	Dose-dependent increase in the percentage of MVH-positive oocytes in primordial follicles Decreased percentage of oocytes in germ cell cysts. No effect on total number of oocytes Increased number of cells positive for Ki67 (proliferation marker) at both doses. Dose dependent increase in proliferation of granulosa cell precursors. Folliculogenesis at PND 21 Increased number of primary follicles and decreased number of secondary and antral follicles at 10 µg/kg-day. Decreased gene and protein expression of GDF9 at 10 µg/kg-day and BMP15 at both doses.
Mao et al. 2020 Female C57BL6J mice, at least six animals per group. Exposure: BPS (> 98% purity) in 70% ethanol (8 to 24 µL/kg) applied and allowed to dry on a small wafer, fed daily at doses of 0, or 200 µg/kg-day for two weeks. After treatment, females were paired with untreated males. Euthanized on GD 12.5; each uterine horn was incised; the uterine position of each conceptus was delineated, and placenta samples were obtained and saved half in liquid nitrogen and the other half was fixed for histological analyses.	Pregnancy outcomes Placental histology: measurement of the labyrinth, spongiotrophoblast, and trophoblast giant cell (GC) areas DEGs analyses in placental tissues on GD 12.5 Metabolomics: Effects on fatty acids Neurotransmitters: Effects on 5-HT and dopamine tissue distribution by immunohistochemistry Placental hormone levels: E2, estrone (E1), Corticosterone, T, and P Placenta morphometric analyses	No effects on pregnancy success, maternal gestational weight, implantation sites, and number or sex ratio of fetuses Effects in GD 12.5 placenta: Decreased ratio of spongiotrophoblast zone to GC area. DEGs: 11 genes were differentially expressed with upregulation of Actn2 (actinin α2) with a 2.17-fold change. The other 10 genes were downregulated, such as Calm4 (calmodulin 4) with -22.52-fold change and Guca2a (guanylate cyclase activator 2a, guanylin) with -26.72-fold change. Metabolomics: Effect on fatty acids: Decreased stearic acid, palmitic acid, docosahexaenoic acid, octadecenoic acid, and hexadecanoic (palmitic) acid. Neurotransmitters: Increase in dopamine concentration Increased percentage of dopamine-positive GCs. Decrease in serotonin concentration and the percentage of serotonin-positive GCs Ten-fold increase in the ratio of 5-hydroxyindoleacetic acid (primary 5-HT metabolite) to 5-HT. Decreased percentage of serotonin-positive GCs. Placental steroids: NS effect on any steroid hormone analyzed
Naderi et al. 2014 Zebrafish (wild-type) embryos at blastula stage 2 days post fertilization (dpf), 700 embryos in total. Treatment: BPS (> 98% purity) in 0.01% acetone was added to 5 L tanks to a final concentration of 0, 0.1, 1, 10 and 100 µg/L (50% of exposure solution was renewed daily) for 75 days Males and females from the same treatments were randomly assigned to spawning tanks; 2 males per 2 female colonies)	Fish development, reproduction, plasma vitellogenin (VTG), GSI, and HSI Plasma hormones: E2, T, T4 and T3 Numbers of eggs spawned Hatching rate and time to hatching (hours) General toxicity: Survival rate, body weight and length	Skew in the sex ratio towards females in adults: percentage of females at 10 and 100 µg/L BPS was 58.8% and 66.7%, respectively (46.3% in the control) Decreased GSI at 100 µg/L (in females), (males lower at 10 µg/L) Increased HSI at 10 and 100 µg/L Increased VTG induction at 10 and 100 µg/L Endocrine effects: Increased plasma E2 at 10 and 100 µg/L Decreased plasma T3 and T4 at 100 µg/L Reproductive effects:

Study Design	Outcomes Assessed	Major Findings
Blood samples from 4 to 6 fish of the same sex and same treatment were pooled to generate a total of three replicate pooled blood samples for each treatment.		Decreased number of eggs during the 7-day spawning period at 10 and 100 µg/L. Lower hatching rate and increased time to hatching of embryos at 10 and 100 µg/L. General toxicity: Decreased survival rate at 100 µg/L, no effect on body length or body weight.
Nevoral et al. 2018 Female ICR mice at puberty (five weeks-old), at least 4 animals per group. Treatment: BPS (purity not reported) in drinking water (solvent vehicle not reported) at doses of 0, 0.001, 0.1, 10, or 100 µg/kg-day for 4 weeks Seven to eight animals were sacrificed at the end of treatment, ovaries were fixed for histological evaluation (left ovary) and proteomic analysis (right ovary). Other group of animals was treated with gonadotropin to retrieve the mature oocytes or mated with untreated males for embryo analysis.	Ovarian histology on seven left ovaries: primary, preantral, antral and atretic follicles and their volumes Ovaries from BPS treated at (0.1 µg/kg--day) were used for proteomic analysis by liquid chromatography-mass spectrometry. Number and percentage of matured oocytes were counted and used for immunocytochemistry for α-tubulin, pericentrin, H3K27me2, and methyl cytosine (5meC). Embryo quality assessed after *in vivo* fertilization. Serum hormone levels	Decreased relative ovary weight at all doses and total ovarian volume at 10 and 100 µg/kg-day. Decreased medulla volumes at all doses Decreased corpora lutea volume (no data presented) Increased volume of antral follicles at 0.001 and 0.1 µg/kg-day Decreased volume of atretic follicles at 0.1 and 10 µg/kg-day Dose-dependent decreased numbers of primary, preantral, and antral follicles Decreased quantity of granulosa cell layers and decreased serum E2 at 10 and 100 µg/kg-day Increased incidence of spindle malformation at 1 and 100 µg/kg-day associated with α-tubulin and pericentrin alterations Increased numbers of abnormal oocytes at 0.1 and 10 µg/kg-day NS increase in number of metaphase II (MII) oocytes retrieved (flushed). Increased methylation of H3K27em2 at 10 and 100 µg/kg-day; no effects on 5meC Decreased fertilization rate in apparent dose-dependent manner at the three lower doses but only significant at 10 µg/kg-day Increased fertilization rate at 100 µg/kg-day
Nevoral et al. 2021 Pregnant ICR mice, at least 4 animals per group. *In vivo* (lactational exposure) Treatment: BPS (purity not reported) in 0.1% ethanol added to drinking water at a concentration of 0, 0.375 ng/mL or 37.5 ng/mL to a calculated dose of 0, 0.2 or 20 µg/kg-day during lactation for 15 days (PND 0 to PND 15). Assessments on F1 female ovaries isolated on PND 15 and PND 60 *In vitro* maturation studies Immature oocytes at the germinal vesicle (GV) stage (from F1 females treated perinatally) that were in proestrus or on estrus day (ovulation). GV-oocytes were used for *in vitro* maturation to obtain matured MII oocytes. Another group of F1 females were gonadotropin stimulated to retrieve mature oocytes that were used for *in vitro* fertilization or immunocytochemistry.	AGD and day of vaginal opening (puberty) Histology on PND 15 and 60 ovaries Stereology and follicle classification count (primordial, primary, preantral, antral, and atretic follicles) *In vitro* GV oocyte maturation Immunocytochemistry: α-tubulin for spindle visualization, and demethylated histone H3 on lysine K27 (H3K27me2) heterochromatin marker were measured in matured oocytes (*in vitro* and *in vivo*) Parthenogenetic activation: *in vivo*-matured oocytes without the cumulus cells. Cultured for 24 hours (cleaved embryos) and 96 hours (blastocysts)	No effect on: AGD, or day of vaginal opening Primordial follicle reserve in young ovaries (not measured on PND 60) Primary, preantral, and antral follicles pool on PND 60 Oocyte maturation: No effects on number of GV oocytes per ovary, germinal vesicle breakdown, maturation stage, number of flushed *in vivo* matured oocytes, and the number of atretic or fragmented oocytes. Immunocytochemistry: Increased occurrence of spindle malformation and frequency of chromosome misalignment in *in vitro*-matured oocytes at 0.2 µg/kg-day Decreased H3K27me2 in mature ovulated oocytes at 0.2 µg/kg-day *In vivo* mature oocytes: No effect on number of flushed oocytes Increased proportion of oocytes with spindle damage (α-tubulin) and chromosome misalignment at 0.2 µg/kg-day No effect on histone heterochromatin marker methylation status (H3K27me2) Parthenogenetic activation: Increased rate of activated eggs and increased cleavage rate; decreased blastocyst rate at 0.2 µg/kg-day
Nourian et al. 2017 Adult, same cycling stage, female mice (strain not specified), at least five animals per group.	Ovaries were used for biochemical analyses, and measurement of lipid peroxidation, using malondialdehyde (MDA) as an indicator.	Decreased fertilization and blastocyst development rates at 10, 50 and 100 µg/kg-day. Dose-dependent decreases in the percentages of two-cell embryos and blastocysts Increased type I embryo arrests at 50 and 100 µg/kg-day

Study Design	Outcomes Assessed	Major Findings
Treatment: BPS (99% purity) in ethanol (ethanol % and vehicle not specified), daily ip injections (volume not specified) at doses of 0, 1, 5,10, 50 or 100 µg/kg-day for 21 consecutive days. 24 hours after the last treatment, five mice in each group where euthanized and the ovaries were collected. Oocytes collected for IVF assays were obtained from five super-ovulated female mice randomly selected from each group. Mouse caudal epididymis sperm were capacitated and used for metaphase II arrested oocyte insemination.	Zygotes were evaluated 24 hours, 48 hours, and five days after insemination.	Lipid peroxidation: dose dependent increase in MDA
Nourian et al. 2020 Adult, regular estrus cycle, female NMRI mice, at least 5 animals per group. Treatment: BPS (99% purity) in PBS solution (0.1 to 0.5% ethanol), daily sc injections (volume not specified) at doses of 0, 1, 5, 10, 50 and 100 µg/kg-day for 21 consecutive days Blood samples from five mice per group were collected at one day after last treatment. Animals were sacrificed and ovaries collected for analysis. IVF: BPS treated females were super-ovulated by gonadotropin treatment (5 mice per group) and oocytes were inseminated with capacitated sperm from one untreated mouse. Blastocysts (10-20 per group) were collected four days later	Serum hormone levels of LH and FSH Ovary RNA quantity and integrity Oxidative stress: total antioxidant capacity, SOD, glutathione peroxidase, CAT IVF embryos Gene expression: estrogen signaling pathway (Erα and Erβ) and apoptosis induction pathway (P53, Bax, Nrf2 and E2F1)	Dose-dependent decreased serum LH and FSH levels Ovary: Induced oxidative stress at all doses (except for glutathione peroxidase that is significant at doses ≥ 5 µg/kg-day) Blastocysts: Increased gene expression of *P53, Bax, E2f1, Erα, and Erβ* at doses > 5 µg/kg-day Decreased gene expression of P53 and F2f1 at 50 and 100 µg/kg-day IVF parameters: Positive correlation with antioxidant enzymes but not MDA, the marker for lipid peroxidation Negative correlation with all genes except *ERα* Positive correlation for LH, FSH, *ERα* Hatch percentage: associated with Nrf2, E2f1, MDA, and CAT
Pal et al. 2023 Adult female golden hamsters, 12 animals per group Treatment: BPS (purity not reported) dissolved in corn oil, daily oral exposure at doses of 0 (vehicle), or 150 mg/kg-day for 28 days Twenty-four hours after treatment, animals were sacrificed. Serum samples were used for further analysis. Ovaries of one side: weighed and fixed for histological and immunohistochemical studies. The other ovaries were used for western blot, qPCR and biochemical analyses.	Serum hormone levels: E2, P, LH, FSH, T3, T4, melatonin Serum inflammatory factor levels: TNFα, CRP, and nitrite–nitrate Histology in one ovary Immunohistochemistry for PCNA Western blot in ovary: ERα, aromatase, and TRα; Silent information regulator 1 (SIRT-1), FOXO-1, Nrf2, PI3K and pAkt, PCNA, connexin-43 qPCR in ovary: Melatonin receptor (MT-1), SIRT-1 [involved in redox balance and ovarian functions], NFkB [involved in redox and inflammatory function in ovary], and inducible nitric oxide synthase	Serum hormone levels: Decrease in LH, FSH, E2, P4, T3, T4, and melatonin Increase in TNFα, CRP, and nitrite–nitrate. Ovarian histology: Decreased number of growing follicles and CL; increased number of atretic follicles. Immunohistochemistry: Low immunoreactivity signal for PCNA Western blot analysis in ovary: Decreased expression of ERα, aromatase, and TRα Decreased expression of SIRT-1, FOXO-1, Nrf2, PI3K and pAkt Increased expression of NFkB, COX-2, caspase-3 Decreased PCNA and connexin-43 qPCR in ovary: Decreased mRNA levels for MT-1 and SIRT-1

Study Design	Outcomes Assessed	Major Findings
	Ovarian oxidative load General toxicity: Body and organs weight	Ovarian oxidative load: Decrease in SOD and CAT Increase in lipid peroxidation. General toxicity: Increased body weight and decrease in relative ovarian weight.
Park et al. 2022 Zebrafish (AB strain), 20 embryos per replicate and five female fish per group depending on experiment. Exp 1: Embryo-larval toxicity tests on normal fertilized embryos at 4–6 hpf (20 embryos per replicate) in 60 x 15 mm cell culture dishes. Treatment: BPS (purity not reported) in 0.1 % DMSO, (15 mL final volume) at concentrations of 0, 1.6, 3.1, 6.3, 12.5, 25, 50, 100, 250, 500, 1,000 µg/mL for seven days. Exp 2: Short-term reproductive toxicity test. Female fish (AB strain, 3–4 months old), five female fish per group. Treatment: BPS (purity not reported) in 0.1 % DMSO added to water (350 mL final volume) at concentrations of 0, 8, 40, 200 µg/mL (fresh test solution at 3-day intervals) for 21 days. After the treatment fish were recovered for further analysis.	Fish were sampled and prepared to determine gonadal and liver development: GSI, and HSI, hepatic VTG and ER mRNA levels, Steroid and thyroid hormones (T3 and T4), and residual BPS concentration General toxicity: Embryo-larval toxicity tests, toxicity was determined by recording lethality and teratogenic effects.	Increased HSI and GSI at 40 µg/mL Decreased GSI value at 200 µg/mL relative to the 40 µg/mL group. Oocyte maturation: Normal mature oocytes in control and at 8 µg/mL Regressed oocytes, numerous perinucleolar oocytes and yolk vesicle oocytes at 40 and 200 µg/mL Gene transcription: Decreased liver VTG mRNA at 200 µg/mL and a NS decrease at the two lower concentrations Hepatic ERα mRNA: NS increase at 8 µg/mL and a NS decrease at the two highest doses. No effects on ERβ mRNA. Hormone effects in whole body (Exp 2): Increased P levels at 40 and 200 µg/mL, and increased E2 levels at all doses Concentration-dependent increased thyroid hormone levels at all doses Concentration-dependent increase in mean whole-body BPS concentrations General toxicity: EC_{50} for embryo toxicity test was 332.6 µg/mL and the LOEL was 250 µg/mL (supplemental information).
Pollock et al. 2019 Female CF-1 mice 3–4 months old, at least seven animals per group Treatment: BPS (≥ 98% purity) in peanut oil (0.1 mL final volume), single sc injection on diestrus day 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 at doses of 0, 36.7, 114.6, or 330 mg/kg. Exp. 2: BPS at doses of 0, 27.7, 78.5, or 252.7 mg/kg. 30 min after BPS injection, animals were fed 0.2 g peanut butter containing 50 µg/kg ^{14}C-BPA (Exp. 1) or 14.5 ng of 5 µCi ^{3}H-E2 (Exp. 2). One hour after oral administration (in food) of radiolabeled compound (Exp. 1 and 2), blood was collected by cardiac puncture from anesthetized animals, and tissues were collected for further analysis of the radiolabel. Exp. 3: BPS at doses of 0, 25.2, or 79.8 mg/kg (only the two lowest BPS doses (1 and 3 mg/animal) tested in this experiment))	Exp 1 and 2: Distribution of ^{3}H-E2 and ^{14}C-BPA in various organs (heart, lung, muscle, adipose, uterus, ovaries), and serum Exp 3: Urinary E2 levels	Exp. 1: Increased radiolabeled BPA levels in uterus at 114.6, or 330 mg/kg and in serum at 330 mg/kg. No increased radiolabel detected in the other tissues and organs analyzed. Exp.2: Dose-dependent decrease in serum ^{3}H-E2 significant at 78.5 and 252.7 mg/kg and NS increase in ^{3}H- E2 levels in the tissue and organs analyzed above control Exp 3: Decreased urinary E2 at 10 h after treatment at 25.2 or 79.8 mg/kg.

Study Design	Outcomes Assessed	Major Findings
Urine was collected at 2, 4, 6-, 8-, 10-, and 12-hours post-injection for hormone measurement.		
Prokešová et al. 2020 Adult (8 weeks old), female ICR mice, 15 animals per group Treatment: BPS (purity not reported) in 50 µL 50% glycerol containing 0.1 % DMSO, daily by oral gavage at doses of 0, 0.001, 0.1, 10, or 100 µg/kg-day for seven days. After exposure animals were sacrificed and immature oocytes in the GV stage were collected from ovaries for further analysis or cultured for 16 hours to obtain matured MII oocytes	Oocyte development Chromosome misalignment (DNA) and spindle malformations (tubulin) in *in vitro* mature MII oocytes. DNA integrity (apoptosis) by terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay on mature oocytes Immunocytochemistry: heterochromatin markers, 5'-methyl cytosine (5meC) and methylation of histone H3 on lysine K27 (H3K27me2) in both GV and mature MII oocytes. RNA isolation, effects of BPS on gene expression in transcriptionally silenced GV oocytes.	No effect on oocyte quantity or quality Effects on MII oocytes: Increased proportion of abnormal oocytes at all doses based on chromatin and spindle malformations Increased abnormal spindles at 0.01, 10, and 100 µg/kg-day. NS increase in abnormal chromatin. Increased apoptosis at 0.01, 0.1, and 100 µg/kg-day Methylation in immature GV oocytes: Increased H3K27 dimethylation at 10 ng/g-day relative to control (other variations among groups) No effect on 5meC relative to the control group. Increased 5meC levels at 10 and 100 µg/kg-day compared to the 0.1 µg/kg-day group. Methylation in mature MII oocytes: Increased 5meC in metaphase chromosomes at 0.1 µg/kg -day NS effect on H3K27me2 levels at any dose. Transcription effects in GV oocytes at 0.1 µg/kg -day: 102 genes affected: 89 genes were upregulated (cellular stress, and markers of preimplantation and embryonic development) and 13 genes were downregulated
Qin et al. 2021 Healthy adult wild-type AB zebrafish, 200 fertilized (2 hpf) eggs per group. Treatment: BPS (99.9% purity) in 0.002% DMSO, added to 1.5 L test solution at a final concentration of 0, 1 or 100 µg/L for 20 days (two-thirds of the exposure solution was renewed every day). At 20 dpf, larvae were transferred to 4.5 L volumes of test solutions and then moved to 40 L, in a 50 L glass tank, from 30 to 240 dpf After 235 days of treatment, the proportion of males and females was approximately equal, with about 80 females per group. Four female fish per group were allowed to mate with untreated males on the morning (8:30 - 10:30) of the 240th day, and eggs were collected within 2 hpf. After exposure, body sized of female fish was measured, and plasma collected by pooling blood samples per group. Ovaries and liver were collected for further analysis	GSI, HSI Histopathological analysis on left ovaries to determine percentage of oocytes at different developmental stages. Lipid profiles in tissue homogenates: triacylglycerol (TAG), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and VTG content from liver, left ovary and plasma. Gene expression from right ovary General toxicity: Body length and weight, survival rate 24 hpf.	Increased HSI and GSI at 100 µg/L Lipid accumulation in interstitial fibrosis tissue (tissue that surrounds the oocyte, necessary for supplying nutrients to oocytes). Follicle maturation: Fewer primary growth stage and more full-grown stage oocytes at both concentrations Increased number of cortical follicles at 1 µg/L Increased vitellogenic (VS)-stage oocytes 100 µg/L Increased egg production at both concentrations VTG and lipid profiles: VTG: Increased at 1 µg/L in plasma and at both doses in ovary; no effect in liver TAG: Increased at both doses in liver and plasma and at low dose in ovary TC: Increased at 1 µg/L in plasma and at both doses in ovary, no effect in liver LDL: Decreased in liver, increased in plasma and ovary at 1 µg/L HDL: Increased HDL at 1 µg/L in plasma, no effect in liver or ovary Ovarian gene transcription: Upregulation of *pparα* and *pparγ* at 100 µg/L Upregulation of metabolic enzyme-related genes: *acc, fasn, acsl1,* at 100 µg/L Increased fatty acid β-oxidation gene expression (*hsl, cpt2, and hsd17β4*) at both concentrations; and *acadm, acadl* at 100 µg/L General toxicity: Increased body weight and BMI at both concentrations, no effect on survival rate: 87% in the controls, 75% at 1 µg/L and 82% at 100 µg/L

Study Design	Outcomes Assessed	Major Findings
Shi et al. 2017 CD-1 mouse pups, 5-6 litters per group. Treatment: BPS (98% purity) in corn oil, (sc) injection (20 to 50 µl) every three days at doses of 0, 50 µg/kg or 10 mg/kg, all doses for 60 days (PND 0 to 60). Litter size was adjusted to 10 pups (5 per sex) and all pups in the same litter received the same treatment. Half the females were sacrificed on diestrus day (after PND 60). The remaining females were mated with untreated males	Vaginal opening monitored from PND 15 Monitor estrous cyclicity from PND 60 Body and uterine weights on half the F1 females Serum hormone levels: E2 and testosterone (T) on PND 60 One ovary for RNA isolation for steroidogenic enzymes: Star, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b1, and Cyp19a1 The other ovary for histological analysis Mated females (n = 10–12/group): gestational length, pup numbers, weights, and sex ratio.	No effect on vaginal opening, time to plug, or body and uterine weight. Increased serum E2 at both doses Increased serum T at 10 mg/kg No effect on steroidogenic enzyme mRNA expression. Three animals in the BPS 50 µg/kg dose group took over 10 days (up to 16 days) to become pregnant. No effects on gestational days, litter size, sex ratio or pup weights
Shi et al. 2019 CD-1 pregnant mice, number of animals not specified but analysis was made on 4–5 litters/group. Treatment: BPS (purity not reported) in corn oil, daily oral administration by placing a pipette tip in the mouth at doses of 0, 0.5, 20, or 50 µg/kg-day from GD 11 to birth One female pup from each litter was euthanized on PND 4 and ovaries fixed for histological analysis. All other F1 females were mated at 3, 6, and 9 months of age with untreated CD-1 males Dams were sacrificed on PND 14 and ovaries saved for RNA and histological analysis	Histological analysis of ovaries on one female pup per litter at PND 4: germ cells in nests and folliculogenesis Puberty: vaginal opening assessed from PND 17 Estrous cyclicity for 30 days after vaginal opening Serum E2 and T in F1 females at 3, 6, and 9 months of age Ovarian steroidogenic enzymes: Star, Cyp11a1, Cyp17a1, Hsd3b1, Hsd17b1, and cyp19a1 at 3, 6 and 9 months of age In F1 mated females: Time to vaginal plug Pregnancy rate, pup numbers, weights, and sex ratio.	Ovarian histology: Retention of germ cells in nests at 0.5 and 20 µg/kg-day Decreased ratio of primary follicles to total oocytes at 0.5, 20, and 50 ug/kg; no effects on secondary follicles NS decreases in the ratio of secondary follicles to total oocytes No effects on the ratio of primordial follicles to total oocytes. Vaginal opening (F1) and estrus cycle: 1–2 days earlier at 0.5 µg/kg-day 2.5 days delayed at 20 µg/kg-day Irregular estrous cyclicity: several days in estrus and diestrus at all doses. F1 female serum hormones: Increased T at 50 µg/kg-day at 9 months No effect on T at other doses or on E2 levels at any dose or age Steroidogenic enzymes: Increased Cyp11a1 mRNA at 0.5 µg/kg-day (at 3 months of age); no effect on other enzymes analyzed. Mating F1 at 6 and 9 months of age: Increased time to vaginal plug for all dose groups. Decreased mating rate: At 6 months: 25% in the low and mid dose groups At 9 months: 34% at the low dose, and 60% at the two higher doses (100% in control) Decreased number of F2 pups/litter at 20 µg/kg-day (NS decrease at 6 months) No effects on F2 pup body weight or sex ratio.
Silva et al. 2019 Adult female gerbils (*Meriones unguiculatus*; 90 days old), 10 animals per group N = 5 – 8 / analysis	Serum levels of E2 and T Stereological (three-dimensional measurements) analysis Histology of prostatic complexes Histopathological analysis of prostate: frequency of prostatic disorders	No effects on serum E2 or T levels Three-dimensional analysis of prostate: Increased epithelium and muscular stroma; decreased ductal lumen. Histology of alveolar prostatic morphology: Decreased % normal cells; increased hyperplasia; increased intraepithelial neoplasia. No effects on PAS-positive prostatic secretion area.

Study Design	Outcomes Assessed	Major Findings
Treatment: BPS (purity not reported) in mineral oil, daily oral exposure (100 µl total volume), at doses of 0 or 40 µg/kg-day for 28 days Twenty-four hours after treatment animals were sacrificed. Blood samples and prostatic complexes (vaginal segment, corresponding urethral segment and prostatic tissue) and ovaries were saved for further analysis.	Histological sections stained by periodic acid-Schiff test (PAS) reaction (secretion assessment). Immunohistochemistry analysis: AR, ERα and PCNA protein expression General toxicity: Body and organs weight	Immunohistochemistry: Increased frequency of AR-positive epithelial and stromal cells Increased ERα immunolabeling in stromal cells Increased PCNA-positive cell frequency in the epithelium and stroma General toxicity: No alterations in body, prostate, or gonadal weight
Téteau et al. 2022 2.5 years old ewes (Ile-de-France), divided in two diet groups: Restricted feed (RF) and well fed (WF, 165 % of food requirement). Twenty animals per BPS group Treatment: BPS (purity not reported) daily administration in the diet at doses of 0 or 50 µg/kg-day for at least three months. Ewes were synchronized for estrus and treated with gonadotropins (pregnant mare serum globulin, PMSG) to induce follicular growth. Two days after PMSG administration (preovulatory time), ewes were sacrificed. Blood, and reproductive tract samples were collected and saved for further analysis.	BPS and BPS glucuronide measured in plasma and in follicular fluid (FF). Steroids in plasma, FF, and oviduct fluid (OF). General toxicity: Body weight, body condition score (BCS)	NS increase in ovary weight (p=0.051) No effect on follicle number (any size) BPS effects on steroid levels in plasma: Decreased P and 5α-dihydroprogesterone (P metabolite) in RF group Decreased E2 and increased E1 in RF group BPS effects on steroid levels in preovulatory FF: Decreased dihydrotestosterone (DHT) and 11-dehydrocorticosterone levels in RF group. Increased E2 levels in WF (no effect in the RF group) Decreased 17α-hydroxyprogesterone and increased 5β-dihydrotestosterone in RF group. There was an interaction between BPS and diet for: E2 (p =0.020), hydroxy pregnenolone (p = 0.028) and DHT (p = 0.001), and E2 (when measured separate p= 0.002) BPS effects on steroid levels in OF: Decreased E2 in the WF group and E1 in the RF group General toxicity: WF ewes had higher body weight and BCS compared to RF (but they were not overweight). There was a BPS effect on BCS (p = 0.026).
Tucker et al. 2018 Pregnant CD-1 mice (GD 8.5), at least 11 animals per group Treatment: BPS (≥ 97:5% purity) in sesame oil, twice daily (every 7h) by oral gavage (10 µL/g body weight) at doses of 0, 0.05, 0.5, or 5 mg/kg twice a day (equivalent to 0.10, 1.0, and 10 mg/kg-day, respectively) from GD 10.5 to GD 17.5 (Parturition GD 18.5 — 19) Analysis on F1 females at PND 20 to 14 months (1 or 2 per litter)	Observations were made on F1 females at PND 20, 35, 56, and at 3, 8, and 14 months of age. Female pubertal indices: vaginal opening and estrus cyclicity; mammary development/score changes in mammary gland whole mounts. In addition, changes in gene expression and mammary gland histopathology were analyzed at 3, 8 and 14 months. Serum hormone levels: E2, P, T, and DHEA mRNA for steroid receptor gene expression at 8 and 14 months General toxicity: Body weight	No differences in age at vaginal opening; first estrus (PND 25 – 27) or estrus cyclicity (3 – 4 days) Mammary gland PND 20: Dose-dependent increases in TEB length and TEB counts Increased branching density at 0.05 and 5 mg/kg twice a day Increased developmental score at 5 mg/kg twice a day No effects on mammary epithelial area PND 35: Increased developmental score at all doses PND 56: Increased developmental score at 0.5 mg/kg twice a day. 3 months of age: Increased TEB counts at 0.5 mg/kg twice a day Increased incidence of diagnoses of mixed cell inflammation at 0.5 mg/kg twice a day (25% inflammation and 50% increased TEB) 14 months of age: Increased incidence of perivascular lymphoplasmacytic inflammation at 0.5 mg/kg twice a day Increases in all types of inflammation diagnoses at 0.5 mg/kg twice a day Increased non-neoplastic lesions at 0.5 mg/kg twice a day Increased incidence of lobuloalveolar hyperplasia at 0.5 mg/kg twice a day Serum hormone analysis:

Study Design	Outcomes Assessed	Major Findings
		Increased E2 at the mid and high doses on PND 20 (no effects at other doses/times) Increased P and DHEA at 0.05 mg/kg twice a day on PND 20 (NS increase at mid and high dose on PND 56); decreased P at 5 mg/kg twice a day at 3 months of age No effect on P, DHEA or E2 levels on PND 28 Increased T at 0.05 mg/kg twice a day on PND 28 Decreased T at mid and high dose on PND 35 (no effects on PND 56) Decreased E2 at 0.05 mg/kg twice a day. Decreased T and DHEA at 0.5 mg/kg twice a day at 8 months of age No effects at 14 months of age on any hormone analyzed Mammary gland nuclear receptor gene expression: Decreased *Pgr* expression at 5 mg/kg twice a day at 8 months. No other gene expression changes at 8 or 14 months General toxicity: Decreased body weight on PND 56 at 5 mg/kg twice a day (no effects on body weight at other times or doses)
Wang et al. 2020 Six-month-old adult female wild-type (AB strain) zebrafish, 40 females/group Treatment: BPS (≥ 98% purity) in 0.002% DMSO added to 4 L of water (refreshed every 24 hours) at doses of 0, 1, 10, and 100 µg/L for 14 days	Test 1: adult female behavioral analysis and observation of body pigment and gonad structure Test 2: 4 adult females and 4 adult males were paired and spawned for 0.5 d. Oocyte classification: perinucleolar oocytes, cortical alveolar oocytes, late vitellogenic oocytes, and spawning oocytes. General toxicity: Body weight and length	Debilitated female shoaling at 100 µg/L: Decreased proportion of time spent in the zone close to fish (1.23-fold reduction), increased time spent in the zone far from fish (3.01-fold) Ovarian histology (effects at all doses): Increased perinucleolar oocytes Decreased cortical alveolar oocytes Decreased late vitellogenic and spawning oocytes General toxicity: No effects on body weight or body length. Females presented depigmentation at all concentrations. .
Yamasaki et al. 2004 Twenty-day-old SD female rats, six rats per group Treatment: BPS (99.4% purity) in olive oil (vehicle control), daily sc injection at doses of 0, 20, 100, and 500 mg/kg-day on three consecutive days Positive control group: EE in olive oil was also sc injected to some rats at a dose of 0 or 0.6 µg/kg-day on three consecutive days right after BPS administration. Another group was injected with tamoxifen at 1 mg/kg-day plus EE on three consecutive days	Uterus weight (uterotrophic assay) Estrogen receptor assay	Effects on uterine relative weights: Increased at 20 and 500 mg/kg-day BPS Increased with EE positive control Tamoxifen reduced the EE effect on uterine weight BPS + EE effects: BPS at 20 mg/kg-day increased uterine weight further than the EE observed effect BPS at 500 mg/kg-day decreased the EE effect on uterine weight BPS - Estrogen binding affinity assay = 0.0055 % of E2
Yue et al. 2023b Mature female (8 to 9 weeks old) CD-1 (ICR) mice, 9 mice per group	Serum hormone levels: E2, P, FT3, FT4. Histological examination: Ovary follicle analysis Uterus, measurement of endometrial area and glands	No effects on serum hormones analyzed (E2, P, FT3, and FT4) Effects on follicles: (author description) Damaged oocyte structure, wrinkled and depressed zona pellucida, atrophy in immature follicles, increased number of atretic follicles Effects in uterus: Reduced endometrial area, atrophied lamina propria, myometrium and plasma layer, and narrowed uterine cavity. Increase in number of uterine glands

Study Design	Outcomes Assessed	Major Findings
Treatment: BPS (purity not reported) in corn oil (volume not specified) daily by oral gavage at doses of 0 or 300 µg/kg-day for 28 consecutive days. After exposure, blood samples and uterus and ovary were collected for further analysis.	RNA sequencing: Identification of DEG Gene Ontology enrichment analysis. Associations among BPS, hub genes, and diseases using the Comparative Toxicogenomic Database online website (http://ctdbase.org/)	No effects on uterus weight. Gene Ontology analysis in uterus and ovary: Affected metabolic hormone related pathways in uterus and ovary (steroids and sterol metabolic process), immune and inflammatory related pathways in uterus and ovary. Gene - Disease associations: Uterine genes included were associated with mammary gland neoplasms and breast cancer. Ovarian genes were associated with polycystic ovary syndrome, hypercholesterolemia, ovarian neoplasm, ovarian and breast carcinoma, endometriosis, estrogen resistance, and others.
Zhang et al. 2020 ICR 8-week-old pregnant female mice, at least 3 animals per group. Treatment: BPS (purity not reported) in 1% DMSO diluted in normal saline, daily oral administration (volume not specified) at doses of 0, 2, or 10 µg/kg-day for 4 days (GD 12.5 to 15.5) NOTE: In a dose finding study, six animals per group were treated with BPS at doses of 0, 2, 10, 50, 100, or 200 µg/kg-day for 3 days (GD 12.5 to 15.5) following the same treatment protocol. All doses had effect on meiosis prophase I progression, therefore all subsequent studies used BPS doses of 2 or 10 µg/kg-day. Complete oocytes were isolated from female mice aged 5 weeks and used for oocyte culture. MII oocytes from COCs from 5-week old F1 oviducts used for IVF and blastocyst culture	meiosis prophase I progression in ovaries from GD 15.5 fetuses. Gene expression and protein levels for meiosis-related genes in PND 15.5 ovaries: deleted in azoospermia-like (Dazl), stimulated by retinoic acid 8 (Stra8), synaptonemal complex protein 3 (Scp3), dosage suppressor of mck1 homolog (Dmc1) and its related protein levels Gene expression and protein levels for genes related to oocyte quality in PND 21 ovaries: bone morphogenetic protein 15 (Bmp15) and growth differentiation factor 9 (Gdf9) and its related protein levels. Germ cell cyst breakdown assessed in F1 ovaries at PND 3, and folliculogenesis assessed in F1 ovaries at PND 21 Oocyte maturation, fertilization (IVF), and early embryo development of F1 females assessed at five weeks Epigenetic alterations in F1 oocytes Ovarian effects in F2 generation	Acceleration in meiotic progression (at all doses): Decreased percentage of oocytes at zygotene stage, increased percentage at pachytene and diplotene stage Meiosis related gene and protein expression (PND 15.5): Increased expression of *Stra8, Scp3*, and *Dmc1* at both doses and *Dazl* at 10 µg/kg-day Increased protein levels of DAZL and STRA8 at both doses and SCP3 at 10 µg/kg-day Oocyte quality related genes and protein expression: Dose related decreases in gene expression and protein levels of BMP15 and GDF9 Germ cell cyst breakdown (PND 3): Increased MVH positive oocytes in primordial follicles at both doses Follicular development (F1, PND 21): Increased oocytes in primordial follicles and decrease in secondary and antral follicles on PND 21 at both doses No effect on total number of oocytes Decreased rates of germinal vesicle breakdown and release of first polar body at both doses Increased rates of aberrant spindles and misaligned chromosomes at both doses Effects on IVF: Decreased rates of cleavage and blastocysts after *in vitro* fertilization at both doses Decreased rates of 4-cell embryos (dose-dependent manner) at both doses Decreased rate of blastocysts at both doses Epigenetic effects on F1 oocytes at both doses: Decreased levels of trimethyl-histone H3 (Lys4) (H3K4me3) Increased levels of H3K9me3 Effects on F2 generation: No effects on litter size, body weight or sex ratio Acceleration of germ cell cyst breakdown on PND 3 (no change in total number of follicles) No effects at PND 21

4.3 Mechanistic considerations and other data relevant to female reproductive toxicity

Findings from studies on the possible mechanisms of action of bisphenol S (BPS) relevant to considerations of female reproductive toxicity are discussed in this section. These findings, which come from *in vivo* and *in vitro* studies, are then discussed in the context of the key characteristics of female reproductive toxicants. Table 4.3.1, at the end of this section, summarizes key elements of the mechanistic studies, except for those *in vivo* studies already presented in Table 4.2.1.

Several possible mechanisms of action of BPS relevant to its effects on the female reproductive system have been investigated, including mechanisms related to its estrogenic activity. Like bisphenol A (BPA), BPS can mimic and interfere with the action of estrogen. BPS modulates estrogen receptor (ER)-mediated effects through several different ER subtypes, such as ERα, ERβ, and the plasma membrane G-protein-coupled estrogen receptor (GPER), as well as their downstream signaling pathways. These effects have been observed in various studies conducted on human cells *in vitro*, as well as in animal studies conducted *in vivo* and *in vitro*. The evidence supporting these findings has been extensively discussed in multiple reviews (Chen et al. 2016a; NTP 2017; Pelch et al. 2019; Rochester and Bolden 2015).

4.3.1 Effects on ovarian development and maturation of oocytes

The effects of BPS on ovarian physiology and follicle development, including oocyte maturation, were presented in section 4.2. Studies reporting mechanistic data relevant to these physiologic effects are briefly summarized below:

In vitro studies

Oocyte maturation

- Newborn mouse ovaries cultured for 3 days *in vitro* in the presence of BPS at 10 to 100 μM underwent abnormal germ cell cyst breakdown, starting at 10 μM. This effect was blocked by co-incubation with 1 μM tamoxifen, a selective estrogen receptor modulator (SERM) with the potential for both ER agonism and antagonism, suggesting an estrogenic mode of action for BPS (Liu et al. 2021).
- Pig ovary cumulus–oocyte complexes (COCs) incubated with BPS *in vitro* resulted in alterations in oocyte maturation. A concentration dependent decrease in oocytes reaching metaphase I (MI) and a failure to resume meiosis occurred after 24 hours at 300 nanomolar (nM) and 30 micromolar (μM) BPS,

concentrations that had no effect on oocyte or cumulus cell viability after 24 or 48 hours. There was also a concentration dependent decrease in progression to metaphase II (MII) at 48 and 72 hours. All BPS-treated oocytes matured to at least the MI stage by 48 hours but by 72 hours the maturation process was delayed, disrupted and/or blocked at all concentrations (Žalmanová et al. 2017).

Spindle morphology and chromosome alignment

- Cow oocytes treated with BPS *in vitro* from 1 femtomolar (fM) to 50 µM resulted in alterations in spindle morphology and chromosome alignment in MII oocytes at BPS concentrations greater than 1 nM without altering the proportion of oocytes entering MII (Campen et al. 2018a).
- Pig ovary COCs incubated with BPS at 3 nM, 300 nM or 30 µM for 24 or 48 hours resulted in α-tubulin assembly alterations, including a concentration dependent decrease in the number of tubulin filaments. This translates into swollen chromosomes and irregular organization, spindles in circular formation, elongated metaphase plates, and reduced spindle size at all doses in MI and MII oocytes (Žalmanová et al. 2017).
- Pig ovary COCs incubated with BPS at 300 picomolar (pM), 30 nM or 3 µM for 48 hours resulted in a concentration dependent decrease in the proportion of oocytes reaching MII, with increases in degenerated oocytes, retention in the germinal vesicle (GV) stage, arrest in the MI stage, spindle disorganization, and chromosome misalignment at all concentrations (Žalmanová et al. 2023).

Follicular cells (granulosa and theca cells)

- Adult ewe ovarian granulosa cells incubated with BPS at 200 µM for 48 hours resulted in a decrease in granulosa cell proliferation (Téteau et al. 2020).
- In human and sheep primary cultures of ovarian theca cells, BPS treatment for 24 hours at up to 1000 ng/mL in sheep cells, and at 200 ng/ml in human cells increased theca cell gap junction intercellular communication (GJIC) at concentrations above 100 ng/mL (Gingrich et al. 2021).
- Signaling pathways such as those involving protein kinase C (PKC), protein kinase A (PKA), mitogen-activated protein kinase (MAPK), phosphatidyl choline specific phospholipase C (PC-PLC), and phosphatidylinositol-specific phospholipase C (PI-PLC) may be required for proper cellular proliferation. Incubating human and sheep theca cells with specific inhibitors for these pathways to assess effects on BPS stimulated GJIC resulted in full inhibition (back to control values) by a MAPK inhibitor (human and sheep theca cells); partial inhibition with inhibitors of PI-PLC, PC-PLC, and ERK-MAPK; and no

effect with PKA or PKC inhibitors (sheep theca cells only). These findings indicate that BPS can modulate GJIC through the MAPK pathway and partially through PC-PLC and PI-PLC and that these effects are independent of PKA and PKC activation (Gingrich et al. 2021).

- Cow ovary COCs, denuded oocytes, and cumulus cells (comprised of granulosa cells) incubated with BPS at 0, or 0.05 mg/mL for 24 hours resulted in changes in some COC cells in the expression of connexins (Cx – gap junctional proteins): Cx37 and Cx43. There was an increase in Cx37 mRNA expression in cumulus cells with a NS increase in Cx37 protein expression, and no effects on Cx43 or Cx37 mRNA or protein expression in COCs as a whole (Sabry et al. 2021).

Mitochondria

- Pig ovary COCs incubated with BPS at 30 nM and 3 μM for 48 hours resulted in lower relative intensity in fluorescence (about a 30% decrease) for mitochondria fusion protein markers, MFN1 and MFN2, and a relative increase in fluorescence at 30 nM (Žalmanová et al. 2023).

In vivo studies

Relevant mechanistic data from *in vivo* studies of BPS on ovarian development and maturation of oocytes presented in Section 4.2 and Table 4.2.1 include the following:

- Chromosome misalignment and/or incidence of spindle malformation in mice (Nevoral et al. 2018; Zhang et al. 2020), and *C. elegans* (Chen et al. 2016b)
- Altered gene expression in mice (Liu et al. 2021; Prokešová et al. 2020; Yue et al. 2023b; Zhang et al. 2020), chickens (Eldefrawy et al. 2021), and zebrafish (Qin et al. 2021)
- Apoptosis in mice (Nourian et al. 2020) and *C. elegans* (Chen et al. 2016b)
- Epigenetic effects in mice (Nevoral et al. 2018; Prokešová et al. 2020; Yue et al. 2023b; Zhang et al. 2020)
- Effects on the signaling pathway involving Notch2 and Jagged1, which is important in cyst breakdown and primordial follicle assembly in mice (Liu et al. 2021)
- Effects on lipid profiles in mice (Yue et al. 2023b), and zebrafish (Qin et al. 2021)
- Indicators of oxidative stress in mice (Nevoral et al. 2018; Nourian et al. 2017, 2020) and rats (Ijaz et al. 2020).

4.3.2 Effects in placenta

The effects of BPS on the placenta were presented in section 4.2. Studies reporting mechanistic data relevant to these physiologic effects are briefly summarized below:

In vitro studies

- BPS treatment of a human cell line (HTR-8/SVneo) derived from extravillous trophoblast cells from a first trimester pregnancy at concentrations ranging from 10^{-9} M to 10^{-7} M resulted in increased cell proliferation (at all concentrations). This increased cell proliferation was mediated through the ER and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways. Inhibition of ERK1/2 phosphorylation by BPS induced secretion of the inflammatory cytokines interleukins (IL) 6 and 8 (Profita et al. 2021).
- In the human trophoblastic 3A placental cell line CRL-1584 BPS altered ABCB1 promoter activity in a haplotype-dependent manner. Changes in ABCB1 promoter activity affects P-glycoprotein levels (P-gp, placental transporter); in the human placenta, efflux of P-gp affects trophoblasts and fluctuations in P-gp levels affect fetal development (Speidel et al. 2018).

In vivo studies

Relevant mechanistic data from *in vivo* studies of BPS on the placenta presented in Section 4.2 and Table 4.2.1 include the following:

- Findings in mouse placenta (Mao et al. 2020)
  - Gene expression: 11 genes were differentially expressed with one upregulated, *Actn2* (actinin α2), and 10 downregulated including *Calm4* (calmodulin 4) and *Guca2a* (guanylate cyclase activator 2a, guanylin)
  - Effect on fatty acids: Decreased stearic acid, palmitic acid, docosahexaenoic acid, octadecenoic acid, and hexadecanoic (palmitic acid)
  - Effects on neurotransmitters: Increased dopamine levels, increased percentage of dopamine-positive giant cells; decreased serotonin levels and decreased percentage of serotonin-positive giant cells
- Findings in sheep placenta (Gingrich et al. 2018)
  - 50% lower e-cadherin protein expression in GD 120 placentomes
  - 20% decrease in trophoblast derived binucleate cells
  - Decreased expression of fusogenic genes: JSRV and HYAL2Increased transcription factor glial cell missing factor 1

4.3.3 Effects on the endocrine system

Endocrine effects of BPS observed *in vivo* in animal studies were presented in section 4.2. Endocrine effects observed *in vitro*, as well as other relevant mechanistic data are briefly summarized below:

In vitro studies

Progesterone

- Decreases in progesterone (P) levels were reported in primary cultures of human granulosa cells, at BPS doses of 10 and 50 µM (Amar et al. 2020).
- A concentration dependent decrease in P secretion was reported in adult ewe granulosa cells incubated with BPS exposure at concentrations ranging from 10 to 200 µM (Téteau et al. 2020).
- Decreases in P levels were reported in ewe granulosa cells treated with BPS at 10 or 50 µM for up to 48 hours (Téteau et al. 2023).
- Increased P and 17-OH-progesterone levels were reported in H295R cells (a human adrenal derived cell line) treated with BPS from 0.8 to 50 µM (Rosenmai et al. 2014).
- In cow theca cells isolated from antral follicles, *in vitro* treatment with BPS from 1 fM to 100 µM did not affect P secretion (Campen et al. 2018b).

Androgens

- In cow theca cells isolated from antral follicles, *in vitro* treatment with BPS from 1 fM to 100 µM did not affect androstenedione secretion (Campen et al. 2018b).
- In the human adrenal cell derived cell line H295R there was a decrease in testosterone (T), androstenedione and dehydroepiandrosterone (DHEA) after BPS exposure (Rosenmai et al. 2014).

Estradiol

- Decreases in estradiol (E2) levels were reported in primary cultures of human granulosa cells exposed to 50 µM BPS for 48 hours (Amar et al. 2020).
- Decreases in E2 levels were reported in ewe granulosa cells treated with BPS at 10 and 50 µM for 48 hours (Téteau et al. 2023).
- A concentration dependent increase in E2 levels was reported in ewe granulosa cells treated with BPS from 1 nM to 200 µM for 48 hours (Téteau et al. 2020).

- Increases in E2 secretion were reported in cow granulosa cells isolated from antral follicles after BPS exposure at 100 μM (Campen et al. 2018b; Žalmanová et al. 2017).
- No effects on E2 or estrone (E1) levels were reported in H295R cells (a human adrenal derived cell line) after treatment with BPS from 0.8 μM to 50 μM (Rosenmai et al. 2014).

Estrogen receptor

- In pig ovary COCs treated with BPS at 3 nM, 300 nM or 30 μM there were decreases in *Erα* gene expression in oocytes at all doses, decreases in *Erβ* mRNA in cumulus cells at 30 μM, and increases in ER (α and β) protein levels at 300 nM (Žalmanová et al. 2017).
- Exposure of Ishikawa cells (a human endometrial epithelial cell line) to 100 nM BPS increased gene expression of *ESR2*, but not *ESR1* (Benjamin et al. 2023).
- In ovaries from newborn mice that were cultured for 3 days in the presence of BPS at 10 μM increases in ER (α and β) mRNA and protein levels were observed (Liu et al. 2021).
- Granulosa cells from adult ewes treated with BPS from 1 nM to 200 μM for 48 hours increased *Esr2* expression at 50 μM and a two-fold increase in *Esr1* and *Esr2* expression at 100 μM (Téteau et al. 2020).
- In H295R cells treated with BPS from 0.8 μM to 50 μM, there was an increase in ER activity (Rosenmai et al. 2014).
- No effect on the *in vitro* response of ewe granulosa cells to BPS was reported in the presence of the GPER antagonist G-15, suggesting the decreases in P and E2 levels observed in BPS treated cells (discussed above) were not mediated by GPER (Téteau et al. 2023).

Other hormone receptors

- In granulosa cells from adult ewes treated with BPS from 1 nM to 200 μM for 48 hours no effects were observed on androgen receptor or progesterone receptor gene expression (Téteau et al. 2020).
- In H295R cells treated with BPS from 0.8 μM to 50 μM, there was an apparent concentration-related decrease (NS) in androgen receptor (AR) activity (Rosenmai et al. 2014).
- In cow COCs and *in vitro* fertilized cow embryos incubated with BPS at 0.05 mg/mL there was an increase in anti-müllerian hormone receptor II (AMHRII) mRNA levels and a non-significant increase in protein levels reported in COCs, and an increase in AMHRII protein levels in day-8 blastocysts (Saleh et al. 2021).

Steroidogenic enzymes

- In pig ovary COCs, treatment with BPS at 3 nM, 300 nM or 30 μM resulted in decreased aromatase gene expression in oocytes (at 3 nM and 300 nM) and cumulus cells (at 30 μM) and decreased aromatase protein levels in oocytes (at 3 nM) (Žalmanová et al. 2017).
- In primary cultures of human granulosa cells there was a non-significant decrease in CYP11A1 (P450 side chain cleavage [P450scc]) protein levels (37.7%; p= 0.09) at 10 μM (Amar et al. 2020).

In vivo studies

Relevant mechanistic data from *in vivo* studies of BPS and its effects on the endocrine system presented in Section 4.2 and Table 4.2.1 include the following:

Hormone levels

- Decreased gonadotropin levels (follicle stimulating hormone and luteinizing hormone) in rats (Ahsan et al. 2018; Ijaz et al. 2020) and mice (Nourian et al. 2020).
- Decreased P plasma levels in rats (Ahsan et al. 2018; Ijaz et al. 2020) and mice (at 5 mg/kg twice a day) (Tucker et al. 2018)
- Increased P levels in zebrafish (Park et al. 2022) and in mice at 0.05 mg/kg twice a day a dose that it was notably smaller than the dose that resulted in decreased P in the same study (Tucker et al. 2018).
- Increased plasma T levels in rats (Ahsan et al. 2018; Ijaz et al. 2020) and mice (Shi et al. 2017; Shi et al. 2019) (Tucker et al. 2018)
- Decreased plasma T levels in mice (at 0.5 and 5 mg/kg twice a day on PND 35) (Tucker et al. 2018).
- Decreased preovulatory follicular fluid dihydrotestosterone and 11-dehydrocorticosterone levels in ewes (restricted feed diet) (Téteau et al. 2022).
- Decreased plasma E2 levels in mice (LaPlante et al. 2017; Nevoral et al. 2018; Pollock et al. 2019), rats (Ijaz et al. 2020), and ewes (restricted feed diet) (and increased plasma E1) (Téteau et al. 2022).
- Increased E2 levels in mice (Shi et al. 2017; Tucker et al. 2018) and zebrafish (Ji et al. 2013; Naderi et al. 2014; Park et al. 2022).
- Increased preovulatory follicular fluid E2 levels in ewes (well fed diet) (Téteau et al. 2022)

Hormone receptors

- Increase in P receptor (PR) protein levels in mammary glands of pubertal mice (Kolla et al. 2018)
- Decrease in PR gene expression in mammary glands of adult mice (Tucker et al. 2018)
- Increase in AR positive epithelial and stromal cells in the female prostate in gerbils (Silva et al. 2019).
- Increased ERα gene or protein expression in the mammary glands of mice (Kolla and Vandenberg 2019; LaPlante et al. 2017), stromal cells of the female prostate in gerbils (Silva et al. 2019), and *in vitro* fertilized embryos from BPS exposed mice (Nourian et al. 2020).
- An ethinyl estradiol (EE) challenge altered ovarian *ERα* and *Erβ* gene expression in BPS exposed mice: non-significant increases in ERα and Erβ mRNA levels occurred with BPS alone, while decreases in Erβ mRNA and non-significant decreases in ERα mRNA occurred with BPS + EE (Hill et al. 2017).
- BPS exposure of mice was associated with a greater number of secondary follicles, with no effects on other follicle types. Co-exposure with BPS + EE was observed to inhibit the increases in total follicles induced by EE alone (Hill et al. 2017).
- BPS exposure was associated with a classical response in female rats indicative of ER-mediated estrogenic activity, increasing uterine weight. Co-exposure with EE and BPS at 20 mg/kg-day was observed to increase the uterotrophic effect of EE alone, while co-exposure with EE and BPS at 500 mg/kg-day was observed to diminish the uterotrophic effect of EE alone (Yamasaki et al. 2004).
- In a rat model of induction of PCOS by DHEA, BPS + DHEA was observed to decrease the average number of cysts per ovary compared to treatment with DHEA alone (Demacopulo and Kreimann 2019).
- BPS exposure was observed to alter a classical ER-mediated response in fish, i.e., hepatic vitellogenin (VTG) expression. BPS was observed to decrease hepatic VTG mRNA levels in zebrafish, indicating altered ER signaling (Park et al. 2022).

Steroidogenic enzymes

- Increased ovarian *Cyp11a1* (P450scc) mRNA levels in mice (Shi et al. 2019)
- Decreased brain mRNA levels of one isozyme of 5α-reductase in rats (Castro et al. 2015).

4.3.4 Key characteristics of female reproductive toxicants

The key characteristics (KCs) of female reproductive toxicants, as described in Luderer et al. 2019, are listed in Table 4.3.1. One or more of these key characteristics are frequently exhibited by exogenous agents that cause female reproductive toxicity (Luderer et al. 2019).

Table 4.3.1 Key characteristics of female reproductive toxicants (source: Luderer et al. (2019)

- **KC 1 Alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism**
- **KC 2 Chemical or metabolite is genotoxic**
- **KC 3 Induces epigenetic alterations**
- **KC 4 Causes mitochondrial dysfunction**
- **KC 5 Induces oxidative stress**
- **KC 6 Alters immune function**
- **KC 7 Alters cell signal transduction**
- **KC 8 Alters direct cell–cell interactions**
- **KC 9 Alters survival, proliferation, cell death, or metabolic pathways**
- **KC 10 Alters microtubules and associated structures**

A related set of key characteristics, those of endocrine-disrupting chemicals, has been described in La Merrill et al. (2020), and is presented in Table 4.3.2.

Table 4.3.2 Key characteristics of endocrine-disrupting chemicals (source: (La Merrill et al. 2020).

- **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**

Relevant findings from studies of BPS can be considered in the context of the key characteristics of female reproductive toxicants and of endocrine-disrupting chemicals. Here we focus on the key characteristics of female reproductive toxicants, and summarize findings related to KC 1, KC 2, KC 8, and KC 10.

KC 1 Alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism.

- A large number of studies, all of which are presented in section 4.3.3, above, report findings relevant to KC 1. Please see section 4.3.3.

KC 2 Chemical or metabolite is genotoxic.

- In pig ovary COCs, BPS treatment resulted in spindle disorganization, chromosome misalignment, and increased aneuploidy in oocytes (Žalmanová et al. 2023).

KC 8 Alters Direct Cell-Cell Interactions

- In cow ovary cumulus cells (i.e., granulosa cells), incubation with BPS altered expression of connexin (Cx) 37, one of multiple connexins that regulate gap junctional intracellular communication. Specifically, an increase in Cx37 mRNA expression and a non-significant increase in Cx37 protein expression was reported, with no effect of BPS on Cx43 mRNA or protein expression (Sabry et al. 2021).
- Mice ovaries cultured for 3 days *in vitro* with BPS from 10 to 100 µM resulted in abnormal germ cell cyst breakdown starting at 10 µM. Germ cell cyst (or nest) breakdown is a normal physiological step in the development of the ovary, and it is mediated by activation of the ER. Activated ER stimulates the c-Jun N-terminal kinases (JNK) signaling pathway to modulate the expression of E-cadherin, which is a cell adhesion protein and is involved in cell-to-cell interactions between oocytes in the germ cell cysts. The BPS effect on the normal cyst breakdown, was blocked by co incubation with SERM tamoxifen, suggesting an estrogenic mode of action for BPS (Liu et al. 2021).
- In human and sheep primary ovarian theca cell cultures, BPS treatment increased of theca cell gap junction intercellular communication (GJIC) (Gingrich et al. 2021).

KC 10 Alters microtubules and associated structures.

- BPS treatment of oocyte from cows *in vitro* from 1 fM to 50 µM resulted in alterations on spindle morphology and chromosome alignment in MII oocytes at

BPS concentrations greater than 1nM without altering the proportion of oocytes entering MII (Campen et al. 2018a).

- Pig ovary COCs incubated with BPS at 3 nM, 300 nM or 30 µM for 24 hours or 48 hours, resulted in α-tubulin assembly alterations including a concentration dependent decrease in the number of tubulin filaments. This translates into swollen chromosomes and irregular organization, spindles in a circular formation, elongated metaphase plates, and reduced spindle size at all doses in metaphase I and II (Žalmanová et al. 2017).
- In COCs from pig ovaries, there was a concentration dependent decrease in proportion of cells reaching MII: retention in GV stage, degenerated oocytes, arrested in MI stage, spindle disorganization and chromosome misalignment at all concentrations (Žalmanová et al. 2023).
- Chromosome misalignment and incidence of spindle malformation have been reported in BPS exposed mice (Nevoral et al. 2018; Zhang et al. 2020), and *C. elegans* (Chen et al. 2016b).

Summary Table

Data discussed above on female reproductive toxicity from studies with mechanistic data not already included in Table 4.2.1 are summarized in Table 4.3.3 below. The results presented are statistically significant ($p<0.05$), unless otherwise stated (i.e., non-significant [NS] change). The studies are organized alphabetically by first author.

Table 4.3.3 Female reproductive toxicity: studies with mechanistic data not already included in Table 4.2.1

Study Design	Outcomes Assessed	Major Findings
Amar et al. 2020 Human granulosa cells *in vitro* from 59 women Treatment: BPS (purity not reported) in the culture medium at 10 nanomolar (nM), 100 nM, 1 micromolar (µM), 10 µM or 50 µM Cell cultures were assessed 48 hours after treatment began for viability, steroidogenesis, cell proliferation and gene and protein expression	Hormone levels: progesterone (P), estradiol (E2) Steroidogenesis: Western Blot Analysis of BPS 10 and 50 µM treatment groups for 3-beta-hydroxysteroid dehydrogenase (HSD3B1), cytochrome P450 family 11 subfamily (CYP11A1), and cytochrome P450 family 19 (CYP19A1) proteins Gene expression Mitogen-activated protein kinase (MAPK 3/1) signaling pathway assays (at 5, 10, 30 or 60 min) in the presence or absence of 10 µM BPS BPS glucuronide was quantified in follicular fluid General toxicity: Viability, cell proliferation	**Hormone levels:** Decreased P at 10 µM (by 16%) and at 50 µM BPS (by 64%) Decreased E2 at 50 µM BPS (by 46%) **Steroidogenesis** NS decrease in CYP11A1 expression levels (37.7%; p=0.0947) at 10 µM BPS No effect on protein levels of HSD3B1 (at 10 µM) or CYP19A1 (at 50 µM) **Gene Expression** Increased ESRRG (estrogen-related receptor γ) expression (1.9-fold) at 50 µM. BPS treatment had no effect on the other analyzed genes (*STAR*, *CYP17A1*, androgen receptor (*AR*), progesterone receptor (*PR*), and *ERs*) Signaling Pathways No effect on MAPK3/1 phosphorylation (at 10 µM) General toxicity: No effects on cell viability or cell proliferation
Benjamin et al. 2023 Ishikawa (ECACC 99040201) cells (human endometrial epithelial cells). 250,000 cells/well on a 6-well plate Treatment: BPS (purity not reported) dissolved in dimethyl sulfoxide (DMSO, vehicle control) then diluted in distilled water to a concentration of 0, 1 nM, or 100 nM for 72 hours	Gene expression: Estrogen receptors (ESR1, ESR2) Proliferation genes (CCND1, CCNB1, GREB1A, CMYC) Migratory gene (*VIM*) Wound healing assay: estimation of time for cells to reach confluence, assessed 2 hours after scraping the cell monolayer in the presence of mitomycin C (DNA synthesis inhibitor) General toxicity: Cell viability	Increase in cell number: 3 to 8-fold increase in cell proliferation Wound healing assay: Increased cell migration capacity at 1 nM Increased gene expression of *ESR2* at 100 nM No effect on proliferation genes Increased expression of migratory gene *VIM* General toxicity: Increased cell viability
Campen et al. 2018a *In vitro:* Cumulus–oocyte complexes (COCs) from cow ovaries (Holstein-Friesian and Jersey heifers). Treatment: BPS (purity not reported) in culture medium at concentration of 0 or from 1 femtomolar (fM) to 100 nM (in 10-fold increments), or 50µM A total of 432 oocytes were analyzed for meiotic stage, including 110 from the vehicle- control group. Of the 432 oocytes analyzed, 350 were at metaphase II (MII) (91 MII	Number of oocytes that reached MII Analysis of spindle and chromosome configuration. Microfilaments, microtubules, and DNA were detected by immunocytochemistry analyses of each oocyte	No effect on the proportion of oocytes that had matured to MII by 24 hours. In the control group, 85.7% of MII oocytes had a bipolar spindle and 93.4% had aligned chromosomes. BPS had overall effects on spindle morphology and chromosome alignment: Reduced proportion of oocytes with a bipolar spindle at 10 fM, 1 picomolar (pM), 100 pM, 1 nM,10 nM, 100 nM or 50 µM. (No effect on spindle morphology at 1 fM, 100 fM or 10 pM). Increased flattened spindles with broad poles representing 17% of abnormalities at 100 nM.

Study Design	Outcomes Assessed	Major Findings
oocytes were in the control group). MII oocytes were analyzed for spindle and chromosome configuration.		Decreased proportion of oocytes with properly aligned chromosomes at 10 fM, 100 pM, 10 nM, 100 nM and 50 µM
Campen et al. 2018b Antral follicles (3–7 mm in diameter) from cow ovaries with an active corpus luteum. Pooled cells from two to three animals per replicate. Granulosa and theca cells were obtained from antral follicles. Treatment: BPS (purity not reported) in culture medium containing 0.05% DMSO, at concentrations of 0, and from 1 fM to 100 µM (in 10-fold increments), in the presence or absence of 0.33 ng/mL follicle stimulating hormone (FSH) (granulosa cells) or 100 pg/mL luteinizing hormone (LH) (theca cells)	P, measured in conditioned medium collected from either granulosa or theca cell cultures. E2 from granulosa cells Androstenedione from theca cells General toxicity: Cell viability	Granulosa cells: Increased basal E2 secretion at 100 µM No effect of BPS under FSH -stimulated conditions No effect on P secretion under any condition (including FSH) Theca cells: No effect on secretion of P or androstenedione in basal condition or in the presence of LH General toxicity: No effect on granulosa or theca cell viability
Gingrich et al. 2021 Sheep primary ovarian theca cells Isolation of theca cells was performed on eight multiparous Polypay × Dorsett breed sheep at gestational day 120 of pregnancy. The theca internal cell layer from antral follicles was isolated by microdissection and plated. N=8 primary cell cultures with 3 replicates per experiment Treatment: BPS (99.7% purity) in 0.1% DMSO (vehicle control) at concentrations of 0, 1, 10, 100, 200, 500, or 1,000 ng/mL, incubated for 24 hours Human ovarian theca cells Human ovaries collected from healthy non pregnant women (n=3, aged 31-46 years) Treatment: BPS (99.7% purity) in 0.1% DMSO (vehicle control) at concentrations 0, or 200 ng/mL, incubated for 24 hours, in the presence or absence of MAPK inhibitor (1 µM)	Theca cell GJIC quantification: Rhodamine-dextran dye used to measure GJIC at different stages of theca cell development (pre-luteinized, luteinizing, and luteinized) Signaling pathway analysis using specific signal transduction pathway inhibitors (D609, ET-18-OCH3, GF109203X, H89, SB202190, and U0126) for: phosphatidyl choline specific phospholipase C (PC-PLC), phosphatidyl inositol specific phospholipase C (PI-PLC), protein kinase C (PKC), protein kinase A (PKA), extracellular receptor kinase-mitogen activated protein kinase (ERK-MAPK) General Toxicity: Cell viability via MTT assay	Theca cell GJIC quantification in both cell types (sheep and human) increased GJIC >100 ng/mL (pre-luteinized) Increased GJIC >100 ng/mL (luteinizing) Non monotonic response (luteinized) Signaling pathway analysis: None of the pathway inhibitors tested individually reduced GJIC below that of the vehicle control. Effects of protein kinase inhibitors on BPS-stimulated GJIC: Sheep theca cells: No effect of PKA or PKC inhibitors Full inhibition (= to control): MAPK inhibitor Partial inhibition with PI-PLC, PC-PLC, and ERK-MAPK inhibitors Human theca cells: Full inhibition (= to control): MAPK inhibitor General toxicity: No effect on cell viability or morphology in sheep or human cells.

Study Design	Outcomes Assessed	Major Findings
PKA activator (CW008) used to evaluate molecular pathways involved in BPS-induced upregulation of gap junctional intercellular communication (GJIC) Nonspecific negative control: Phorbol 12-myristate 13-acetate (TPA) (inhibits GJIC) by internalizing gap junction channels through PCK/ER1/2) dependent mechanisms		
Liu et al. 2021 *In vitro*: PND 0 CD-1 mice ovaries in culture medium, number of animals (or ovaries) was not reported but in the statistical analysis section it is stated that each experiment was repeated at least three times. Treatment: BPS (purity not reported) in culture medium at concentrations of 0, 10, 50, or 100 μM for 3 days. Co-treatment: A selective estrogen receptor (ER) modulator, tamoxifen, in culture medium at a final concentration of 0 or 1 μM. JNK inhibitor SP600125 in culture medium at a final concentration of 0 or 5 μM for 3 days. BPS treated ovaries (10 μM BPS) were incubated with a thymidine analog (proliferation marker) in the culture medium at 50 nM for 2 hours before assessment.	Follicular cell proliferation in *in vitro* treated ovaries at 10 μM Immunofluorescence and immunohistochemistry Gene and protein expression: ER (α and β); c-Jun N-terminal kinases (*JNKs*), *Notch2, Jagged1*, and *Cx43* (genes involved in germ cell nest (cyst) breakdown and the formation of primary follicles) Western blot analysis for JNK (phosphorylation) and E-cadherin (a key protein in the JNK signaling pathway)	Effects in follicles: Dose-dependent increase in percentage of MVH-positive cells (oocytes) in primary follicles BPS at all doses increased germ cell cyst breakdown, this effect was blocked by 1 μM tamoxifen and by 5 μM SP600125 Decreased percentage of oocytes in germ cell cysts No effect on total number of oocytes Gene and protein expression at 10 μM BPS: Increased *Er-α* and *Er-β* mRNA and protein levels Increased protein and gene expression of *Notch2*, *Jagged1*, and *Cx43* (granulosa cell proliferation) Decreased E-cadherin protein detection, effect reverted by 5 μM JNK inhibitor SP600125 Activation of JNK phosphorylation (counteracted by 1 μM tamoxifen) Components of JNK pathway: increased expression of MDM2 and decreased expression of E-cadherin; adding SP6000125 had the opposite effect
Profita et al. 2021 HTR-8/SVneo human trophoblast cell line (derived from human first-trimester placenta), used to measure adhesion, migration, and invasion Treatment: BPS (purity not reported) in DMSO at concentrations of 0 or 0.1 μM Co-exposure studies: Tamoxifen at 0.01 μM U0126 (MEK1,2 inhibitor) at 0.01 μM	Cell proliferation: E-SCREEN assay used to evaluate proliferative effect of BPS on HTR-8/SV cells Cell Migration Phosphorylation Inflammatory cytokine analyses Viability: MTT assay Western blot analysis to measure MAPK activity as phosphorylated / total ERK1/2 (% of phosphorylation with respect to control) Luminex assay (analysis of inflammatory cytokines and cytokine-induced vascular endothelial growth factor (VEGF)	E-SCREEN: Increased cell proliferation at 10^{-11}-10^{-7} M Increase in ERK1/2 phosphorylation at 10^{-9} M and 10^{-7} M Increase in IL-6 and IL-8 secretion at 10^{-11} M that was counteracted with U0126 inhibitor

Study Design	Outcomes Assessed	Major Findings
Pre-exposure to E2: Incubated with E2 (10^{-12}M-10^{-8} M) and then exposed to BPS for 3 days	Secretions of IL-6 (interleukin 6), IL-8 (interleukin 8), IL-1β (interleukin 1β), TNF-α (tumor necrosis factor-α), chemokine CCL2 (Chemokine Ligand 2), and VEGF	
Rosenmai et al. 2014 H295R cell line (human adrenal cell line) Treatment: BPS (purity not reported) in DMSO at concentrations of 0, or from 0.8 to 50 µM in 2-fold dilutions QSAR modeling	Cell viability H295R steroidogenesis assay Time-resolved fluorimmunoassay: E2, testosterone (T), and P HPLC/MS: P, 17β-OH P, dehydroepiandrosterone (DHEA), androstenedione T, dihydrotestosterone, corticosterone, cortisol, E2, and estrone (E1) ER activity: luciferase reporter gene assay. AR reporter gene assay Aryl hydrocarbon receptor reporter gene assay	No effect on cell viability Steroidogenesis: Increased P and 17-OH-P levels Decreased levels of T, androstenedione, and DHEA Decreased cortisol and corticosterone levels No effect on E2 or E1 levels Receptor activity assays: Increased ER activity NS trend on decreased AR activity; predicted negative in the QSAR modeling for AR activity.
Sabry et al. 2021 *In vitro*: cumulus–oocyte complexes (COCs), denuded oocytes, and cumulus cells from bovine ovaries. n= 40/group. Exp. 1 Oocyte culture. Treatment: BPS (purity not reported) in media with 0.1% ethanol at concentrations of 0 or 0.05 mg/mL for 24 hours. After 24 hours maturation, oocytes were used for imaging or for *in vitro* fertilization (IVF) incubation with untreated bovine sperm. After 18 hours, likely zygotes were separated from cumulus cells and incubated until at the 8-cell blastocyst stage. Exp. 2 Cumulus cell culture. Treatment: BPS in media. After 24 hours cells were used for mRNA and protein detection.	Confocal microscopy examination of COCs. IVF Gene and protein expression of connexins (Cx), i.e., gap junctional proteins: Cx37 and Cx43 in COCs, oocytes, and cumulus cells by mRNA and western blot analyses.	No change in COC morphology Accelerated embryo development, with hatched blastocysts at 8 days Increased Cx37 mRNA levels, with NS increase in protein levels in cumulus cells No effect on Cx43 or Cx37 mRNA levels in COCs No effect on Cx43 mRNA levels in oocytes or cumulus cells No effect on Cx37 mRNA levels in oocytes No changes in Cx37 protein levels in COCs or localization in cumulus cells (confocal microscopy)
Saleh et al. 2021 *In vitro*: COCs from bovine ovaries, 60 COCs per group Treatment: BPS (purity not stated) in 2.5 µl of 0.1% ethanol added to the *in vitro* maturation medium	On COCS, oocytes and cumulus cells: Gene and protein detection for anti-müllerian hormone (AMH) and its receptor (AMHRII) on each frozen sample.	**COCs, oocytes and cumulus cells** Increased AMHRII mRNA levels in COCs; no effects in oocytes or cumulus cells. NS increase in AMHRII protein expression in COCs; no effects in oocytes or cumulus cells

Study Design	Outcomes Assessed	Major Findings
supplemented with gonadotropins and E2 (2.5 mL final volume) at a BPS final concentration of 0 or 0.05 mg/mL for 24 hours. After incubation, 30 COCs were removed and washed with PBS and snap frozen. 30 denuded oocytes and the cumulus cells were isolated and snap frozen for further analysis. IVF produced embryos: After maturation, COCs from each group were incubated with bovine sperm for 18 hours. Presumptive zygotes were incubated until day-8 blastocysts.	In IVF embryos: Rates of development determined for: cleavage rate at 40–45 hpf 2–4 cells between 45 and 50 hpf 8–16 cells between 75 and 80 hpf blastocyst rates at 8 days post-fertilization Apoptosis rate Gene and protein expression of AMH and AMHRII Determination of sex ratio via real-time polymerase chain reaction (qPCR): expression of two transcripts that are only expressed in males (DDX3Y and USP9Y), blastocysts that did not express any of these transcripts were classified as female	No effect on AMH mRNA or protein level in COCs; no effects on protein levels in oocytes or cumulus cells AMH: No effect in mRNA levels on cumulus cells, oocytes or COCs **IVF embryos** No effects on development rates in IVF embryos. Apoptosis: Increased average percentage of DNA fragmentation (a marker for apoptosis) in arrested 2–4 cells; 8-16 cells and day-8 blastocysts Increased protein levels of AMH and AMHRII in day-8 blastocysts. No effect on sex ratio
Speidel et al. 2018 Human trophoblastic 3A placental cell line (CRL-1584) (This cell type represents the human placenta and is well characterized, and capable of synthesizing human chorionic gonadotropin in culture.) Treatment: BPS (99% purity) dissolved in 50% ethanol in culture medium, final ethanol concentration of 0.01%, at concentrations as described below: **Acute BPS exposure** (0 or 0.5 nM) for 90 min with samples at 0, 15, 30, 45, 60, and 90 mins. **Chronic BPS exposure** (0 or 0.3 nM) for 12 days. After 12 days of BPS exposure, cells were transferred to 6-well plates with exposure for 24 hours after transfection. Cells were harvested 36-48 hours after transfection with ABCB1 promoter haplotype luciferase reporter generation in CRL-1584 cells (luciferase constructs represents different haplotypes)	Cell viability Acute and chronic effects of BPS on ABCB1 promoter activity using 4 haplotypes: 1, 4, 29 and 30	No effect on cell viability after acute exposure ***Acute exposure*** Decrease in *ABCB1* promoter activity of haplotypes 4, 29 and 30 ***Chronic exposure*** Increase in *ABCB1* promoter activity of haplotypes 1, 29 and 30
Téteau et al. 2020 *In vitro:* Granulosa cells isolated from adult ewes; 1 × 10^5 viable cells/well Treatment: BPS (purity not reported) in ethanol added to	Cell proliferation Hormone secretion: P and E2 levels were measured in culture medium.	Decreased cell proliferation at 200 μM. Hormone secretion: Dose related decreased P secretion from 10 μM to 200 μM Increased E2 secretion at concentrations ≥ 10 μM.

Study Design	Outcomes Assessed	Major Findings
culture medium (150 µL/well) at concentrations of 0, 1 nM, 10 nM, 100 nM, 1 µM, 10 µM, 50 µM, 100 µM or 200 µM for 48 hours Cell proliferation: BPS treatment (as above) was supplemented with 10 µM bromodeoxyuridine/5-bromo-2'-deoxyuridine (BrdU) for 48 hours to determine effects on cell proliferation	Immunoblotting on protein lysates in six independent cultures for identification of steroidogenic enzymes (CYP19A1, CYP11A1 and HSD3B1) Gene expression in six independent cultures by qPCR for AR, PR, ER1, ER2, CYP19A1, CYP11A1, HSD3B1 and STAR	Steroidogenic enzymes: No effects on mRNA or protein levels for the three enzymes analyzed. Gene expression of hormone receptors: No effect on *AR* or *PR* mRNA levels Increased *ESR2* at 50µM and a significant two-fold increase in *ESR1* and *ESR2* at 100 µM
Téteau et al. 2023 *In vitro:* Granulosa cells isolated from adult ewes, 100,000 viable cells/well in 150 µL medium/well Treatment: BPS (purity not reported) in ethanol (1/1111) added to culture medium (150 µL/well) at concentrations of 0, 10 or 50 µM for 1, 12, 24, or 48 hours (time depending on the experiment). Granulosa cells were cultured with the plasma membrane ER (GPER) agonist, G-1, at 1 or 10 µM (positive control) or co-cultured with the GPER antagonist, G-15, at 10 µM in ethanol (same ethanol concentration as used for BPS treatment) At the end of culture time, supernatant and cells were separated for further analysis	Cell viability at 1 and 48 hours Hormone levels: P and E2 were measured in the supernatant after 48 hours of incubation with BPS (with or without 10 µM G-15) Gene expression after 1 hour incubation with 50 µM BPS qPCR and gene expression on differentially expressed genes (DEG) on granulosa cells treated for 12, 24 or 48 hours with BPS and/or 10 µM G-15	No effect on cell viability Decreased P levels at both BPS concentrations compared to control and compared to G-15 + BPS Decreased E2 levels at both BPS doses compared to control and compared to G-15 + BPS. Gene expression: 12 DEGs were identified at 50 µM BPS. Four genes were downregulated, and eight genes were upregulated. These DEG are involved mostly in cell communication.
Žalmanová et al. 2017 *In vitro*: COCs from pig ovary Treatment: BPS in 01% DMSO added to the culture medium at concentrations of 0, 3 nM, 300 nM or 30 µM for 24, 48 or 72 hours. After treatment, oocytes were separated from cumulus cells and fixed and the oocyte stage determined: germinal vesicle (GV), late diakinesis, metaphase I (MI), anaphase I, telophase I , and MII	Oocyte maturation stages: progression to MI and MII α-tubulin assembly Gene expression (mRNA) of *ERα, ERβ*, and aromatase in oocytes and cumulus cells treated for 48 hours. Content of hyaluronic acid (abundant extracellular compound produced by cumulus cells, and an indicator of chemical damage to cumulus cells or of cumulus cell expansion) Protein detection of ERα, ERβ, and aromatase in oocytes by immunofluorescence Cell viability	**Oocyte maturation:** Decreased MI stage achievement after 24 hours (dose dependent) Meiosis was not resumed after 24 hours at 300 nM and 30 µM Decreased MII stage achievement after 48 and 72 hours (concentration dependent) At 48 hours: all BPS-treated oocytes mature to at least MI At 72 hours: delayed, disrupted and blocked oocyte maturation at all concentrations tested α-tubulin assembly: Swollen chromosomes and irregular organization, Decreased numbers of tubulin filaments, spindles in a circular formation, elongated metaphase plates, and reduced spindle size at all doses in MI and MII oocytes **Gene expression:** Decreased Erα mRNA levels in oocytes at all concentrations; no effect on

Study Design	Outcomes Assessed	Major Findings
		cumulus cells Decreased *Erβ* mRNA levels at 30 μM in cumulus cells; no changes in oocytes Decreased aromatase mRNA levels at 3 and 300 nM in oocytes and at 30 μM in cumulus cells Increased hyaluronic acid content at 300 nM at 24 and 48 hours **Immunofluorescence in oocytes:** Increased ERα at 3 and 300 nM (24 and 48 hours) Increased ERβ at 300 nM and 30 μM (24 hours) Decreased ERβ at 300 nM (48 hours) Decreased aromatase at 3 nM (24 hours) No effects on viability of oocytes or cumulus cells after 24 and 48 hours
Žalmanová et al. 2023 *In vitro:* COCs from pig ovaries. Oocytes were cultured in modified M199 medium in the presence of gonadotropins to stimulate oocyte maturation. Treatment: BPS (purity not reported), dissolved in 0.1% DMSO, (control) at concentrations of 0, 300 pM, 30nM, or 3 μM for 48 hours. MII oocytes with extrusion of first polar body were selected for IVF. 15-20 MII oocytes were incubated in modified Tris-buffered medium with sperm from untreated males.	Cell viability Meiotic progression of porcine oocytes Spindle organization and chromosome alignment Chromosome spreads of MII pig oocytes (aneuploidy) TUNEL assay (DNA integrity, apoptosis) Mitochondria status (by immunofluorescence): number and distribution Mitochondrial fusion proteins (mitofusins, MFN1 and MNF2) Glycolytic activity of cumulus cells	Concentration dependent decrease in proportion of cells reaching MII: retention in GV stage, degenerated oocytes, arrested in MI stage. No effect on the number or quality of blastocysts that completed MII maturation. Spindle organization: Spindle disorganization and chromosome misalignment at all concentrations. Number of chromosomes: Increased number of aneuploidy oocytes at all concentrations. DNA integrity: No effects on DNA damage or apoptosis No effects on the quality of blastocysts or in the number of double strand breaks (DSBs) in blastocysts Mitochondria number and distribution: Lower relative intensity (about 30% decrease) of the marker for mitochondria at 30 nM and 3 μM Relative increase in fluorescence signal for MFN1 and MFN2 at 30 nM No effects on glucose metabolism Increased cell viability at both BPS doses (in cultures without serum)

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APPENDIX A. LITERATURE SEARCH APPROACH ON THE FEMALE 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 and July 2023. The goal was to identify peer- 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.

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 1/2023 7/2023	#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

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

Search Dates	Search step	Search Concepts
1/2022 1/2023 7/2023	#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	(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	643	583	596	131	9	816
Animal DART	422	499	397	102	16	584
ADME	236	489	505	none	none	759

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 female reproductive toxicity

For purposes of identifying the available evidence on the female reproductive toxicity of BPS, citations identified as having a reasonable possibility of containing information relevant to female reproductive toxicity 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).

One hundred and twenty-four references were cited in this document.

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	PubMed DART strategy

SET #	STRATEGY	CONCEPT GROUP
	[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] 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	Additional DART terms (OEHHA Strategy)

SET #	STRATEGY	CONCEPT GROUP
	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 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	

SET #	STRATEGY	CONCEPT GROUP
	"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	"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	Key Characteristics of Male Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	"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]) 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	

SET #	STRATEGY	CONCEPT GROUP
	"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	Key Characteristics of Female Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	"plasma membrane receptor*"[tiab] OR "prolactin receptor*"[tiab] OR "reproductive hormone*"[tiab] OR "sex 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	

SET #	STRATEGY	CONCEPT GROUP
	"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 ("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	

SET #	STRATEGY	CONCEPT GROUP
	"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 "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	PubMed DART strategy

SET #	STRATEGY	CONCEPT GROUP
	[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] 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	

SET #	STRATEGY	CONCEPT GROUP
	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	Additional DART terms (OEHHA Strategy)

SET #	STRATEGY	CONCEPT GROUP
	"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 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	

SET #	STRATEGY	CONCEPT GROUP
	"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	"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	Key Characteristics of Male Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	"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]) 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]	

SET #	STRATEGY	CONCEPT GROUP
	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	Key Characteristics of Female Reproductive Toxicity

SET #	STRATEGY	CONCEPT GROUP
	"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 "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	

SET #	STRATEGY	CONCEPT GROUP
	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 "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-	

SET #	STRATEGY	CONCEPT GROUP
	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 "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])	

SET #	STRATEGY	CONCEPT GROUP
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 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])	Experimental Animals (RoC modified)
9	#7 AND #8	Combine Chemical +

SET #	STRATEGY	CONCEPT GROUP
		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

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

Study/ Design*	Date of Collection/ Sample size	LOD (ng/mL)/ Adjustment for <LOD	% samples with BPS detected	Matrix, Median BPS (ng/mL) **	25th, 75th Percentile (ng/mL)	Results***
Gestational Diabetes Mellitus						
Tang et al. 2021 Case-control	2015-2021 n = 500	0.046 LOD÷√2	82.2	Serum, 0.097	0.05, 0.107	Higher odds of GDM for women with female fetuses or high BMI
Zhang et al. 2019 Cohort	2013-2015 n = 1,841	0.2 LOD÷√2	90.1	Urine, 0.31	0.14, 0.81	No significant results with GDM
Zhu et al. 2022 Case-control	2015-2017 n = 333	0.1 LOD÷√2	Cases: 83.8 (TM1) 88.2 (TM2) Controls: 84.2 (TM1) 81.7 (TM2)	Urine, Cases: 0.6 (TM1) 0.5 (TM2) Controls: 0.4 (TM1) 0.4 (TM2)	Cases: 0.3, 1.3 (TM1) 0.2, 1.2 (TM2) Controls: 0.2, 0.9 (TM1) 0.2, 1.1 (TM2)	Higher odds of GDM for total population, with even higher odds for women identifying as non-Asian/Pacific Islanders
Thyroid Hormones During Pregnancy						
Aker et al. 2018 Case-control	2006-2008 n = 439	0.1 ‡ LOD÷√2	26.2 ‡	Urine, <LOD	<LOD, no mention	Exposure dichotomized above/below LOD; FT4 lower at < 15 weeks, TSH higher for term births at < 15 weeks
Derakhshan et al. 2019 Cohort	2007-2010 n = 1,996	0.03 Reported concentrations < LOD used	80.2	Urine, 0.08	No mention	No significant results with thyroid hormones
Derakhshan et al. 2021 Cohort	2002-2006 n = 1,267	0.05 LOD÷√2	66.6 (TM1) 29.8 (TM2) 18.9 (TM3)	Urine, 0.34 (TM1) 0.24 (TM2) NA (TM3)	< LOD, 8.83 (TM1) < LOD, 1.69 (TM2) NA (TM3)	Higher TT4 in early pregnancy
Huang et al. 2022 Cohort	2015-2018 n = 446	0.046 LOD÷√2	86.3	Serum, 0.097	0.096, 0.104	Lower TT3 in TM1, and higher FT3 in TM2

Study/ Design*	Date of Collection/ Sample size	LOD (ng/mL)/ Adjustment for <LOD	% samples with BPS detected	Matrix, Median BPS (ng/mL) **	25th, 75th Percentile (ng/mL)	Results***
Sex Steroid Hormones and Related Proteins During Pregnancy						
Aker et al. 2019 Cohort	2012-2017 n = 602	0.1 LOD÷√2	96.6 (TM2a) 91.4 (TM2b)	Urine 0.50 (TM2a) 0.47 (TM2b)	0.23, 1.07 (TM2a) 0.23, 1.06 (TM2b)	Lower CRH
Wang et al. 2022 Cohort	2012 n = 528	0.004 LOD÷2	24.2	Urine, 0.01 µg/g	< LOD, 0.03 µg/g	No significant results with kisspeptin
Sex Steroid Hormones and Related Proteins in Young Girls						
Hu et al. 2022 Cross-sectional	2013-2016 n = 1,179	0.1 LOD÷√2	88.3	Urine, 0.37	0.19, 0.74	No significant results with sex hormones
Wang et al. 2021 Cross-sectional	2013-2016 n = 1,317	0.1 LOD÷√2	88.4	Urine, 0.3	IQR = 0.6	Higher TT/E2 ratio, inverted U-shape dose-response
Wang et al. 2022 Cohort	2012 n = 195	0.004 LOD÷2	24.2	Urine, 0.01 µg/g	< LOD, 0.03 µg/g	No significant results with kisspeptin
Sex Steroid Hormones in Women						
Zhan et al. 2023 Case-control	2014-2016 n = 733	0.003 LOD÷√2	93.2	Urine, 0.12	0.02, 0.49	Higher levels of TT
Polycystic Ovary Syndrome						
Jurewicz et al. 2020 Case-control	2017 n = 357	0.022 LOD÷√2	95	Urine, GM 0.14 (cases) GM 0.08 (controls)	Not mentioned	Higher odds of PCOS (inference not interpretable)
Zhan et al. 2023 Case-control	2014-2016 n = 733	0.003 LOD÷√2	93.2	Urine, 0.12	0.02, 0.49	Higher odds of PCOS
Other Female Reproductive Outcomes						
Ao et al. 2022 Case-control	2014-2016 n = 1,751	0.003 LOD÷√2	82.5	Urine, 0.11 µg/g	0.01, 0.48 µg/g	Higher odds of unexplained recurrent miscarriage for women at or over 30 years of age

Study/ Design*	Date of Collection/ Sample size	LOD (ng/mL)/ Adjustment for <LOD	% samples with BPS detected	Matrix, Median BPS (ng/mL) **	25th, 75th Percentile (ng/mL)	Results***
Blaauwendraad et al. 2022 Cohort	2004 – 2005 n = 524	0.15 LOD÷√2	68.5 (TM1) 29.7 (TM2) 20 (TM3)	Urine, 0.6 (TM1) < LOD (TM2) < LOD (TM3)	< LOD, 2.4 (TM1) < LOD, 0.3 (TM2) < LOD, < LOD (TM3)	Increased age at first menstruation
Peinado et al. 2020 Case-control	2018-2019 n = 124	0.2 LOD÷2	14.8	Urine, < LOD	< LOD, < LOD	No significant results with endometriosis
Philips et al. 2018 Cohort	2002-2006 n = 877	0.05 LOD÷√2	70.5	Urine, 0.35	0.17, 1.03 ‡‡	No significant results with fecundability
Philips et al. 2019 Cohort	2004-2005 n = 1,233	0.05 LOD÷√2	69.5	Urine, 0.35	0.17, 1.03 ‡‡	No significant results with gestational hypertension measures
Philips et al. 2020 Cohort	2002-2006 n = 1,213	0.05 LOD÷√2	68.1 (TM1) 29.1 (TM2)	Urine, 0.35 (TM1) 0.24 (TM2)	0.17, 1.09 (TM1) 0.12, 0.49 (TM2)	Lower gestational weight gain in early pregnancy
Wesselink et al. 2021 Case-cohort	2010-2012 n = 754	0.1 Reported concentrations < LOD used	95.4 (Visit 1) 94.3 (Visit 2) 95.9 (Visit 3)	Urine, 0.5 (Visit 1) 0.6 (Visit 2) 0.8 (Visit 3)	IQR not reported 90th 2.6 (Visit 1) 90th 2.5 (Visit 2) 90th 3.5 (Visit 3)	Reduced fibroid risk
Yue et al. 2023a Cross-sectional	2017 n = 86	0.002 LOD value used	97.7	Urine, 0.07	0.03, 0.15	No significant results with adult thyroid hormones
Zhan et al. 2022 Cross-sectional	2013-2016 n = 857	0.1 LOD÷√2	90.4	Urine, 0.60	0.20, 1.30	No significant results with infertility
Zhang et al. 2023 Cross-sectional Study	2020-2021 n = 111	0.1 LOD÷√2	60 > LOQ	Urine, 0.32 µg/g	0.19, 0.55 µg/g	Decreased AMH and increased odds of diminished ovarian reserve

Abbreviations: AMH = Anti-Müllerian hormone, BPS = bisphenol S, BMI = body mass index, CRH = corticotropin-releasing hormone, GDM = gestational diabetes mellitus, GM = geometric mean, FT3 = free triiodothyronine , FT4 = free thyroxine, E2 = estradiol, IQR = interquartile range, LOD = limit of detection, LOQ = level of quantification, PCOS= polycystic ovary syndrome , NA = not applicable, TSH = thyroid-stimulating hormone, TM1 = trimester 1, TM2 = trimester 2, TM3 = trimester 3, TT = total testosterone, TT4 = total thyroxine, TT3 = total triiodothyronine.

*Studies ordered by outcome, then by author.

** Mean is reported only when median wasn't available.

*** Brief overview of summary results focused on statistically significant findings. See Table 4.1 for complete reporting of methodology and results for each study.

‡ Mistakes were noted in the methods section where the BPS LOD is given as 0.1 ng/mL in text and 0.4 ng/mL in Table 2 of the study. The percentage of samples with BPS above the LOD was given as <25% in text and given as 26.2% in Table 2.

‡‡ Median, 25th, and 75th percentile values reported to be the same in Phillips et al (2018) and Phillips et al (2019) despite differing analytic sample size