Evidence on the Female Reproductive Toxicity of Bisphenol S (BPS)

Developmental and Reproductive Toxicant Identification Committee

December 12, 2023

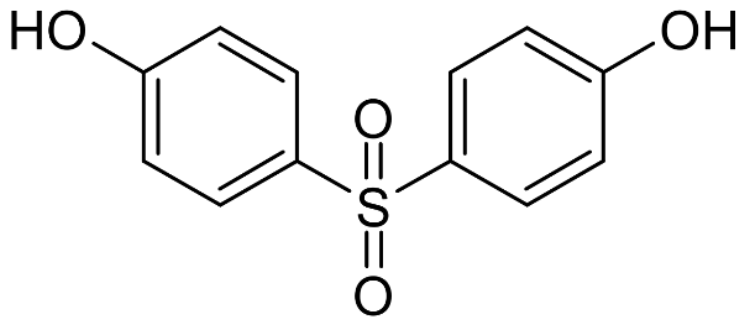
Reproductive Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment, CalEPA

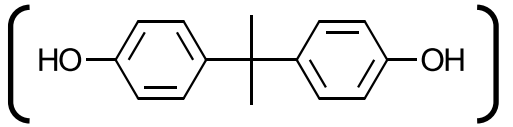
CalEPA
California Environmental Protection Agency

Outline

- Introduction
- Pharmacokinetics of BPS
- Female reproductive toxicity data
  - Animal studies
  - Mechanistic data
    - Key characteristics of female reproductive toxicants
  - Human studies
- Summary

Bisphenol S (BPS)



- Synonym: 4,4’-Sulfonyldiphenol
- BPS is an analog of Bisphenol A 

Uses, occurrence, and exposure

Use:

- Thermal paper, hard plastic items, synthetic fibers for clothing and textiles

Production:

- As of 2019, 1 to 10 million pounds per year in US
- Manufacturers have been gradually replacing BPA with BPS

Exposure:

- BPS has been detected in cash register receipts, food, personal care products, and environmental samples of dust and sediment
- Detection frequency in Biomonitoring California studies (2018 – 2020): 64.4% - 76.7%

Literature search and screening

- Conducted literature searches on the developmental and reproductive toxicity of BPS
- Used HAWC (Health Assessment Workspace Collaborative) as a tool for multi-level screening of literature search results
  - Level 1: title and abstract
  - Level 2: full text
  - Focused on literature relevant to female reproductive toxicity

Pharmacokinetics of BPS

- BPS is rapid absorbed
- Distributed throughout the body:
  - Detected in human cord blood, amniotic fluid, breast milk and placenta
  - Detected in rats in gastrointestinal track tissues, liver, kidney, heart, spleen, lung, and muscle
- Metabolized via glucuronidation, sulfation, and hydroxylation to form BPS-glucuronide, BPS-sulfate, and hydroxylated BPS
- Excreted in urine, feces, breast milk in humans

Estimated half-life	Hours
Humans	**7 – 9**
Mice	**3 – 4**
Rats	**4 – 12**

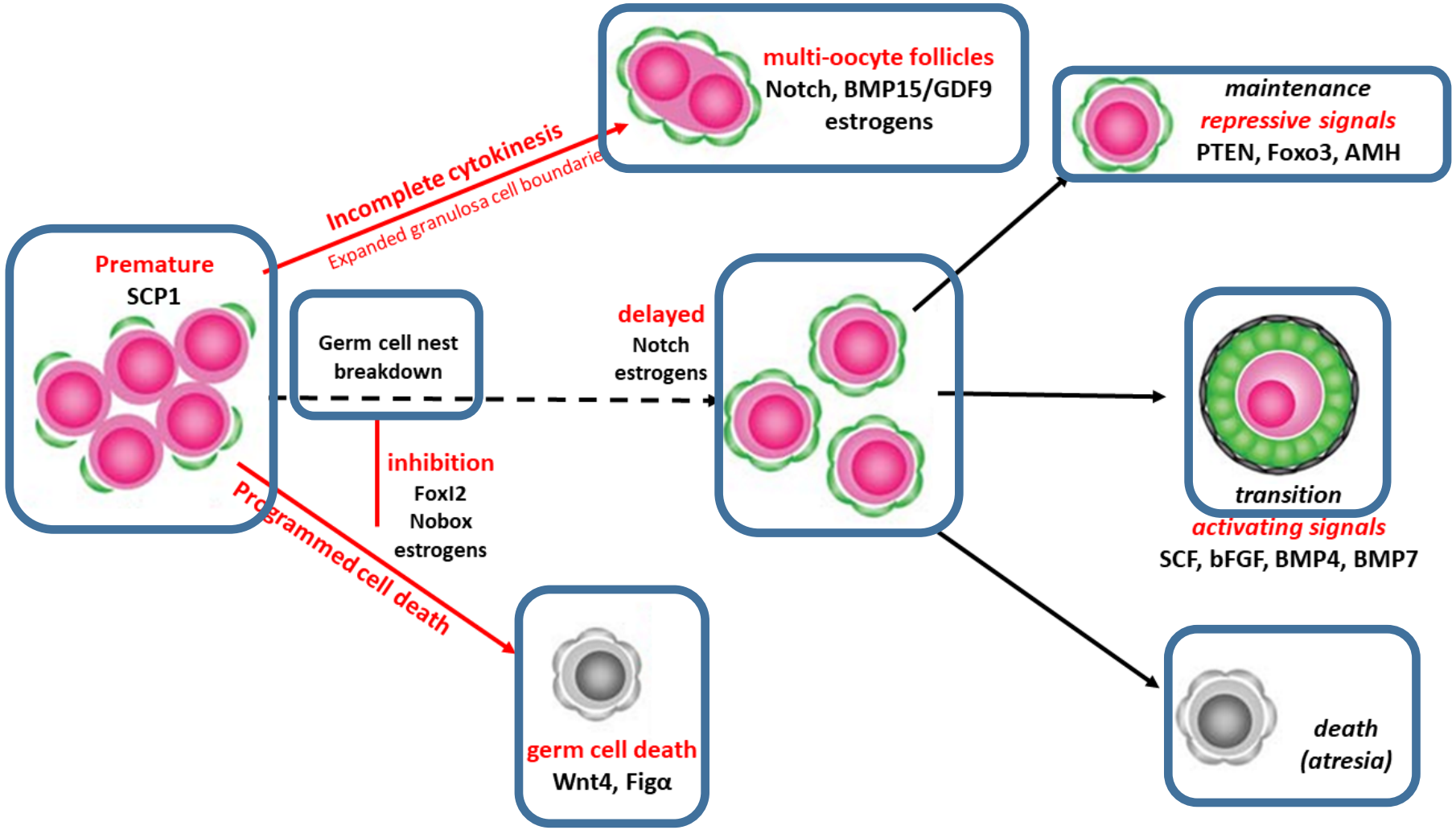
Effects of BPS on Female Reproductive Toxicity

Animal Studies

BPS: Animal findings

- *Ovarian histology*
- *Uterine effects*
- *Endocrine effects*
- *Reproductive performance*
- *Mammary gland development*
- *Alteration in puberty onset*

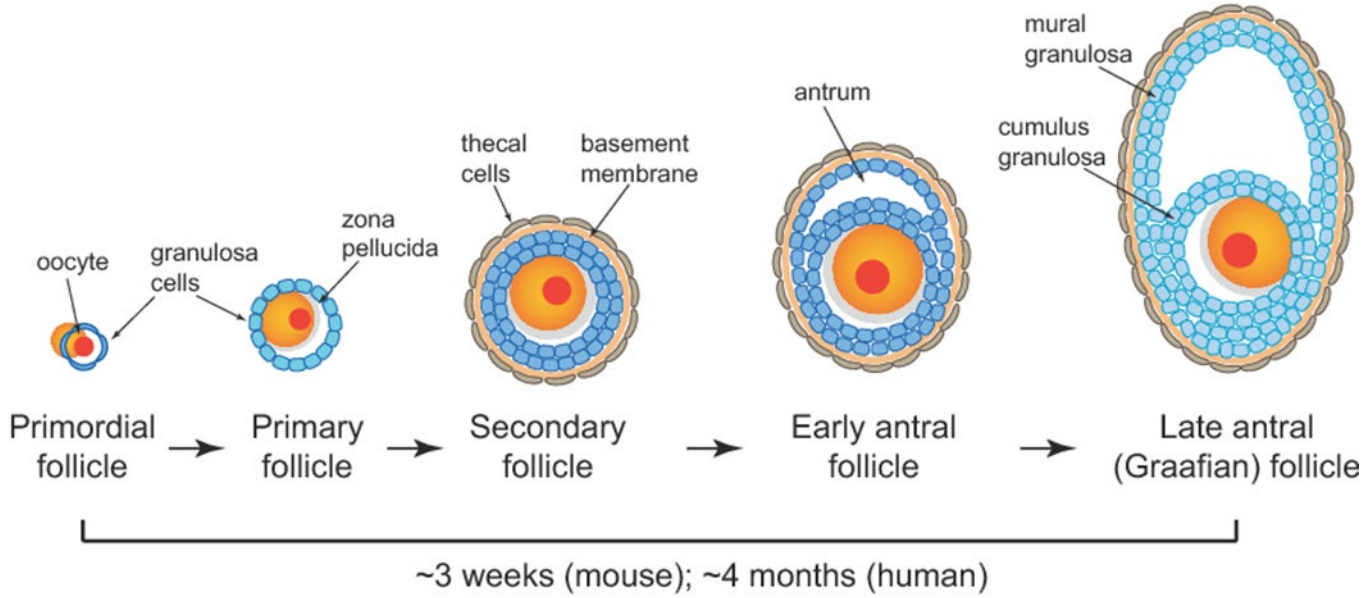
Ovarian follicle development



(Adapted from Tingen et al.: Mol Hum Reprod. 2009 Dec; 15(12): 795–803.)

Oocyte development in the ovary

Oocyte and follicular growth



Adapted from Clarke H. 2017.
Results Probl Cell Differ 63:17-41.

BPS: Animal findings

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Ovarian histology

BPS effects on ovarian structures:

- Germ cell nest breakdown
- Primary, secondary, antral, or atretic follicles
- Granulosa cell layers
- Corpora lutea

BPS effects on oocytes:

- Meiotic progression
- Damaged oocyte structure, spindle malformations

Ovarian histology: Alterations in follicle development

- Timing of germ cell nest breakdown in mice from 0.5 to 20 µg/kg-day (*ip injection,* Liu et al. 2021; Shi et al. 2019a; Zhang et al. 2020)
- ↓ Number of primary, secondary, and antral follicles:
  - Mice at 10 µg/kg-day (*ip injection,* Liu et al. 2021),
  - Hamsters at 150 mg/kg-day (Pal et al. 2023)
- ↓ Preovulatory follicles in chickens at 50 µg/kg-day (Eldefrawy et al. 2021)
- ↑ Number of secondary follicles in mice at 200 µg/kg-day (Hill et al. 2017)
- ↑ Number of atretic follicles, and immature follicle atrophy in mice at 300 µg/kg-day (Yue et al. 2023)
- ↑ Number of atretic follicles in rats at 50 mg/kg-day (*sc injection*, Ashan et al. 2018) and hamsters at 150 mg/kg-day (Pal et al. 2023)

Ovarian histology (continued)

- ↓ Number of granulosa cell layers in mice at 10 and 100 µg/kg-day (Nevoral et al. 2018)
- ↓ Number of *corpora lutea* in:
  - Rats at 5 µg/kg-day (Kaimal et al. 2021; *sc injection*, Ashan et al. 2018; *ip injection,* Ijaz et al. 2020 at 500 µg/kg-day)
  - Hamsters at 150 mg/kg-day (Pal et al. 2023)

Ovarian histology (continued)

Effects on oocytes in mice:

- Accelerated meiotic progression from 2 to 200 µg/kg-day (Zhang et al. 2020)
- ↑ Number of abnormal oocytes at 0.1 and 10 µg/kg-day (Prokešová et al. 2020; Nevoral et al. 2018)
- Damaged oocyte structure at 300 µg/kg-day (Yue et al. 2023)
- Spindle damage and spindle malformations from 0.01 to 100 µg/kg-day (Nevoral et al. 2018; Prokešová et al. 2020; Zhang et al. 2020)

Ovarian histology (continued)

Effects on oocytes in Zebrafish:

- Altered oocyte maturation at 1, 10 and 100 µg/L (Wang et al. 2020; Qin et al. 2021)
- Oocyte degeneration at 40 and 200 µg/mL (Park et al. 2022)

Also:

- ↓ Gonadosomatic index in treated adult females at 0.5, 5, and 50 µg/L (Ji et al. 2013) and when treated as embryos at 100 µg/L (Naderi et al. 2014)

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Uterine effects

- Narrowing of the uterine cavity,
- ↓ Endometrial area,
- ↑ Number of uterine glands, in mice at 300 µg/kg-day (Yue et al. 2023), and at 30 mg/kg-day (Benjamin et al. 2023)
- Squamous metaplasia in rats at 1,000 mg/kg-day (BASF 2014a)
- Cell vacuolization in rats at 1 µg/kg-day (*sc injection,* Demacopulo and Kreimann 2019)
- Alteration in relative uterine weights in rats:
  - ↓ at 50 mg/kg-day (*sc injection,* Ahsan et al. 2018)
  - ↑ at 300 mg/kg-day (ECHA 2019) and at 20 and 500 mg/kg-day (*sc injection,* Yamasaki et al. 2004)

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Endocrine effects

Gonadotropins:

- ↓ Levels of luteinizing hormone (LH) and follicle-stimulating hormone (FSH):
  - Rats at 50 mg/kg-day (*sc injection*, Ahsan et al. 2018; *ip injection,* Ijaz et al. 2020),
  - Mice from 1 to 100 µg/kg-day (*sc injection,* Nourian et al. 2020)
  - Hamsters at 150 mg/kg-day (Pal et al. 2023)
- ↓ *gnrh3* and *fshβ* gene expression in zebrafish brain at 50 µg/L (Ji et al. 2013)

Progesterone:

- Changes in progesterone levels were observed in rats, mice, ewes, golden hamsters, and zebrafish
- ↑ in progesterone receptor (PR) expression in mammary gland of mice exposed during gestation and lactation at 2 or 200 µg/kg-day (Kolla et al. 2018)

Endocrine effects: Estradiol (E2)

- ↓ Serum E2 in:
  - Mice from 2 to 200 µg/kg-day (LaPlante et al. 2017; Nevoral et al. 2018)
  - Hamsters at 150 mg/kg-day (Pal et al. 2023)
- ↓ Plasma E2 in:
  - Rats at 5 and 50 mg/kg-day (*ip injection,* Ijaz et al. 2020),
  - Ewes at 50 µg/kg-day (Téteau et al. 2022)
- ↓ Urinary E2 levels in mice at 25.2 or 79.8 mg/kg (*sc injection*, Pollock et al. 2019)
- ↑ Serum E2 levels in mice on PND 60 at 50 µg/kg or 10 mg/kg every three days (*sc injection,* Shi et al. 2017), and on PND 20 at 0.5 and 5 mg/kg twice a day (Tucker et al. 2018)
- ↑ Mammary gland expression of estrogen receptor alpha (Erα) in mice (Kolla and Vandenberg 2019)

E2 effects (Continued)

Zebrafish:

- ↑ Plasma E2 levels at 10 or 100 µg/L (and vitellogenin) (Naderi et al. 2014); at 50 µg/L (Ji et al. 2013)
- ↑ Whole-body E2 levels at 8, 40, and 200 µg/mL (Park et al. 2022)
- ↓ ERα (and vitellogenin) mRNA levels at 200 µg/mL, and non-significant changes in ER beta (ERβ) mRNA levels (Park et al. 2022)

Endocrine effects (Continued)

Testosterone (T) in females

- ↑ Serum T levels in mice:
  - On postnatal day (PND) 28 at 0.05 mg/kg twice a day (Tucker et al. 2018), and at 9 months of age (daily dose) (Shi et al. 2019)
  - On PND 60 at 10 mg/kg - every three days (*sc injection,* Shi et al. 2017)
- ↑ Plasma T levels in rats at 50 mg/kg-day (Ahsan et al. 2018), at 5 and 50 mg/kg-day (*ip injection,* Ijaz et al. 2020)
- ↓ Serum T levels on PND 35 in mice at 0.5 and 5 mg/kg twice a day (Tucker et al. 2018)

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Reproductive performance

Estrous cycle irregularity (gestational exposure):

- In pubertal mice at 0.5, 20, or 50 µg/kg-day (Shi et al. 2019)
- In adult rats at 180 mg/kg-day (BASF 2019a) and at 300 mg/kg-day (ECHA 2019)

Fertility:

- ↓ Percentage of female rats that conceived (60% vs 100% in controls) at 50 mg/kg-day (*sc injection,* Ahsan et al. 2018)
- ↓ Fertilization at 10 µg/kg-day and ↑ fertilization at 100 µg/kg-day in mice exposed during puberty (Nevoral et al. 2018)
- ↓ Fertilization (IVF) and blastocyst development rates, from mice exposed at 10, 50 and 100 µg/kg-day (*ip injection*, Nourian et al. 2017)

Implantation:

- ↓ Mean number of implantation sites in rats at 300 mg/kg-day (ECHA 2019)
- ↑ Rate of post-implantation loss in rats exposed for 14 weeks from premating to lactation at 300 mg/kg-day (ECHA 2019)

Reproductive performance (Continued)

Effects on placenta:

- ↓ Ratio of spongiotrophoblast to giant cell area in mice at 200 µg/kg-day for 2 weeks prior to mating and through GD 12.5 (Mao et al. 2020)
- Non-significant ↑ in placental weight in sheep at GD 120, and no effect on placental stereology or number of placentomes per placenta at 0.5 mg/kg-day (*sc injection,* Gingrich et al. 2018)

Reproductive performance (Continued)

Zebrafish:

- ↓ Number of eggs and ↓ hatching rate,
- ↑ Time to hatching of embryos at 10 and 100 µg/L (Naderi et al. 2014)
- Altered spawning behavior, i.e., debilitated female shoaling at 100 µg/L (Wang et al. 2020)

C. elegans:

- ↑ Embryonic lethality
- ↓ Brood size at 125 and 500 µM (Chen et al. 2016b)

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Mammary gland development in mice

Growth of terminal end buds (TEB):

- ↑ TEB counts on PND 20 at 0.05 - 5 mg/kg twice a day and at 3 months at 0.5 mg/kg twice a day (Tucker et al. 2018)
- Larger TEB area at 2 µg/kg-day; ↑ average TEB size on PND 24 (Kolla et al. 2018)
- ↑ TEB-like structures at 9 weeks at 2 and 200 µg/kg-day (Kolla et al. 2018)

Mammary gland development in mice (continued)

Alveolar buds:

- ↑ Number of alveolar buds at 200 µg/kg-day and ↑ incidence of intraductal hyperplasia at 2 and 200 µg/kg-day at 9 weeks (Kolla et al. 2018)
- Inflammation at 3 and 14 months, non-neoplastic lesions, and lobuloalveolar hyperplasia at 0.5 mg/kg twice a day (Tucker et al. 2018)

Mammary gland development in mice (continued)

- ↓ Cell proliferation on PND 24 at 2 µg/kg-day,
- ↑ Proliferation in adults at 2 µg/kg-day (Kolla et al. 2018)
- ↓ Ductal area at 2 µg/kg-day on PND 31 (Kolla and Vandenberg 2019)
- ↑ Mammary gland developmental score on PND 20 (5 mg/kg twice a day), on PND 35 at all doses, and on PND 56 (0.5 mg/kg twice a day) (Tucker et al. 2018)
- ↓ Volume of lobules,
- ↑ Volume of adipose tissue at 200 µg/kg-day (LaPlante et al. 2017)

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Alteration in puberty onset

- Delayed puberty onset in mice at 20 µg/kg-day,
- Earlier onset in mice at 0.5 µg/kg-day (Shi et al. 2019)
- Delayed onset in rats exposed perinatally at 50 - 60 mg/kg-day (*sc injection,* Ahsan et al. 2018) and (BASF 2019a)
- No effects in other studies with similar dosing strategies

Effects of BPS on Female Reproductive Toxicity

Mechanistic Studies

Mechanistic considerations and other relevant data

Effects on ovarian development and maturation of oocytes

- Oocyte maturation
- Spindle morphology and chromosome alignment
- Follicular cells

Effects on the placenta

Effects on the endocrine system (hormone levels, receptor levels, mRNA)

Ovarian development and oocyte maturation: *in vivo* evidence

- Chromosome misalignment and/or spindle malformation
  - Mice (Nevoral et al. 2018; Zhang et al. 2020) and *C. elegans* (Chen et al. 2016b)
- Altered gene expression
  - Mice (Liu et al. 2021; Prokešová et al. 2020; Yue et al. 2023b; Zhang et al. 2020)
  - Chickens (Eldefrawy et al. 2021)
  - Zebrafish (Qin et al. 2021)
- Oxidative stress in rodents (Nevoral et al. 2018; Nourian et al. 2017, 2020; Ijaz et al. 2020)
- Germline 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 signaling pathways in mice related to cyst breakdown and primordial follicle assembly (Liu et al. 2021)
- Effects on lipid profiles in Zebrafish (Qin et al. 2021)

Oocyte maturation: *In vitro evidence*

BPS altered germ cell nest breakdown and meiotic progression in oocytes

- Newborn mouse ovaries cultured for 3 days with 10-100 µM BPS (Liu et al. 2021)
  - Blocked by Tamoxifen
- Pig ovary cumulus-oocyte complexes exposed to 300 nM or 30 µM BPS (Žalmanová et al. 2017)
  - Concentration-dependent decrease in oocytes reaching metaphase I (MI)
  - Concentration-dependent decrease in progression to metaphase II at 48 and 72 hours
  - All BPS-treated oocytes matured to at least the MI stage by 48 hours; at 72 hours the maturation process was delayed, disrupted, and/or blocked at all concentrations

Spindle morphology and chromosome alignment: *In vitro* evidence

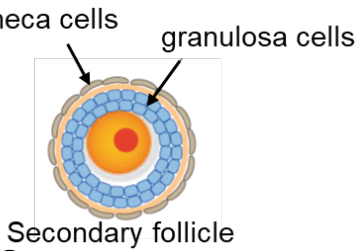
BPS caused alterations in spindle morphology and chromosome alignment in oocytes

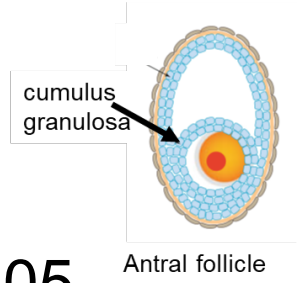
- In cow oocytes treated with 1 fM-50 µM BPS: in metaphase 2 oocytes (above 1 nM) (Campen et al. 2018a)
- α-Tubulin assembly alterations in pig ovary cumulus-oocyte complexes treated with BPS at 3 nM, 300 nM or 30 µM for 24 or 48 hours (Žalmanová et al. 2017)
  - ↓ Number of tubulin filaments leading to swollen and misalignment chromosomes, elongated metaphase plates, and ↓ spindle size at all doses in MI and MII oocytes
  - ↓ Proportion of oocytes reaching MII in 48-hour group
  - ↑ Degenerated oocytes, retention in the germinal vesicle (GV) stage, spindle disorganization

Follicular cells: *In vitro evidence*

BPS caused changes in follicular cell communication and proliferation

- Ovarian theca cells from humans and sheep:
  - ↑ Gap junction intercellular communication (GJIC; >100 ng/mL) up to 1000 ng/mL (sheep) or 200 ng/mL (humans) (Gingrich et al. 2021)
  - Full inhibition of BPS effect by a MAPK inhibitor; partial inhibition with inhibitors of PI-PLC, PC-PLC, and ERK-MAPK (Gingrich et al. 2021)
- ↓ Granulosa cell proliferation in sheep cells at 200 µM for 48 hours (Téteau et al. 2020)
- ↑ Cx37 mRNA expression in cumulus cells from cow ovaries at 0, or 0.05 mg/mL for 24 hours (Sabry et al. 2021)
- No effect on Cx43 or Cx37 mRNA or protein expression in cumulus-oocyte complexes (Sabry et al. 2021)





Effects on the placenta: *In vivo* evidence

BPS altered autocrine and paracrine signaling in the placenta

- Mice (Mao et al. 2020)
  - Altered gene expression:
    - Upregulation of *Actn2* (actinin α2)
    - Downregulation of 10 genes, including *Calm4* (calmodulin 4) and *Guca2a* (guanylate cyclase activator 2a, guanylin)
  - ↓ Fatty acids including stearic, palmitic, docosahexaenoic, octadecenoic, and palmitic acid
  - Altered neurotransmitters:
    - ↑ Dopamine levels and dopamine-positive giant cells
    - ↓ Serotonin levels and serotonin-positive giant cells
- Sheep (Gingrich et al. 2018)
  - ↓ e-cadherin protein in GD 120 placentomes
  - ↓ Trophoblast derived binucleate cells
  - ↓ Expression of fusogenic genes: JSRV and HYAL2
  - ↑ Transcription factor glial cell missing factor 1

Effects on the placenta: *In vitro* evidence (continued)

The ERK1/2 pathway has been identified as one of the pathways that affects cell proliferation after BPS exposure

- HTR-8/SVneo cell line (human trophoblast cells) (Profita et al. 2021)
  - ↑ Cell proliferation mediated through the ER and ERK1/2 pathways
  - Inhibition of ERK1/2 phosphorylation induced secretion of IL-6 and IL-8
- CRL-1584 cell line (trophoblastic 3A human placenta) (Speidel et al. 2018)
  - Altered ABCB1 promotor activity (ABCB1 encodes placental transporter P-glycoprotein)

Effects on the endocrine system: *In vivo evidence*

BPS altered hormones involved in reproduction and development in whole animals

- ↓ Gonadotropins (FSH and LH) in rodents
- ↓ P levels in rodents
- ↑ P in zebrafish and mice
- Altered plasma T in rodents
- ↓ Dihydrotestosterone and 11-dehydrocorticosterone in preovulatory follicular fluid levels in ewes
- ↓ Plasma E2 in rodents and ewe and ↑ plasma E1 in ewes
- ↑ E2 levels in mice (serum) and zebrafish (plasma, whole body)
- ↑ E2 preovulatory follicular fluid levels in ewes

Effects on the endocrine system: *In vitro* evidence

BPS altered hormones involved in reproduction

- ↓ Progesterone:
  - Human granulosa cells, 10 and 50 µM (Amar et al. 2020)
  - Ewe granulosa cells, 10-200 µM (Téteau et al. 2020)
  - Ewe granulosa cells, 10 or 50 µM (Téteau et al. 2023)
- ↑ Progesterone:
  - Human adrenal-derived H295R cells, 0.8-50 µM (Rosenmai et al. 2014)
- No change in progesterone secretion
  - Cow theca cells (Campen et al. 2018)
- Androgens
  - ↓ Testosterone, androstenedione, and dehydroepiandrosterone (DHEA) in H295R cells (Rosenmai et al. 2014)
  - No change in androstenedione secretion in cow theca cells (Campen et al. 2018)

Effects on the endocrine system: *In vitro* evidence (continued)

BPS altered hormones involved in reproduction

- ↓ Estradiol (E2)
  - Human granulosa cells, 50 µM (Amar et al. 2020)
  - Ewe granulosa cells, 10 or 50 µM (Téteau et al. 2023)
- ↑ Estradiol
  - Ewe granulosa cells, 1 nM to 200 µM (Téteau et al. 2020)
  - Cow granulosa cells, 100 µM (Campen et al. 2018b; Žalmanová et al. 2017)
- No change (estradiol; estrone (E1))
  - Human H295R cells, 0.8-50 µM (Rosenmai et al. 2014)

Hormone receptor: *In vivo* evidence

BPS induced changes in steroid hormone receptor expression in vivo

- ↑ Progesterone receptor (PR) expression in mammary gland of mice exposed during gestation and lactation
- ↑ Estrogen receptor alpha (Erα) expression in mammary gland of mice
- ↓ ERα (and vitellogenin) mRNA levels at 200 µg/mL, and non-significant changes in ER beta (ERβ) mRNA levels in zebrafish

Hormone receptors: *In vitro* evidence

BPS induced changes in hormone receptor expression in vitro

- ↓ ERα mRNA expression in oocytes of pig ovary cumulus-oocyte complexes at all concentrations (Žalmanová et al. 2017)
- ↓ ERβ mRNA expression in cumulus cells of pig ovary cumulus-oocyte complexes at concentrations > 30μM (Žalmanová et al. 2017)
- ↑ ERα and ERβ protein levels in pig ovary cumulus-oocyte complexes (Žalmanová et al. 2017)
- ↑ ERα and ERβ mRNA and protein expression in mouse ovaries (Liu et al. 2021)
- ↑ Expression of ERβ mRNA, but not ERα in human Ishikawa cells (Benjamin et al. 2023)
- ↑ mRNA expression of ERβ at 50 and 100 μM and ERα at 100 μM in ewe granulosa cells (Téteau et al. 2020)
- ↑ AMH receptor mRNA levels in cow COCs at 0.05 mg/mL (Saleh et al. 2021)

Key characteristics (KCs) of female reproductive toxicants and endocrine-disrupting chemicals

KCs of female reproductive toxicants

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

KC1: Alters hormone receptor signaling; alters reproductive hormone production, secretion, or metabolism

Many studies of BPS report changes in hormones and hormone receptor expression

Changes hormone levels in vivo

- ↓ Gonadotropins (FSH and LH) in rodents
- ↓ P levels in rodents
- ↑ P levels in zebrafish and mice
- Altered plasma and serum T in rodents
- ↓ Dihydrotestosterone and 11-dehydrocorticosterone in preovulatory follicular fluid in sheep
- ↓ Plasma estradiol (E2) in rodents and sheep and increased plasma estrone (E1) in sheep
- ↑ E2 in mice (serum) and zebrafish (plasma, whole body)
- ↑ E2 in preovulatory follicular fluid in sheep
- ↑ Progesterone receptor (PR) in mice
- ↑ Estrogen receptor (ERα) in mice and ↓ in zebrafish

Changes in vitro

- ↓ E2
  - Human granulosa cells (Amar et al. 2020)
  - Sheep granulosa cells (Téteau et al. 2023)
- ↑ E2
  - Sheep granulosa cells (Téteau et al. 2020)
  - Cow granulosa cells (Campen et al. 2018b; Žalmanová et al. 2017)
- No change (E1 or E2)
  - H295R cells (Rosenmai et al. 2014)
- Alterations in ER gene expression in cell lines and ovarian cell cultures from multiple species
- ↑ AMH receptor mRNA levels in cow COCs

KC2: **Chemical or metabolite is genotoxic**

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

KC8: **Alters direct cell-cell interactions**

- ↑ in theca cell gap junction intercellular communication in human and sheep primary ovarian theca cell cultures (Gingrich et al. 2021)
- ↑ in Cx37 mRNA expression in cow ovary cumulus cells of the granulosa (Sabry et al. 2021)
- Abnormal germ cell nest breakdown in cultured mouse ovaries. Nest breakdown involves ER, the JNK pathway, and cell adhesion proteins, e.g., E-cadherin (Liu et al. 2021)

KC10: **Alters microtubules and associated structures**

- Alterations in spindle morphology and chromosome alignment in cow oocytes (Campen et al. 2018a)
- ↓ Number of tubulin filaments in pig ovary cumulus–oocyte complexes (Zalmanova et al. 2017)
- Incidence of spindle malformation in mice (Nevoral et al. 2018; Zhang et al. 2020)
- Spindle disorganization and chromosome misalignment in pig ovary cumulus–oocyte complexes (Žalmanová et al. 2023), and *C. elegans* (Chen et al. 2016b)

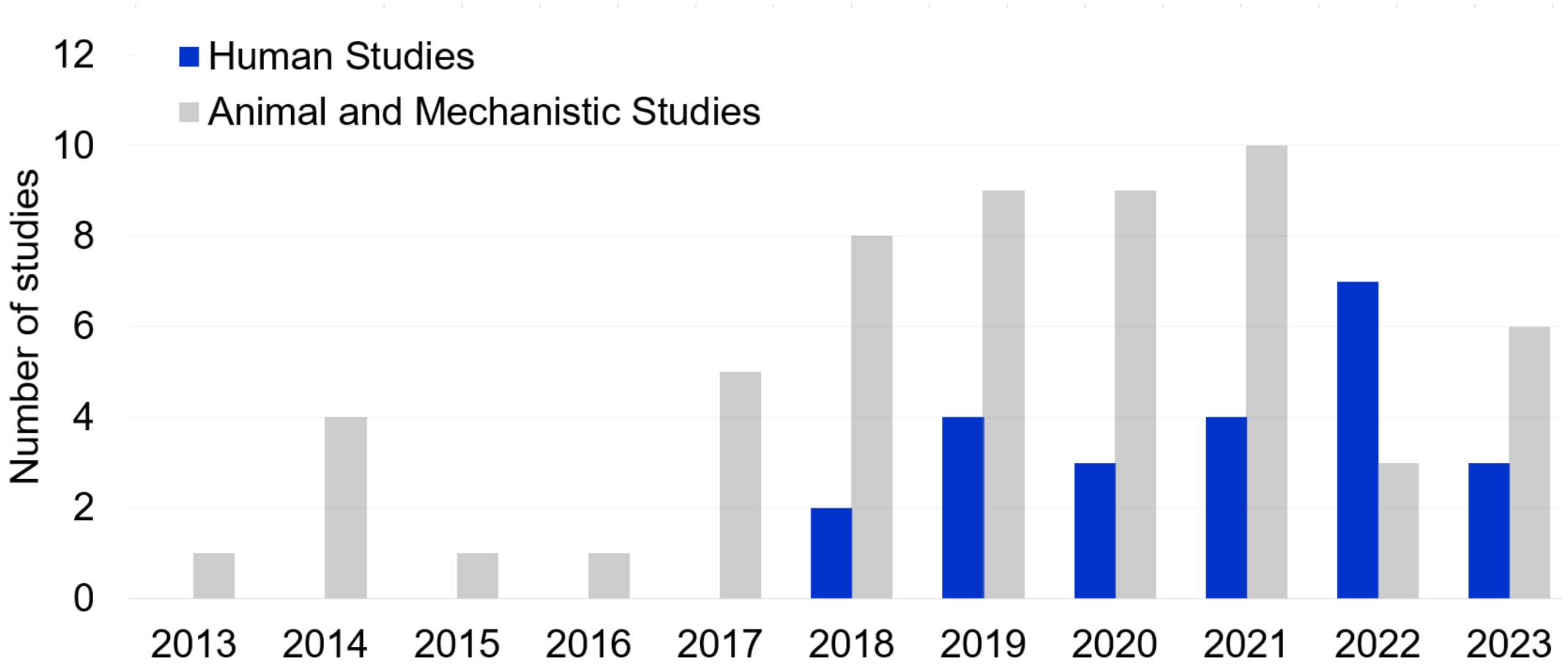
Break for Questions from the DARTIC

DARTIC Meeting - December 12, 2023

Female Reproductive Outcomes Examined in

Epidemiologic Studies

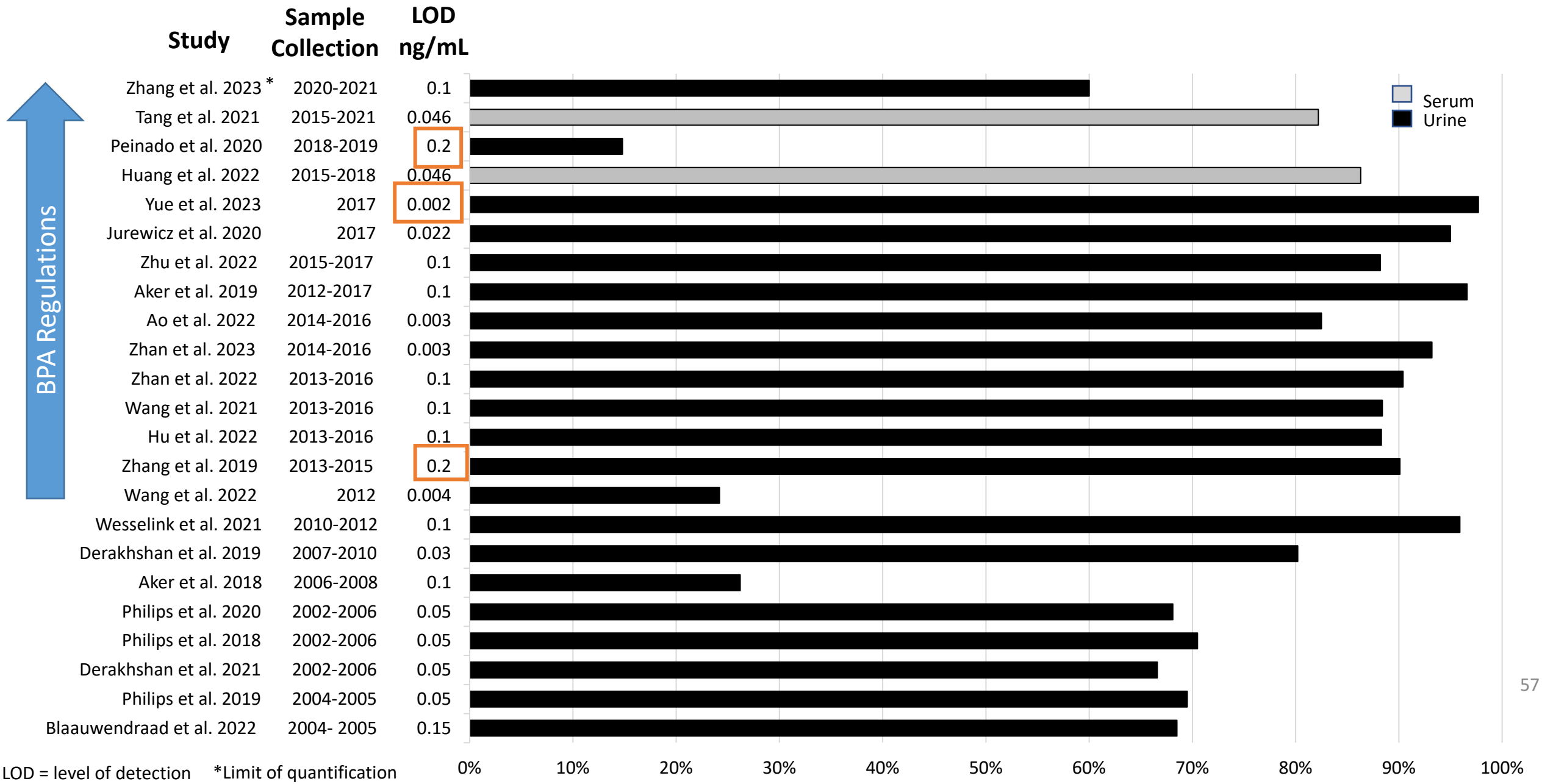
BPS: Number of studies published by year



Epidemiologic studies of BPS and female reproductive toxicity: Methods and key issues

- Study designs: cross-sectional, prospective cohort, case-control
- Limit of detection varied across studies
- Percentage of sample with detectable BPS levels varied across studies from 14.8% to 96.6%
- Multiple bisphenols and other chemical exposures
  - Most correlations between BPS and other bisphenols were in the range of 0.1 to 0.3

Percent of samples with BPS detected in epidemiologic studies



LOD = level of detection *Limit of quantification

Female reproductive outcomes examined in epidemiologic studies of BPS

- Female reproductive outcomes (2 or more studies):
  - Gestational diabetes mellitus (GDM)
  - Polycystic ovary syndrome (PCOS)
  - Thyroid hormones during pregnancy
  - Sex steroid hormones and related proteins during pregnancy and in young females
- Other female reproductive outcomes (one study)

Gestational diabetes mellitus (GDM)

- 1st trimester BPS associated with GDM in two studies, fasting plasma glucose in one study:
  - Prospective birth cohort in China (Tang et al. 2021):
    - Tertile 2 vs 1, OR: 1.77 (95% CI: 1.01, 3.13);
    - Tertile 3 vs 1, OR: 1.68 (95% CI: 0.95, 2.99)
    - Associations stronger when BMI ≥ 23 kg/m^2 or for pregnancies with a female fetus
  - Nested case-control study in California (Zhu et al. 2022):
    - Tertile 2 vs 1, OR: 1.86 (95% CI: 0.94, 3.67);
    - Tertile 3 vs 1, OR: 2.12 (95% CI: 1.00, 4.50)
    - Associations stronger for those who identified as non-Asian/Pacific Islander (race/ethnicities of White, Black, Hispanic or other)
  - Prospective cohort study in China (Zhang et al. 2019)
    - No significant associations with GDM
    - Higher fasting plasma glucose; stronger for pregnancies with a female fetus

Polycystic ovary syndrome (PCOS)

- BPS associated with PCOS in two case-control studies
  - Case-control study China (Zhan et al. 2023)
    - Higher odds of PCOS:
      - per unit increase OR: 1.18 (95% CI: 1.10, 1.25)
      - Quartile 2 vs 1, OR: 1.49 (95% CI: 1.12, 1.91)
      - Quartile 3 vs 1, OR: 1.70 (95% CI: 1.28, 2.22)
      - Quartile 4 vs 1, OR: 2.12 (95% CI: 1.58, 2.79)
  - Case-control study in Poland (Jurewicz et al. 2020)
    - Unclear reporting of results, rationale for methodology
    - Analyses stratified by tertiles of BPS exposure:
      - In tertile 1, per unit increase OR: 1.12 (95% CI: 1.03, 3.71)
      - In tertile 2, per unit increase OR: 1.29 (95% CI: 0.79, 6.89)
      - In tertile 3, per unit increase OR: 1.33 (95% CI: 0.58, 4.88)

Thyroid hormones during pregnancy

Study Name	Study Design	Thyroid Hormones Measured	Statistically Significant Results
Derakhshan et al. 2021	Cohort	TSH, FT4, TT4	↑ TT4, per unit increase in ln BPS β = 0.97 (95% CI: 0.03 1.91)
Huang et al. 2022	Cohort	TT3, TT4, FT3, FT4, and TSH	↓ TT3 for tertile 2 vs 1 %Δ = –10.90 (95% CI: –18.16, –2.99)
			↑ FT3 for tertile 3 vs 1 %Δ = 6.07 (95% CI: 1.20, 11.17)
Aker et al. 2018	Nested case-control	FT4, TT4, TT3, TSH, TT3/TT4 ratio	↓ FT4 for those with detectable BPS %Δ = –9.63 (95% CI: –18.10, –0.33)
			↑ TSH for those with detectable BPS among term births %Δ = 11.77 (95% CI: 0.08, 24.8)
Aker et al. 2019	Cohort	TT3, TT4, FT4, TSH, TT3/TT4	None
Derakhshan et al. 2019	Cohort	TSH, FT4, FT3, TT4, and TT3, TT4/TT3 ratio, FT4/FT3 ratio	None

Thyroid stimulating hormone (TSH), free thyroxine (FT4), total thyroxine (TT4), total triiodothyronine (TT3), free triiodothyronine (FT3)

Sex steroid hormones and related proteins

- Prospective cohort study in Puerto Rico (Aker et al. 2019)
  - Higher BPS levels in pregnancy associated with lower corticotropin releasing hormone
  - No associations observed for sex hormone binding globulin (SHBG), estriol, progesterone, testosterone
- Case-control study in China (Zhan et al. 2023)
  - Higher levels of testosterone in women with no PCOS ($\beta = 0.07$, 95% CI: 0.02, 0.12)
- Two cross-sectional studies using NHANES data 2013-2016 from female girls and adolescents
  - Quartile 2 vs 1 BPS associated with higher testosterone/estradiol ratio (Wang et al. 2021)
  - Non-linear associations for free androgen index and SHBG (Wang et al. 2021)
  - No associations with estradiol, testosterone, SHBG (Wang et al. 2021, Hu et al. 2022)

Other female reproductive outcomes

Study	Design	Outcome	Results
Zhang et al. 2023	Cross-sectional	Anti-müllerian hormone (AMH) and diminished ovarian reserve for women at an infertility clinic	Lower AMH (β = -0.29, 95% CI: -0.51, -0.07); Higher odds of diminished ovarian reserve: OR: 6.85 (95% CI: 1.24, 37.82)
Philips et al. 2020	Cohort	Gestational weight gain	Lower gestational weight gain
Wesselink et al. 2021	Case-cohort	Uterine fibroids	Lower risk of uterine fibroids (HR 0.93, 95% CI: 0.87, 0.99); 4.1% increase in existing fibroid growth (95% CI: 0.3%, 8.0%)
Blaauwendraad et al. 2022	Cohort	Onset of menstruation	Delayed onset of menstruation, 0.17 years (95% CI: 0.02, 0.31)
Ao et al. 2023	Case-control	Unexplained recurrent miscarriage	Higher odds of unexplained recurrent miscarriage; stronger for women over 30 years

Summary of evidence for BPS: Ovary

Animal studies:

- Alterations to: Germ cell nest breakdown, follicles, granulosa cells, *corpora lutea*
- Accelerate meiotic progression
- Damaged oocyte structure, spindle malformations

Mechanistic studies:

- Chromosome misalignment
    - Spindle malformations
        - α -Tubulin assembly alterations
- Altered follicular cell communication and proliferation

Human epidemiology:

- Higher odds of diminished ovarian reserve (one study) and PCOS (two studies)

Summary of evidence for BPS: Uterus/Placenta

Animal studies:

- Morphometric and histologic effects in the uterus
- ↓ Implantation sites, ↑ post-implantation loss
- Placenta: ↓ ratio of spongiotrophoblast to giant cell area

Mechanistic studies:

- Altered autocrine and paracrine signaling in the placenta

Human epidemiology:

- Higher odds of unexplained recurrent miscarriage (one study), higher fibroid growth/lower risk of developing fibroids (one study)
- No associations with endometriosis, time to pregnancy or risk of infertility

Summary of evidence for BPS: Hormones

Animal studies:

- ↓ Gonadotropins, changes in progesterone and estradiol
- ↑ Testosterone (low doses), ↓ at higher doses
- Possible pubertal changes

Mechanistic studies:

- Changes in hormone levels and hormone receptor expression

Human epidemiology :

- Lower AMH (one study), corticotropin releasing hormone (one study), inconsistent thyroid hormone associations, higher testosterone (one study)
- Higher odds of GDM
- Delay onset of menstruation in young females (one study)

Thank you!

DARTIC Meeting - December 12, 2023