



Proposal for development of hazard identification materials on *p,p'*-bisphenol chemicals

Developmental and Reproductive Toxicant Identification Committee

October 9, 2025

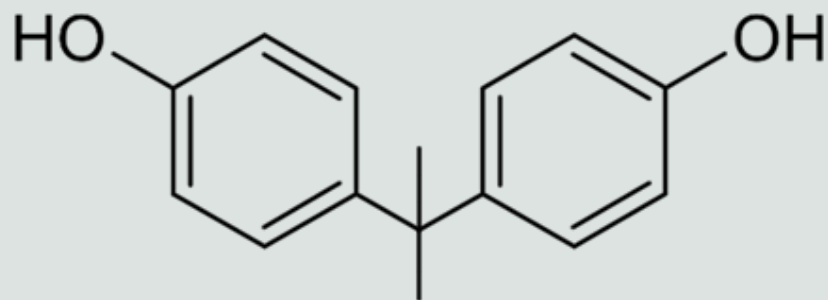
Reproductive Toxicology and Epidemiology Section
Reproductive and Cancer Hazard Assessment Branch
Office of Environmental Health Hazard Assessment, CalEPA

Outline

- Introduction
- Chemical identification
- Method applied for outcome selection
- Exposure
- Summary table example
- Discussion

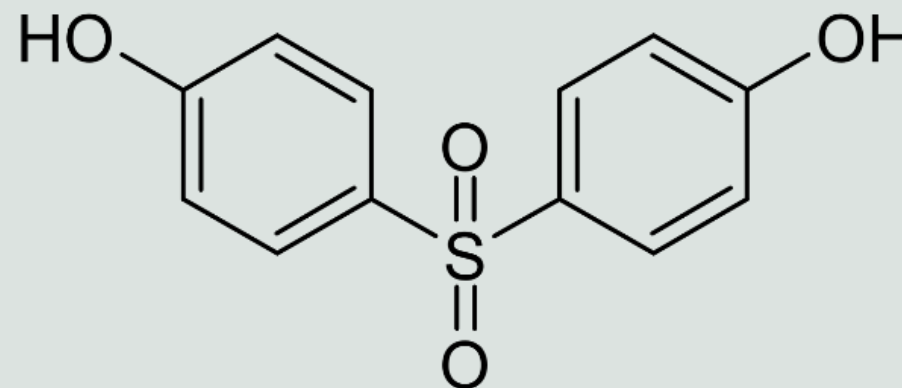


Bisphenols listed on Prop 65 for DART endpoints



4,4'-Isopropylidenediphenol;
Bisphenol A (BPA)

- Female Reproductive Toxicity (2015)
- Developmental Toxicity (2020)



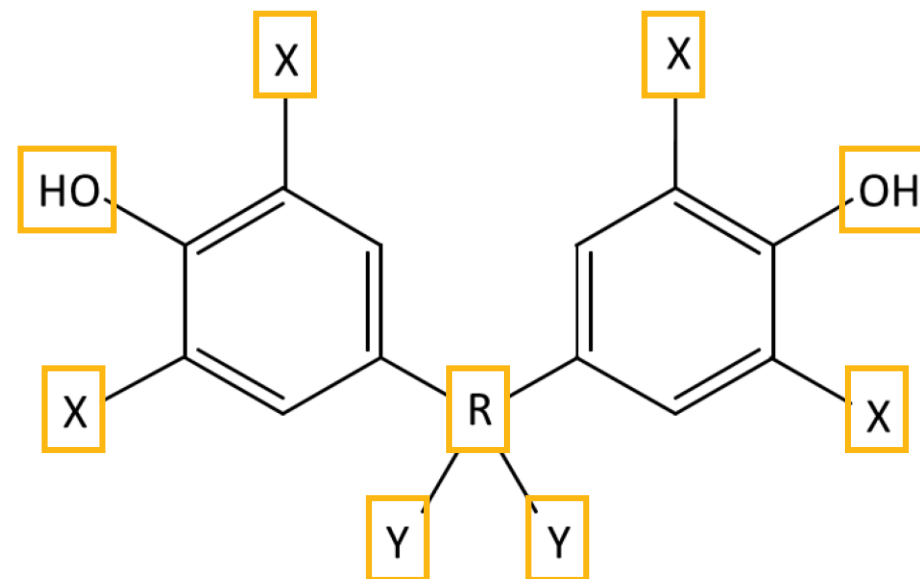
4,4'-Sulfonyldiphenol;
Bisphenol S (BPS)

- Female Reproductive Toxicity (2023)
- Male Reproductive Toxicity (2025)

p,p' -Bisphenol chemicals

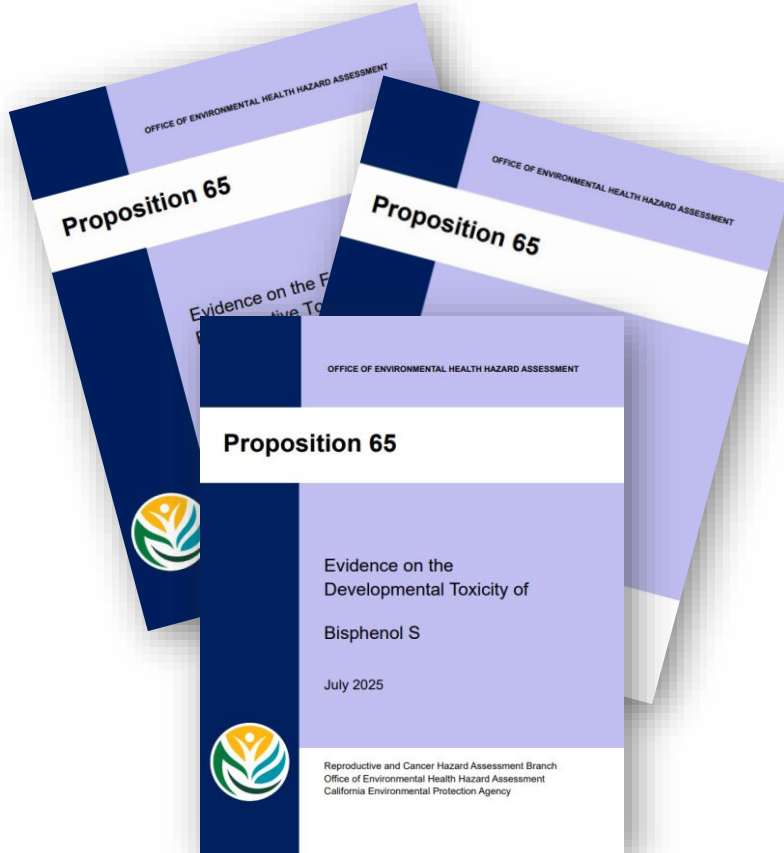
- R** bridging atom linking two phenol groups
- X** substituents attached to a phenol group
- Y** substituents attached to the phenol group bridge

In ethers and esters of p,p' -bisphenols, the hydroxyl groups are replaced by ether and ester groups



*Representative structure of a p,p' -bisphenol
(Adapted from Kitamura et al. 2005)*

Current hazard identification documents



Public health

Regrettable
substitutions

Time
constraints

Proposal

We propose a targeted outcome approach.

1. Focus on limited outcomes per endpoint.
2. Modify the scope and type of information provided in summary tables.

OEHHA is seeking the DARTIC's advice and consultation.

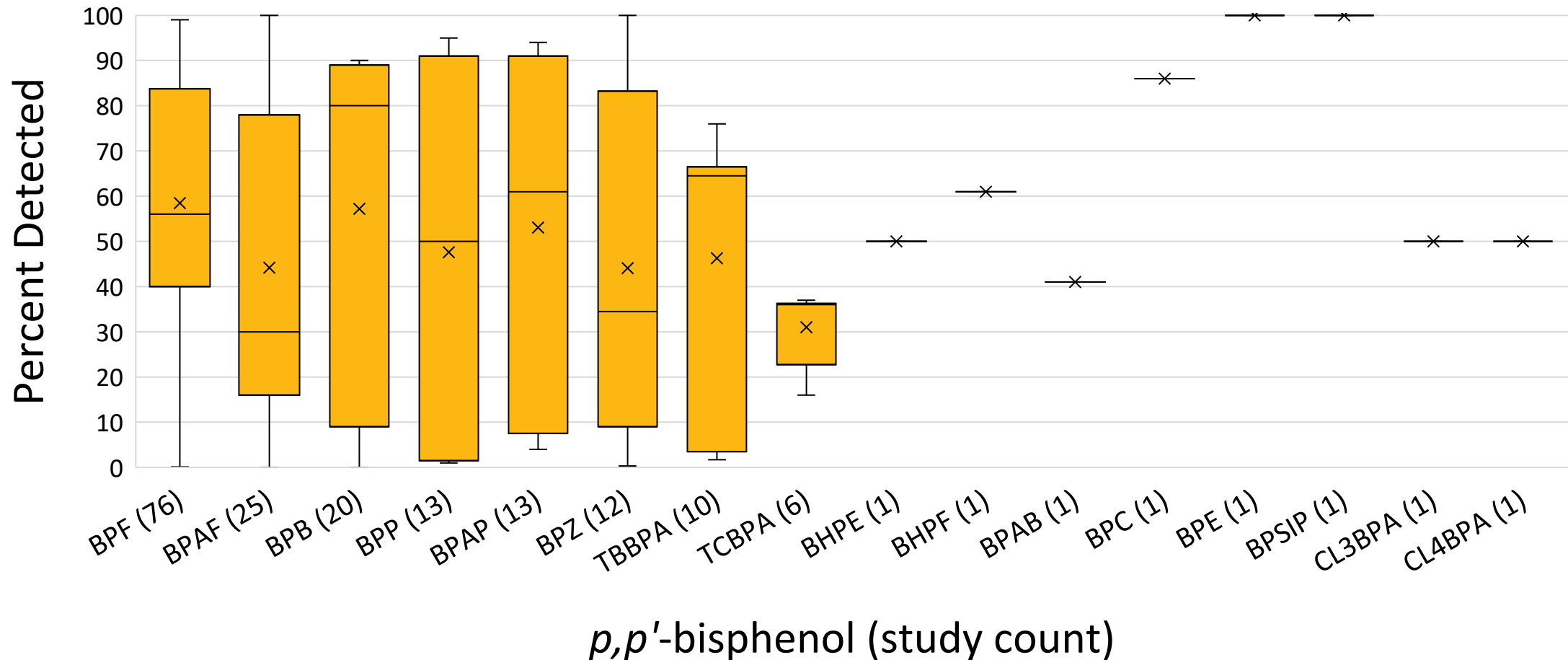


Literature search and screening

- Conducted an **exploratory** literature search using search terms for select p,p' -bisphenol chemicals and DART outcomes
- Screened studies using HAWC (Health Assessment Workspace Collaborative)
 - Title and abstract review
 - Counted studies with findings for each p,p' -bisphenol by outcome
- Visualized the study counts via heat maps



p,p' -Bisphenol chemicals detected in humans: detection frequencies



Visualization of the study counts via heat maps

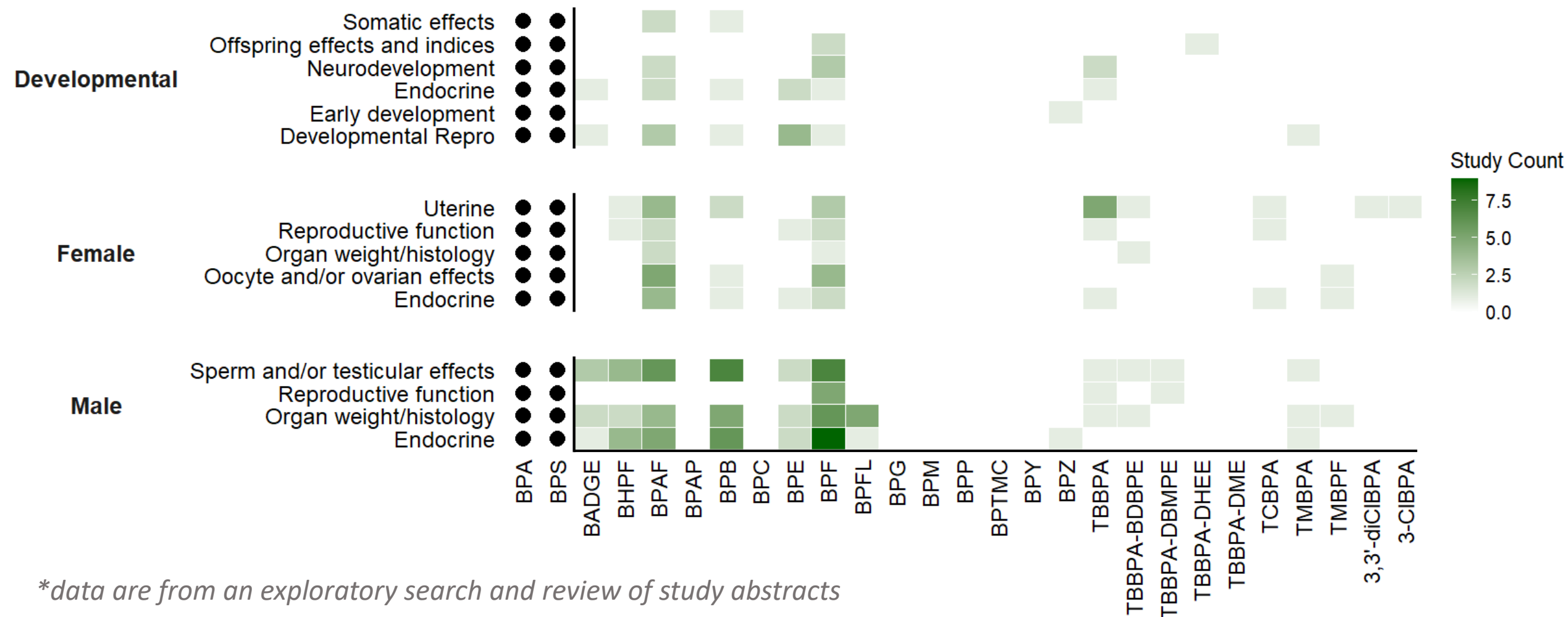
- Preliminary review of study abstracts
- Study counts in human, mammalian, and non-mammalian studies
 - Counted studies with findings for each p,p' -bisphenol by outcome
- Visualize common outcomes among p,p' -bisphenols



Counts of human studies with significant associations



Counts of mammalian studies with effects



Counts of non-mammalian studies with effects



**data are from an exploratory search and review of study abstracts*



Selected outcomes per endpoint

Female reproductive

- Oocyte and ovarian
- Uterine
- Endocrine

Male reproductive

- Sperm and testicular
- Endocrine

Developmental

- Somatic
- Neurodevelopmental
- Reproductive development
- Endocrine



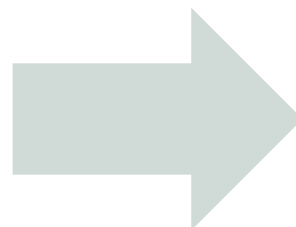
Proposed modifications to study tabulations

Study Design	Outcomes assessed	Major Findings
Bai et al. 2023 Zebrafish (wild-type [WT], AB strain). Treatment: BPS (> 98% purity) in 0.1% DMSO (vehicle control) at 1, 10, or 100 (= EC₂₅) µM (equivalent to 0.25, 2.50, and 25.03 mg/L, calculated by OEHHA), or 5 µM Valproic acid (VPA, positive control) from 8 hours post-fertilization (hpf) to 120 hpf. For neurobehavioral assays, embryos/larvae were exposed from 8 hpf to 4.5 days post-fertilization (dpf) (corresponds to 108 hpf). For immunocytochemistry (ICC) of neuronal markers and qRT-PCR assays on genes encoding ion channels and acetylcholine receptor, only one BPS concentration was used (1 µM). Endpoints assessed from 5 dpf (120 hpf) to 13 dpf. Note: Initial screening for mortality and malformations: embryos were exposed to a “wide range of concentrations.” Concentrations used in main experiment were selected based on computed effective concentration for malformation (EC₂₅).	Larval mortality at 120 hpf (n = 20 larvae, 3 biological replicates); LC₅₀ calculated. Malformations at 120 hpf (n = 20 larvae, 3 biological replicates); 25% effective concentration (EC₂₅) and half-maximal effective concentration (EC₅₀) calculated. Body measurements: body length (BL), intraocular distance (ID), lower jaw length (LJL), and ceratohyal cartilage length (CCL) at 4.5 dpf (n = 30 larvae). Shoaling behavior: nearest neighbor distance (NND, mm) and interindividual distance (IID, mm) at 11 dpf (n = 10 larvae). Motor and social behaviors: spontaneous movement (tail bends/min) at 24 hpf, larval locomotion at 5 dpf, light/dark exploration at 10 dpf, and social contact at 13 dpf (n = 22-44 larvae). ICC and gene expression analyses at single concentration of 1 µM BPS ICC: number of mature newborn neurons and number of neural stem cells in proliferation at 5 dpf (n = 4-10 larvae). qRT-PCR gene expression analysis at 120 hpf (n = 3, 20 larvae pooled as one replicate).	Mortality: LC₅₀ = 224 µM. Malformations: EC₂₅ = 100 µM, EC₅₀ = 144 µM; Bent spine, malformed tail, and yolk sac edema observed at 250 µM. Body measurements: Increased ID, LJL, and CCL (i.e., macrocephaly) at 1 and 100 µM. No effect on body length. Positive control (valproic acid) reported similar effects of increased ID, LJL, and CCL and no effect on body length. Shoaling behavior: increased NND at 10 µM, increased IID at ≥ 10 µM, i.e., reduced prosocial behavior. Motor and social behaviors: 24 hpf: Increased spontaneous movement at ≥ 1 µM BPS. 5 dpf: Hyperactivity during both light and dark periods at ≥ 1 µM BPS. 10 dpf: Increased anxiety-like behavior in light/dark exploration at ≥ 1 µM BPS. 13 dpf: Decreased social contact at ≥ 1 µM BPS. Neurogenesis: Accelerated neurogenesis as indicated by increased number of newborn neurons and proliferating neural stem cells. Gene expression: Upregulated expression of several genes associated with potassium and calcium ion channels; and downregulated expression of calcium ion channel gene <i>tnnc-2</i> and acetylcholine receptor gene <i>chma1</i>.



Prior tabulation

Study Design
Bai et al. 2023 Zebrafish (wild-type [WT], AB strain). Treatment: BPS (> 98% purity) in 0.1% DMSO (vehicle control) at 1, 10, or 100 (= EC₂₅) µM (equivalent to 0.25, 2.50, and 25.03 mg/L, calculated by OEHHA), or 5 µM Valproic acid (VPA, positive control) from 8 hours post-fertilization (hpf) to 120 hpf. For neurobehavioral assays, embryos/larvae were exposed from 8 hpf to 4.5 days post-fertilization (dpf) (corresponds to 108 hpf). For immunocytochemistry (ICC) of neuronal markers and qRT-PCR assays on genes encoding ion channels and acetylcholine receptor, only one BPS concentration was used (1 µM). Endpoints assessed from 5 dpf (120 hpf) to 13 dpf. Note: Initial screening for mortality and malformations: embryos were exposed to a “wide range of concentrations.” Concentrations used in main experiment were selected based on computed effective concentration for malformation (EC₂₅).



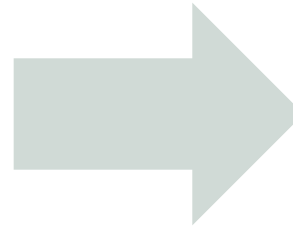
New tabulation

Study Design	<i>p,p'</i> -Bisphenol chemical(s)	Treatment period
Bai et al. 2023 Zebrafish (WT, AB strain) 20 zebrafish per treatment, n = 3	[µM] BPA: 0.2, 2, 20 BPF: 0.3, 3, 30 BPS: 1, 10, 100 The following chemicals were only evaluated for nearest neighbor and interindividual distances: BPAF: 0.05, 0.5, 5 BPAP: 0.04, 0.4, 4 BPB: 0.1, 1, 10 BPC: 0.05, 0.5, 5 BPE: 0.3, 3, 30 BPF: 0.3, 3, 30 BPZ: 0.07, 0.7, 7	In 0.1% DMSO (control) From 8 -108 hpf



Prior tabulation

Outcomes assessed
Larval mortality at 120 hpf (n = 20 larvae, 3 biological replicates); LC ₅₀ calculated.
Malformations at 120 hpf (n = 20 larvae, 3 biological replicates); 25% effective concentration (EC ₂₅) and half-maximal effective concentration (EC ₅₀) calculated.
Body measurements: body length (BL), intraocular distance (ID), lower jaw length (LJL), and ceratohyal cartilage length (CCL) at 4.5 dpf (n = 30 larvae).
Shoaling behavior: nearest neighbor distance (NND, mm) and interindividual distance (IID, mm) at 11 dpf (n = 10 larvae).
Motor and social behaviors: spontaneous movement (tail bends/min) at 24 hpf, larval locomotion at 5 dpf, light/dark exploration at 10 dpf, and social contact at 13 dpf (n = 22-44 larvae).
ICC and gene expression analyses at single concentration of 1 µM BPS
ICC: number of mature newborn neurons and number of neural stem cells in proliferation at 5 dpf (n = 4-10 larvae).
qRT-PCR gene expression analysis at 120 hpf (n = 3, 20 larvae pooled as one replicate).



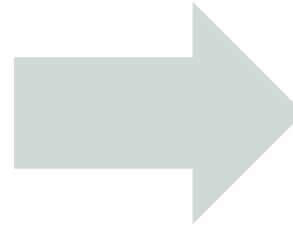
New tabulation

Outcomes assessed
Somatic effects Only for BPA, BPF and BPS
Neurodevelopment Spontaneous movement (24 hpf) Swimming distance (5 dpf) Light/dark activity (10 dpf) Shoaling behavior (11 dpf; nearest neighbor distance, interindividual distance) Social contact (13 dpf) Neurogenesis (108 hpf; 2 µM BPA, 3 µM BPF, 1 µM BPS)



Prior tabulation

Major Findings
Mortality: LC ₅₀ = 224 µM.
Malformations: EC ₂₅ = 100 µM, EC ₅₀ = 144 µM; Bent spine, malformed tail, and yolk sac edema observed at 250 µM.
Body measurements: Increased ID, L JL, and CCL (i.e., macrocephaly) at 1 and 100 µM. No effect on body length.
Positive control (valproic acid) reported similar effects of increased ID, L JL, and CCL and no effect on body length.
Shoaling behavior: increased NND at 10 µM, increased IID at ≥ 10 µM, i.e., reduced prosocial behavior.
Motor and social behaviors: 24 hpf: Increased spontaneous movement at ≥ 1 µM BPS. 5 dpf: Hyperactivity during both light and dark periods at ≥ 1 µM BPS. 10 dpf: Increased anxiety-like behavior in light/dark exploration at ≥ 1 µM BPS. 13 dpf: Decreased social contact at ≥ 1 µM BPS.
Neurogenesis: Accelerated neurogenesis as indicated by increased number of newborn neurons and proliferating neural stem cells.
Gene expression: Upregulated expression of several genes associated with potassium and calcium ion channels; and downregulated expression of calcium ion channel gene <i>tnni-2</i> and acetylcholine receptor gene <i>chra1</i> .



New tabulation

Major Findings
Somatic effects Increased intraocular distance (BPA, BPF, BPS) Increased lower jaw length (BPA, BPF, BPS) Increased ceratohyal cartilage length (BPA, BPF, BPS) No effects on head size
Neurodevelopment BPA, BPS and BPF had an increase in spontaneous movement, dark period swimming distance, time in light area, and decreased light/dark crossings Increased light period swimming distance BPA (0.2 and 2 µM) and all BPS concentrations Decreased light period swimming distance at 20 µM BPA and 0.3 µM BPF Increased nearest neighbor distance (BPA, BPC, BPF, BPS, BPAF, and BPAF) Increased interindividual distance (BPA, BPC, BPF, BPS, BPAF, and BPAF) Decreased time in contact (BPA, BPS, and at 0.3 & 3 µM BPF) Increased time in contact at 30 µM BPF Accelerated neurogenesis and increased neural stem cell proliferation



Updated study tabulation

Study Design	<i>p,p'</i> -Bisphenol chemical(s)	Treatment period	Outcomes assessed	Major Findings
Bai et al. 2023 Zebrafish (WT, AB strain) 20 zebrafish per treatment, n = 3	[μM] BPA: 0.2, 2, 20 BPF: 0.3, 3, 30 BPS: 1, 10, 100 The following chemicals were only evaluated for nearest neighbor and interindividual distances: BPAF: 0.05, 0.5, 5 BPAP: 0.04, 0.4, 4 BPB: 0.1, 1, 10 BPC: 0.05, 0.5, 5 BPE: 0.3, 3, 30 BPF: 0.3, 3, 30 BPZ: 0.07, 0.7, 7	In 0.1% DMSO (control) From 8 -108 hpf	Somatic effects Only for BPA, BPF and BPS Neurodevelopment Spontaneous movement (24 hpf) Swimming distance (5 dpf) Light/dark activity (10 dpf) Shoaling behavior (11 dpf; nearest neighbor distance, interindividual distance) Social contact (13 dpf) Neurogenesis (108 hpf; 2 μ M BPA, 3 μ M BPF, 1 μ M BPS)	Somatic effects Increased intraocular distance (BPA, BPF, BPS) Increased lower jaw length (BPA, BPF, BPS) Increased ceratohyal cartilage length (BPA, BPF, BPS) No effects on head size Neurodevelopment BPA, BPS and BPF had an increase in spontaneous movement, dark period swimming distance, time in light area, and decreased light/dark crossings Increased light period swimming distance BPA (0.2 and 2 μ M) and all BPS concentrations Decreased light period swimming distance at 20 μ M BPA and 0.3 μ M BPF Increased nearest neighbor distance (BPB, BPC, BPF, BPS, BPAF, and BPAP) Increased interindividual distance (BPA, BPC, BPF, BPS, BPAF, and BPAP) Decreased time in contact (BPA, BPS, and at 0.3 & 3 μ M BPF) Increased time in contact at 30 μ M BPF Accelerated neurogenesis and increased neural stem cell proliferation



Questions for discussion

1. Do you have feedback on the approach we used to select a subset of outcomes per DART endpoint?
2. Do you think the proposed modified tables have enough information to inform your future discussion?



Thank you



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