Evidence on the Carcinogenicity of Perfluorooctane Sulfonic Acid (PFOS) and its Salts and Transformation and Degradation Precursors

Carcinogen Identification Committee Meeting

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Office of Environmental Health Hazard Assessment, CalEPA

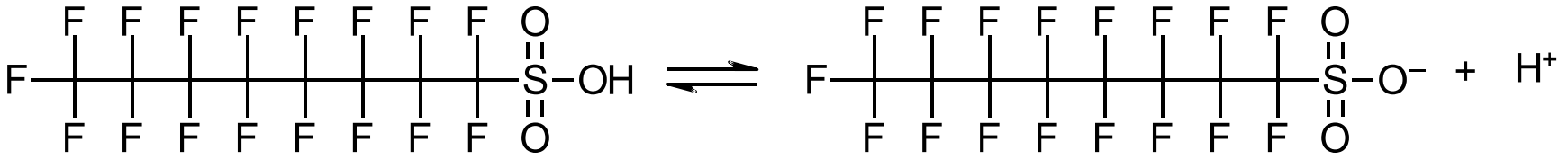
CalEPA
California Environmental Protection Agency

Overview

- Introduction
- Carcinogenicity data on PFOS
  - Epidemiological studies
  - Animal cancer bioassays
  - Mechanistic data
    - Pharmacokinetics
    - Key characteristics (KCs) of carcinogens
    - Comparison with perfluorooctanoic acid (PFOA)
- Summary of evidence

Perfluorooctane sulfonic acid (PFOS)

- Man-made chemical
  - One of many PFASs (per- & poly-fluoroalkyl substances)
  - Fully fluorinated "C8" chemical
- "PFOS and its salts and precursors"
  - *Salts*
    - PFOS potassium salt used in animal bioassays
  - *Precursors*
    - Containing "$\mathbf{C_8F_{17}SO_2}$" moiety that may transform or degrade to PFOS (*e.g.*, PFOSA, Me/Et-PFOSE)
    - Used to produce PFASs



PFOS (CAS RN: 1763-23-1)

PFOS anion (CAS RN: 45298-90-6)

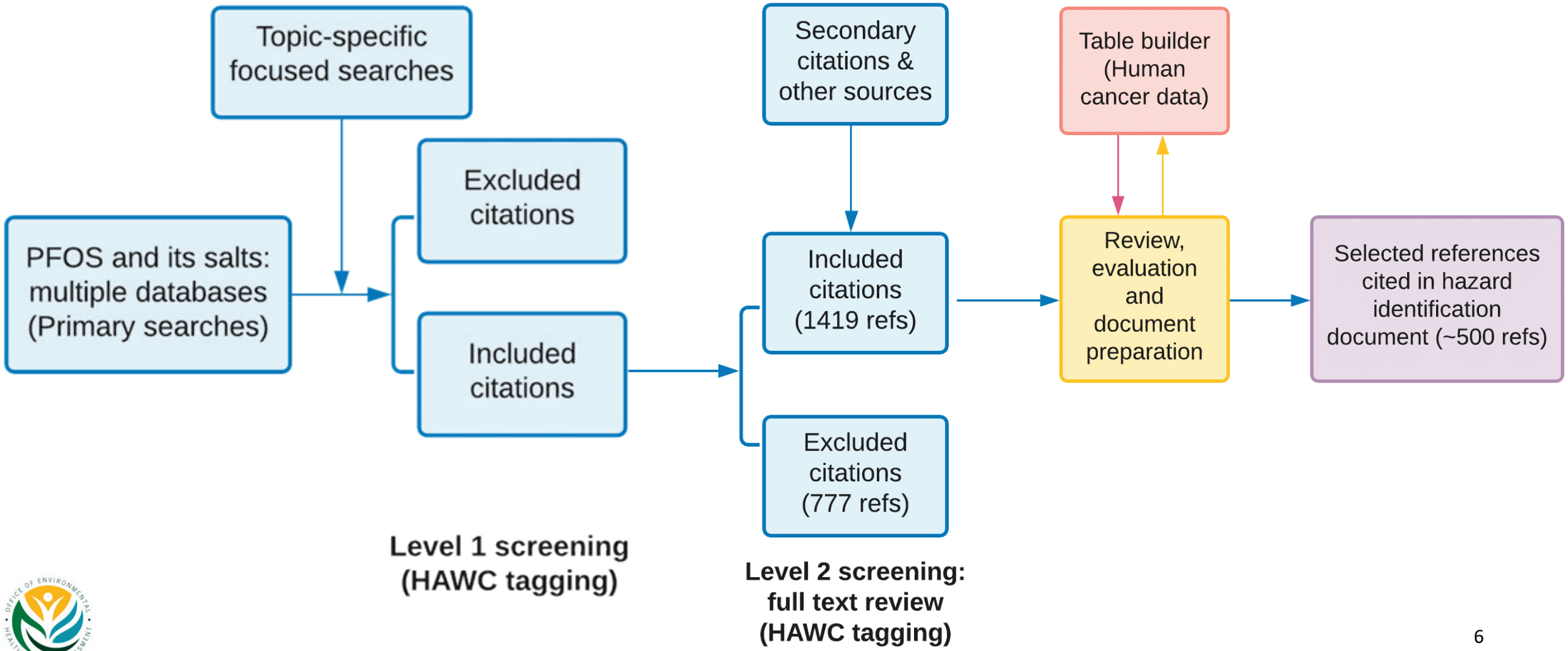
Uses of PFOS and its salts and precursors and exposure to PFOS

- Stain-, grease-, heat- and water-resistant applications
  - *E.g.*, non-stick cookware, waterproof textiles
- Human exposure pathways
  - Contaminated food and water
  - Ingestion of dust
  - Inhalation
- Persistent and bioaccumulative ("forever chemicals")
  - Ubiquitous in the environment and biota
- PFOS detected in almost all human serum samples from national or California biomonitoring studies
  - A decreasing trend since PFOS phase-out, but remain elevated in certain populations (*e.g.*, firefighters in California)

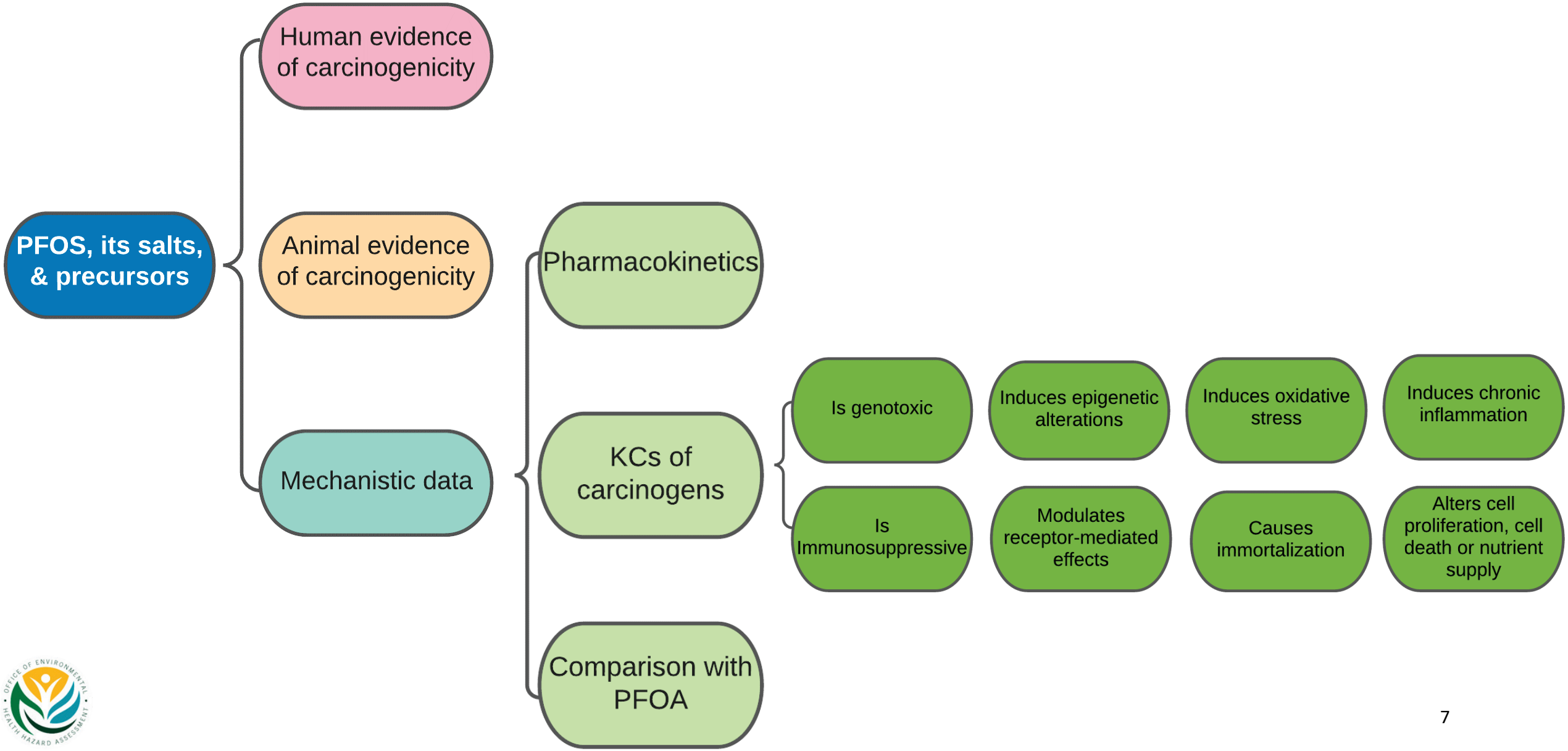
Agency reviews

- CalEPA, OEHHA
  - First public review draft: “Proposed Public Health Goals for PFOA and PFOS in drinking water” (OEHHA 2021)
- Other health agencies
  - US EPA (2016)
    - *“Suggestive evidence of carcinogenic potential”*
  - Agency for Toxic Substances and Disease Registry (ATSDR 2021)
  - Health Canada (2016)
  - European Food and Safety Authority (EFSA 2020)

Literature search and screening process



Summary of data streams



Epidemiology

Epidemiology - Overview

Cancer Site	Number of Studies Included
Breast	11
Prostate	3
Bladder	3
Liver	3
Kidney	2
Colorectum	2
Skin	2
Lung, urinary tract, pancreas, lymphohematopoietic system, pediatric germ cell tumor	1

- Literature: 23 epidemiologic studies identified, 18 included
- Inclusion criteria:
  - Reported risk estimate for PFOS and cancer
  - Cohort, case-control, nested case-control designs
- Exclusion criteria:
  - Cross-sectional or ecologic designs, case-reports, conference abstracts, reviews
- Data were sparse for most cancers, except breast cancer (mixed results)

Epidemiology - Methods

- Each included study was evaluated for its quality
  - Guidance: NTP Report on Carcinogens Handbook (NTP 2015), IARC Monographs Programme Preamble (IARC 2019)
  - Assessed direction and impact of biases (from selection, information, confounding)

- Hill guidelines and other considerations for causal inference
  - Consistency
  - Temporality
  - Magnitude of effect
  - Dose-response

Epidemiology – Key Issues

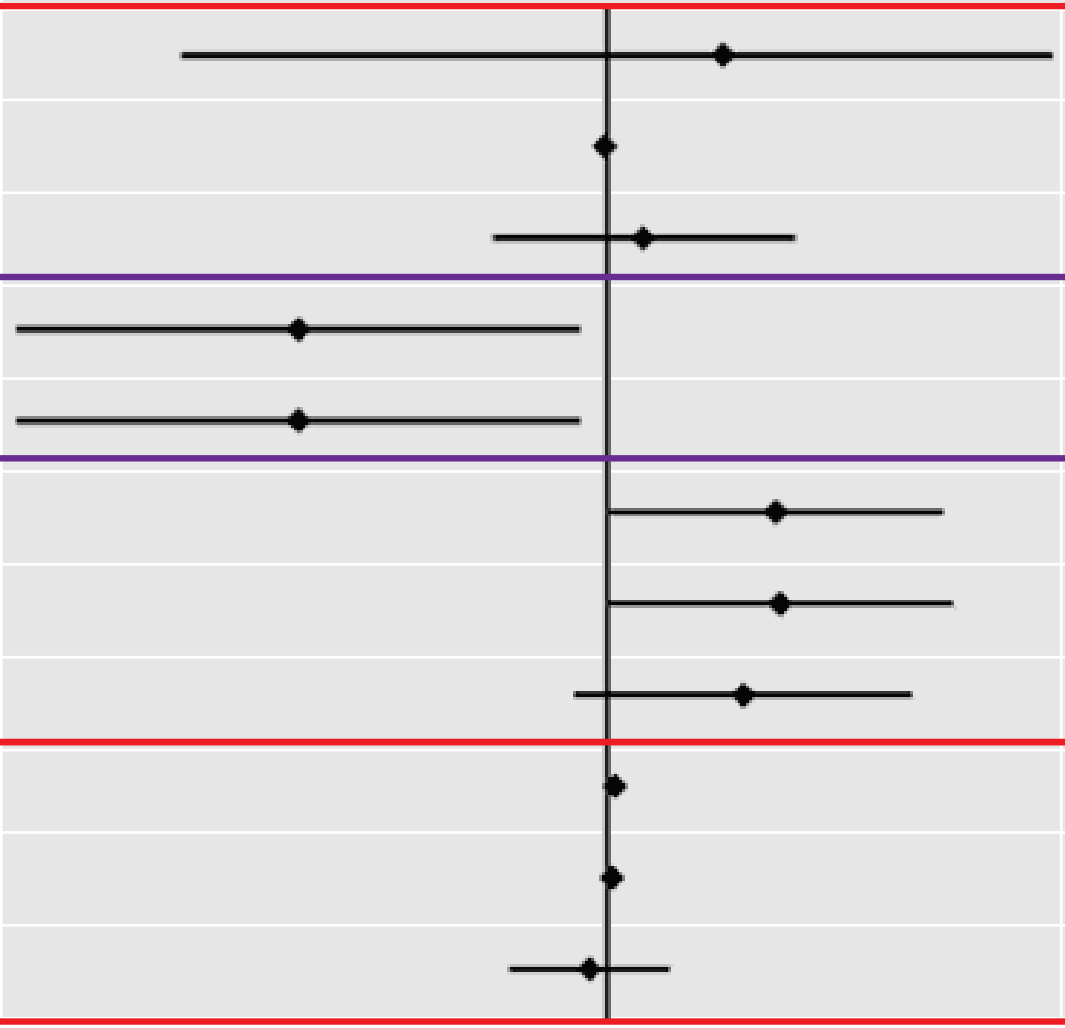
- Timing of exposure assessment
  - Exposure based on a single serum sample or samples collected during a single short window of time could miss long-term exposure changes
- Reverse causation
  - Serum PFOS levels measured at or near the time of diagnosis may be affected by physiological or behavioral changes due to onset of disease
- Confounding
  - Co-exposures to other PFASs were not accounted for in most studies

PFOS and Breast Cancer – Exposure Characterization

Reference	Location	PFOS levels (ng/ml)	Method	Timing PFOS Assessment
Alexander et al. (2003), Olsen et al. (2003b)	Alabama	Chemical plant workers mean: 900 Film plant workers mean: 100	JEM, Serum	Before diagnosis
Bonefeld-Jørgensen et al. (2014), Ghisari et al. (2017)	Denmark	Controls mean: 30.6	Serum	Before diagnosis
Mancini et al. (2020)	France	Overall median: 17.51 Cases: 17.62; Controls: 17.32	Serum	Before diagnosis
Cohn et al. (2020)	California	Cases median: 30.5 Controls median: 32.1	Maternal serum	Before diagnosis [in offspring]
Bonefeld-Jørgensen et al. (2011), Ghisari et al. (2014)	Greenland	Cases median: 45.6 (range: 11.6-124) Controls median: 21.9 (range: 1.5-172)	Serum	At diagnosis
Wielsøe et al. (2017), Wielsøe et al. (2018)	Greenland	Cases median: 35.5 Controls median: 18.2	Serum	At diagnosis
Hurley et al. (2018b)	California	Cases mean: 8.021 Controls mean: 8.320	Serum	After diagnosis

PFOS and Breast Cancer

Reference	Exposure category or level (ng/ml)	Risk estimate	RR (95% CI)
Alexander et al. (2003)	All cohort members		1.57 (0.19, 5.66)
Bonefeld-Jørgensen et al. (2014)	Any PFOS		0.99 (0.98, 1.01)
Ghisari et al. (2017)	All		1.15 (0.64, 2.08)
Cohn et al. (2020)	$\log_2$ PFOS, low cholesterol (Q1)		0.30 (0.10, 0.90)
Cohn et al. (2020)	$\log_2$ PFOS, high cholesterol (Q4)		0.30 (0.10, 0.90)
Mancini et al. (2020)	Quartile 2 (13.6 – 17.3)		1.93 (1.01, 3.70)
Mancini et al. (2020)	Quartile 3 (17.3 – 22.5)		1.96 (1.00, 3.84)
Mancini et al. (2020)	Quartile 4 (22.5 – 85.3)		1.70 (0.88, 3.28)
Bonefeld-Jørgensen et al. (2011)	PFOS, adj.		1.03 (1.00, 1.07)
Wielsøe et al. (2017)	Adj. continuous PFOS		1.02 (1.01, 1.03)
Hurley et al. (2018)	$\log_{10}$ PFOS		0.93 (0.68, 1.28)



Animal bioassays

Animal bioassays - overview

- Two-year carcinogenicity studies in male and female Sprague Dawley [Crl:CD (SD) BR] rats (Thomford 2002; Butenhoff et al. 2012)
  - Dietary administration of K^+PFOS at doses of 0, 0.5, 2, 5, or 20 ppm for two years (50 animals/group/sex)
  - Additional 20 ppm recovery group (40 animals/sex): 20 ppm K^+PFOS in the diet for 52 weeks, and basal diet for 52 weeks before study termination

- Six-month dietary exposure to K^+PFOS as a promoter after initiation with aflatoxin B1 in rainbow trout (Benninghoff et al. 2012)

Two-year dietary study in male rats exposed to K^+PFOS

(Butenhoff et al 2012; Thomford 2002)

Tumor site	Tumor type	Administered dose in feed (ppm)					Increasing trend test p-value
		0	0.5	2	5	20	
Liver	Hepatocellular adenoma	0/41	3/42	3/47	1/44	7/43**	0.006
Pancreas	Islet cell adenoma	4/44	3/44	4/48	4/46	4/44	NS
	Islet cell carcinoma	1/38	2/41	2/44	5/44	5/40	0.048
	Combined adenoma and carcinoma	5/44	5/44	6/48	8/46	9/44	NS

** $p<0.01$, by pairwise comparison; NS-not significant

Tumor findings in “20 ppm recovery” group in male rats

(Butenhoff et al 2012; Thomford 2002)

- 20 ppm K^+PFOS in feed for one year then on basal diet for one year

Tumor site	Tumor type	Administered dose in feed	
		0	20 ppm
Thyroid	Follicular cell adenoma	3/31	9/29*

* $p<0.05$, by pairwise comparison

Two-year dietary study in female rats exposed to K^+PFOS

(Butenhoff et al 2012; Thomford 2002)

Tumor site, cell type	Tumor type	Administered dose in feed (ppm)					Increasing trend test p-value
		0	0.5	2	5	20	
Liver, hepatocellular	Adenoma	0/28	1/26	1/15	1/28	5/31*	p < 0.01
	Carcinoma (rare)	0/28	0/29	0/16	0/31	1/32	NS
	Combined adenoma and carcinoma	0/28	1/29	1/16	1/31	6/32*	p < 0.01
Thyroid, follicular cell	Adenoma (rare)	0/26	0/25	0/14	2/26	1/30	NS
	Carcinoma (rare)	0/24	0/15	0/9	1/15	0/25	NS
	Combined adenoma and carcinoma	0/26	0/25	0/14	3/26	1/30	NS
Mammary	Fibroadenoma	20/60	27/50*	20/48	24/49	11/60	NS

* p<0.05, by pairwise comparison; NS-not significant

Tumor findings in “20 ppm recovery” group in female rats

(Butenhoff et al 2012; Thomford 2002)

- 20 ppm K^+PFOS in feed for one year then on basal diet for one year

Tumor site	Tumor type	Administered dose in feed	
		0	20 ppm
Thyroid	Follicular cell adenoma (rare)	0/24	1/17

Tumor promotion study in rainbow trout

(Benninghoff et al. 2012)

- Six-month dietary exposure to K^+PFOS as a promoter after initiation with aflatoxin B1

Tumor site	Tumor type	AFB_1 0 ppb		AFB_1 10 ppb	
		PFOS 0 ppm	PFOS 100 ppm	PFOS 0 ppm	PFOS 100 ppm
Liver	Adenoma and carcinoma combined	0	0	1%	13%**

** $p < 0.01$ compared with AFB_1 10 ppb/PFOS 0 ppm as determined by logistic regression analysis.

Break for Questions from the Carcinogen Identification Committee

Mechanistic considerations and other relevant data

Pharmacokinetics

- Well absorbed
  - Full absorption in SD rats and NZ rabbits
- Wide distribution
  - Binding to proteins
  - Mainly in liver, plasma, & kidney (less in lung, brain, gonads, bone, and other tissues)
  - Crosses blood-brain barrier and placenta
  - Detected in breastmilk
- Slow excretion
  - Urinary and fecal excretion
  - Enterohepatic circulation
  - In women: pregnancy-related losses; elimination via breast milk or menstrual blood
- Long half-life in humans:
  - Humans: 1.7-8.7 years
  - Cynomolgus monkeys: 110-200 days
  - Rats: 24-83 days
  - Mice: 30-43 days
- Biotransformation of precursors
  - PFOS can be formed from various PFOS precursors (e.g., PFOSA or EtPFOSE)

Key characteristics of carcinogens

1. Is electrophilic or can be metabolically activated
2. **Is genotoxic**
3. Alters DNA repair or causes genomic instability
4. **Induces epigenetic alterations**
5. **Induces oxidative stress**
6. **Induces chronic inflammation**
7. **Is immunosuppressive**
8. **Modulates receptor-mediated effects**
9. **Causes immortalization**
10. **Alters cell proliferation, cell death, or nutrient supply**

KC 2: "Is genotoxic"

Mutation:

- Not mutagenic in bacterial assays
- ↑ in transgenic mice and fish *in vivo*; in transgenic mouse cells *in vitro*

Chromosomal Effects:

- Micronuclei formation
  - No effect in human HepG2 cells
  - ↑ in several *in vivo* studies in rats, with no effect in one study in male SD rats
  - ↑ in hepatocytes of transgenic mice, with no effect in bone marrow in mice
  - ↑ in peripheral blood cells of zebrafish
  - ↑ in mussels and onion
- Chromosomal aberration
  - No effect in human peripheral blood lymphocytes exposed *in vitro*
  - ↑ in onion

KC 2: "Is genotoxic" (cont'd)

DNA damage:

- DNA strand breaks
  - ↑ in 1 of 3 studies in human HepG2 cells; not in human sperm cells *in vitro*
  - ↑ in bone marrow, peripheral blood cells, and hepatocytes of treated rats; not in SHE cells *in vitro*
  - ↑ in primary mouse Leydig cells
  - ↑ in zebrafish and carp, in green mussels, flatworms, water flea, earthworms, and onion, but not in gull eggs or *Paramecium*
- ↑ γ-H2AX in transgenic mouse embryonic fibroblasts *in vitro*
- ↑ DNA damage in germ cells of *C. elegans*
- In 2 of 3 human studies, PFOS was associated with urinary 8-OHdG
- No increase in UDS in rat primary liver cells

KC 4: "Induces epigenetic alterations"

- Epigenetic findings in humans and animals *in vivo,* human and animal cells *in vitro*
  - Altered methylation of regions associated with specific genes
    - *E.g.*, CpGs mapped to *CXADRP3* and *SNAPIN* in human cord blood
  - Global methylation changes
    - *E.g.*, associated with Alu global hypomethylation in human cord blood
  - miRNA changes
    - *E.g.*, altered expression of cancer-related miRNAs in rodents
  - Alterations in expression of DNMTs
    - *E.g.*, ↑ DNMT3a expression in rats (can lead to ↓ expression of tumor suppressor genes)

KC 5: "Induces oxidative stress"

- Evidence from multiple *in vivo* and *in vitro* human and animal studies:
  - **Oxidative DNA damage**
  - **ROS or RNS production**
  - **Lipid peroxidation**
  - Total antioxidant capacity
  - **Changes in antioxidant enzymes or glutathione status**
  - Changes in Nrf2 expression
- Additional support from 'omics' studies:
  - **Perturbation in pathways related to oxidative stress (*e.g.*, glutathione cycle)**
  - Change gene expression related to Nrf2-mediated oxidative stress response or peroxisomal fatty acid β-oxidation

KC 6: “Induces chronic inflammation”

- Pro-inflammatory cytokine production in multiple human cell types *in vitro*
  - ↑ IL-1 in two studies using human bronchial epithelial cells and lymphocytes
  - ↓ IL-10 and IFN-γ using human peripheral blood leukocytes
  - ↓ TNF-α secretion and mRNA expression in human blood cells
  - ↓ C-X-C motif chemokine ligand 10 (CXCL-10) production
  - Unclear: IL-2, IL-4, IL-6, and IL-8 production
- Pro-inflammatory cytokine production in animal models
  - ↑ IL-1 production in mice, rats and zebrafish
  - ↓ IL-2 production in mice
  - ↓ IL-8 mRNA in chicken embryo fibroblasts
  - ↑ IL-15 mRNA in zebrafish
  - ↑ TGF-β mRNA in zebrafish
  - No change in IL-5 production by mouse splenic T cells
  - Unclear: IL-4, IL-5, IL-6, IL-10, TNF-α and IFN-γ production

KC 7: "Is immunosuppressive"

- *IgM levels*
  - ↓ in multiple mouse studies with or without antigen challenge
  - No change in mice (one with and one without antigen challenge)
  - ↑ in rats (without antigen challenge)
- *Effects on T cell and B cell cellularity or proliferation*
  - ↓ number and proliferation of thymocytes and splenocytes in mice
  - No change in two additional studies
  - ↑ proliferation of dolphin T-lymphocytes exposed *in vitro*
- *NK cell activity*
  - ↓ NK cell activity in cultured human blood cells and mice
  - ↑ NK cell activity in mice

KC 8: "Modulates receptor-mediated effects"

- Estrogen receptor (ER) and estradiol (E2)
  - Human observational studies: ↓ E2 in women and girls
  - Human cells *in vitro:* ↑ ERα and ERβ reporter activity; ↑ proliferation of breast epithelial cells; ↓ expression of estrogen-responsive genes; altered E2
  - Rodents *in vivo*: altered ERα, ERβ expression; altered estrous cycle; similar gene expression profile to ERα agonist; altered E2
  - Fish: altered vitellogenin expression; altered ERα and ERβ expression; ↑ E2

KC 8: "Modulates receptor-mediated effects" (cont'd)

- Androgen receptor (AR) and testosterone
  - Human observational studies: altered testosterone levels
  - Human cells *in vitro*: ↓ AR activation by DHT in a reporter gene study; altered testosterone
  - Rodents *in vivo*: altered AR expression
  - Rodents *in vivo* and *in vitro*: ↓ testosterone
  - ↓ human AR activation by DHT in a reporter gene study in CHO cells

KC 8: "Modulates receptor-mediated effects" (cont'd)

- Peroxisome proliferator-activated receptor (PPARα)
  - Human cells *in vitro:* ↑ PPARα-mediated gene expression
  - Rodents *in vivo*: altered expression of genes related to PPARα
  - Animal cells *in vitro*: ↑ expression of genes related to PPARα
  - Fish: altered expression of PPARα
- A weaker agonist of human PPARα compared to rodent PPARα, yet ↑ PPARα-mediated gene expression in human cells
- Two studies found effects independent of PPARα, using PPARα-knockout mice

KC 8: "Modulates receptor-mediated effects" (cont'd)

- PPARγ, PXR, CAR, PPARβ/δ
  - Human cells *in vitro:* alters activity of PPARγ, PXR, CAR
  - Rodents *in vivo*: altered gene expression of PPARγ, PXR, CAR
  - Animal cells *in vitro*: altered gene expression of PXR, CAR; activation of PPARβ/δ, PPARγ
  - Fish: altered expression of PPARγ, PPARβ
- Thyroid hormones
  - No consistent trends in the general human population
  - ↓ thyroid hormone levels in animal studies

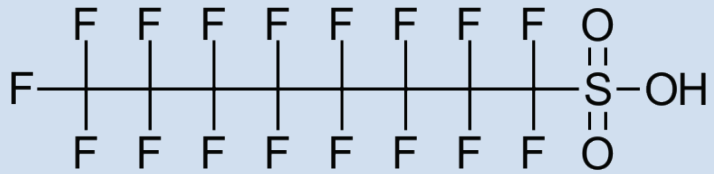
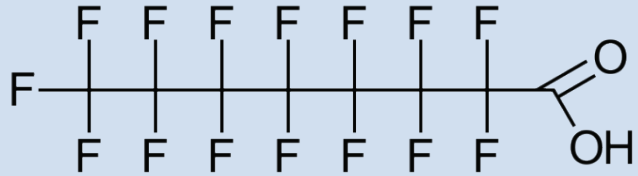
KC 9: “Causes immortalization”

- Studies on PFOS serum levels and telomere length in humans
  - Positive associations in females and the 40-50 year old age group in a US population
  - A weakly positive association with maternal telomere length but not with newborn in a California birth cohort
  - Inverse associations in female newborns from China and 50-65 year olds in a Belgian population
- ↑ transformation frequency of SHE cells
- ↑ malignant transformation of normal human breast epithelial cells

KC 10: "Alters cell proliferation, cell death, or nutrient supply"

- ↑ proliferation from multiple studies of human cells *in vitro*
- ↑ proliferation or ↓ apoptosis in rat liver
- Early transcriptional changes related to cell cycle control, apoptosis, and proliferation in the liver of rats exposed to PFOS *in utero* and via lactation
- Altered expression of proteins linked to cell proliferation, including ↑ levels of regulatory cell cycle proteins and growth factors in a human fetal liver cells
- ↓ GJICs in a rat liver epithelial cell line
- In primary salmon hepatocytes *in vitro*: a slight ↓ in apoptosis, ↓ caspase 3B expression

Comparison of PFOS and PFOA: Treatment-related tumors in rat cancer bioassays

Chemical	PFOS	PFOA
Structure		
Thyroid follicular cell adenoma and/or carcinoma	M, F	Not observed
Liver hepatocellular adenoma and/or carcinoma	**M (adenomas only), F**	**M, F**
Pancreatic tumors	**M (islet cell carcinoma)**	**M, F (acinar cell adenoma and/or carcinoma)**
Testicular Leydig cell adenoma	Not observed	M
Mammary gland fibroadenoma	**F**	**F**
Uterine adenoma and/or adenocarcinoma	Not observed	F

Comparison of PFOS and PFOA: Data-rich endpoints

Both chemicals have evidence for:

- Genotoxic effects
  - *E.g.*, chromosomal effects, DNA damage
- Effects related to oxidative stress and carcinogenesis
  - *E.g.*, oxidative DNA damage, ↑ ROS & RNS, alter total antioxidant capacity
- Immunosuppressive effects related to carcinogenesis
  - *E.g.*, suppress IgM production, reduce T cells & B cells
- Receptor-mediated effects related to carcinogenesis
  - *E.g.*, alter expression of genes regulated by ERα, PPARα, PPARγ, PXR, CAR

Summary of evidence

Summary: Carcinogenicity studies

- Humans
  - Breast cancer: results mixed, regardless of whether PFOS levels were measured before or after diagnosis
  - Other sites: too few studies to draw conclusions
- Animals
  - Long-term carcinogenicity studies in rats
    - Hepatocellular adenomas in males and combined adenomas and carcinomas in females
    - Thyroid follicular cell adenomas in males and rare adenomas and carcinoma in females
    - Pancreatic islet cell carcinomas in males
    - Mammary gland fibroadenomas in females
  - Tumor promotion study in rainbow trout with aflatoxin B1 as initiator: liver adenomas and carcinomas combined

Summary: Key characteristics of carcinogens

- KC 2: ↑ mutagenicity, ↑ chromosomal effects, ↑ DNA damage
- KC 4: altered methylation of specific gene regions, global methylation changes, microRNA changes, alterations in expression of DNMTs
- KC 5: ↑ oxidative DNA damage, ↑ ROS & RNS, ↑ lipid peroxidation
- KC 7: ↓ IgM, ↓ NK cells, ↓ thymocytes & splenocytes
- KC 8: changes in expression of genes regulated by ERα, PPARα, PPARγ, PXR, and CAR, and in AR expression; ↑E2 and ↓ thyroid hormone
- KC 10: ↑ cell proliferation, ↓ apoptosis, ↓ GJICs
- KC 6/KC 9: unclear/inconsistent effects