Application of the Key Characteristics of Carcinogens to PFAS

Alexis Temkin, Ph.D
Environmental Working Group
Center for PFAS and Cancer (CPAC) Joint Virtual Symposium
Thursday, March 7, 2024

Temkin et al. 2020. Application of the Key Characteristics of Carcinogens to Per and Polyfluoroalkyl Substances. *International Journal of Environmental Research and Public Health. 17, 1668*

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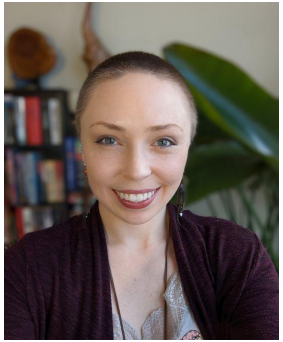

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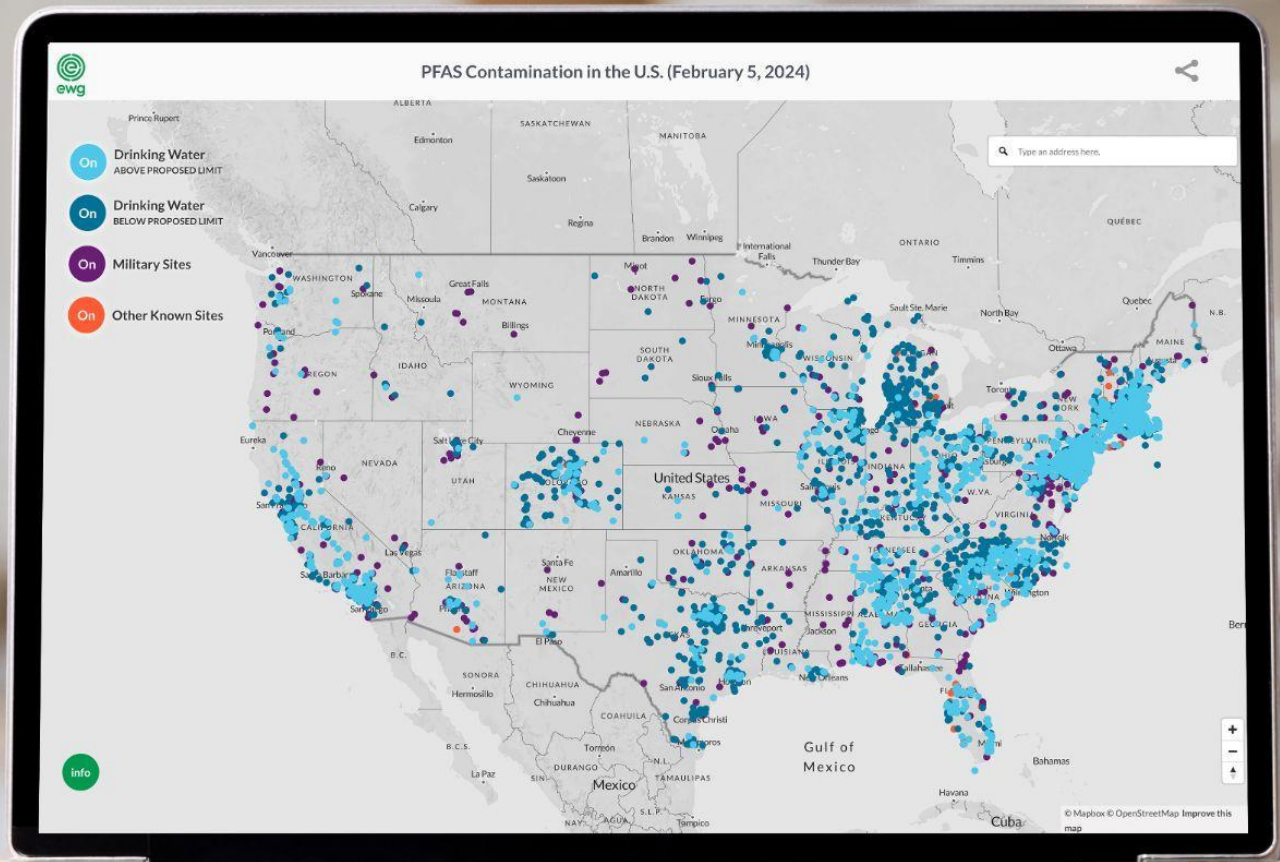
PFAS Contamination in the U.S. (February 5, 2024)
Drinking Water
ABOVE PROPOSED LIMIT
Drinking Water
BELOW PROPOSED LIMIT
Military Sites
Other Known Sites
Type an address here.
United States
Mexico
Gulf of Mexico
Cuba
Bahamas
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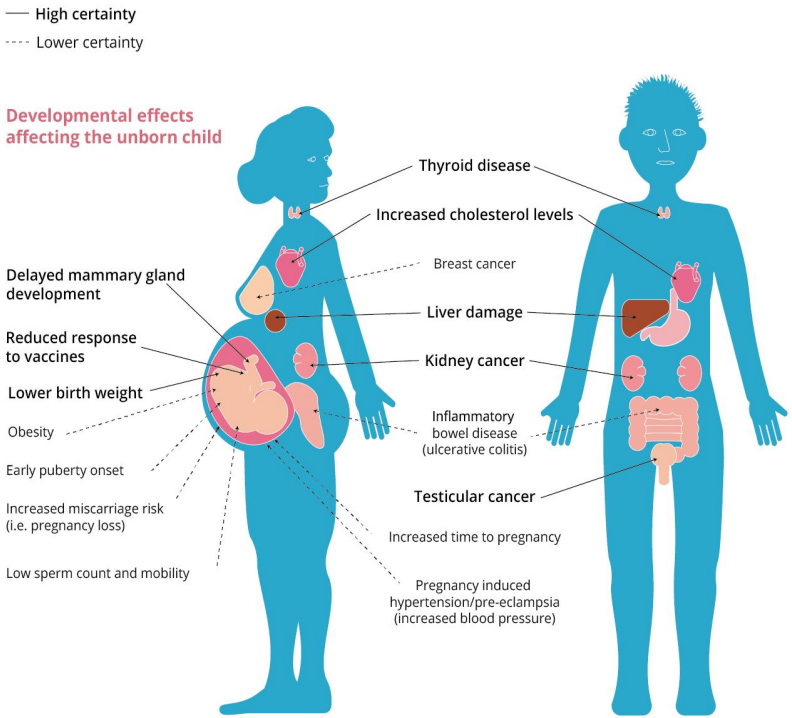
The PFAS Problem(s)

- Widespread global contamination from broad range of uses, industrial dischargers and transport
- Highly persistent and mobile chemicals
- **Growing number of health concerns associated with PFAS exposure**
- **Huge chemical class**

Health Effects of PFAS Exposure



Sources: US National Toxicology Program (2016); C8 Health Project Reports (2012); WHO IARC (2017); Barry et al. (2013); Fenton et al. (2009); and White et al. (2011) apud Emerging chemical risks in Europe — 'PFAS'[1].

Health harms associated with long-chain and short-chain PFAS commonly detected in drinking water and used in consumer or industrial products

Chemical	Harm to the immune system	Harm to development and reproduction	Harm to the endocrine system	Metabolic changes	Changes in the liver	Increased risk of cancer
	Weaker immune response; lower antibody production in response to vaccination; increased allergic response; increased risk of asthma; changes in spleen and thymus	Reduced birth weight; pregnancy-induced hypertension; preeclampsia; reduced fertility; reduced duration of breastfeeding; altered mammary gland development; harm to the male reproductive system	Changes in hormone levels, including thyroid and reproductive hormones; thyroid disease; hormone receptor activation	Increased cholesterol and lipids; weight gain; diabetes	Increased liver weight; changes in liver enzymes	Increased risk of testicular, kidney or breast cancer; increased tumors in laboratory animals; evidence of one or more of the key characteristics of carcinogens
Long-chain PFAS						
PFOA*#	■	■	■	■	■	■
PFOS*#	■	■	■	■	■	■
PFNA*#	■	■	■	■	■	●
PFHxS*#	■	■	■	●	■	●
PFDA*#	■	■	■	■	■	●
PFDoA#	●	●	●	▲	■	●
PFUA#	●	■	●	▲	■	●

The Key Characteristics of Carcinogens

- Characteristics to organize and integrate evidence for chemical hazard identification
- Born out of analysis of know human carcinogens classified by IARC
- Focus on mechanistic data, but evidence can come from epidemiology, animal bioassays, and *in vitro*/NAMs
- Intentionally broad, and less specific that AOPs or MOA frameworks to improve risk assessment

Smith et al. (2016) *Environmental Health Perspectives. 124(6): 713-721*
Guyton et al. (2018) *Chemical Research and Toxicology. 31(12):1290-1292*
Smith et al. (2020) *Cancer Epidemiology, Biomarkers & Prevention. 29(10):1887-1903*

The Key Characteristics of Carcinogens

Key Characteristics	Examples of Relevant Evidence
1—Is electrophilic or can be metabolically activated	Parent compound or metabolite with an electrophilic structure (e.g., epoxide, quinone, etc.), formation of DNA and protein adducts
2—Is genotoxic	DNA damage (DNA strand breaks, DNA-protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, cytogenetic changes (e.g., chromosome aberrations, micronuclei)
3—Alters DNA repair or causes genomic instability	Alterations of DNA replication or repair (e.g., topoisomerase II, base-excision or double-strand break repair
4—Induces epigenetic alterations	DNA methylation, histone modification, microRNAs
5—Induces oxidative stress	Oxygen radicals, oxidative stress, oxidative damage to macromolecules (e.g., DNA, lipids)
6—Induces chronic inflammation	Elevated white blood cells, myeloperoxidase activity, altered cytokine and/or chemokine production
7—Is immunosuppressive	Decreased immunosurveillance, immune system dysfunction
8—Modulates receptor-mediated effects	Receptor in/activation (e.g., ER, PPAR, AhR) or modulation of endogenous ligands (including hormones)
9—Causes immortalization	Inhibition of senescence, cell transformation
10—Alters cell proliferation, cell death or nutrient supply	Increased proliferation, decreased apoptosis, changes in growth factors, energetics and signaling pathways related to cellular replication or cell-cycle control, angiogenesis

Applying the KC Framework to PFAS

Study Objective:

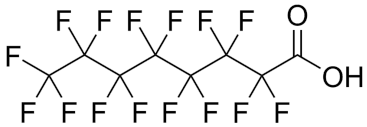
Assemble, organize and evaluate the literature on PFAS through the lens of the key characteristics of carcinogens

Methods:

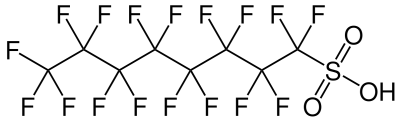
- Selected 26 PFAS
- Literature search of existing reviews supplemented with peer-reviewed articles
- Epidemiology, animal bioassay and mechanistic data
- Strength of evidence assessment

The 26 PFAS we focused on from a huge chemical class

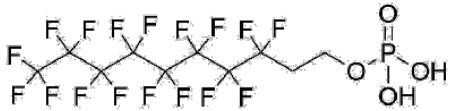
	Perfluoroalkyl carboxylic acid
Long Chain	PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA
Short Chain	PFHxA, PFBA, PFPeA, PFHpA



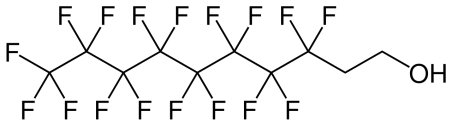
	Perfluoroalkane sulfonic acid/sulfonamid
Long Chain	PFOS, PFHxS, PFOSA*
Short Chain	PFBS



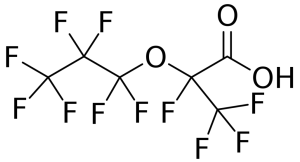
	Polyfluoroalkyl phosphate ester
Long Chain	8:2 monoPAP, 8:2 diPAP, 8:2 triPAP, 10:2 diPAP
Short Chain	4:2 diPAP, 6:2 diPAP



	Fluorotelomer alcohol
Long Chain	8:2 FTOH
Short Chain	4:2 FTOH, 6:2 FTOH



	Fluorinated ether carboxylate
Short Chain	GenX (HFPO-DA); PMOH, PMPP/ADONA



Major Findings:

- Multiple PFAS exhibit several key characteristics of carcinogens
- Many data gaps
- PFOA and PFOS exhibit up to 5 KCS
- KCs 4, 5, 7, 8 and 10 have the strongest evidence
    - 4 - epigenetic alterations
    - 5 - oxidative stress
    - 7 - immune suppression
    - 8 - receptor-mediated effects
    - 10 - cell proliferation

Multiple PFAS exhibit several key characteristics of carcinogens

Key Characteristics	PFOA	PFOS and Long-chain PFAS	Short-chain PFAS	Examples of Relevant Evidence
1—Is electrophilic or can be metabolically activated	PFOA	PFOS, PFHxS, PFNA, PFDA, PFUnA, PFDoA, PFOSA, 8:2 FTOH*, 8:2 diPAP*, 10:2 diPAP	PFBS, PFHxA, PFBA, PFHpA, GenX, 6:2FTOH*, 4:2 diPAP*, 6:2 diPAP	PFAS are quite stable with long half-lives
2—Is genotoxic	PFOA	PFOS, PFNA, PFDA, PFHxS	PFHxA, GenX, PFBS	PFAS are not directly mutagenic; Evidence of DNA damage in some assays likely from secondary event such as oxidative damage
3—Alters DNA repair or causes genomic instability	Data gap	Data gap	Data gap	Data gap
4—Induces epigenetic alterations	PFOA	PFOS, PFHxS, PFNA, PFUnA	Data gap	Observations of differentially methylated regions and changes in global methylation in human cohorts, and *in vitro* assays
5—Induces oxidative stress	PFOA	PFOS, PFHxS, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, PFOSA	PFBS, PFHxA, PFPeA	Evidence primarily from *in vivo* and *in vitro* assays investigating ROS levels, lipid peroxidation, antioxidant enzymes, etc.

No association

Somewhat suggestive evidence

Suggestive evidence

Strong evidence

Multiple PFAS exhibit several key characteristics of carcinogens

Key Characteristics	PFOA	PFOS and Long-chain PFAS	Short-chain PFAS	Examples of Relevant Evidence
6—Induces chronic inflammation	PFOA	PFOA, PFOS, PFHxS, PFDA, PFUnA, 8:2 FTOH	PFBS	Associations with disease characterized by chronic inflammation, measurements of proinflammatory cytokines *in vivo* and *in vitro*
7—Is immunosuppressive	PFOA	PFOS, PFHxS, PFNA, PFDA, PFOSA, 8:FTOH	PFBS	Reduced vaccine response in humans, decreased T cell-dependent antibody response in animals, changes in immune cell populations
8—Modulates receptor-mediated effects	PFOA	PFOS, PFHxS, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA, 8:2 FTOH, 8:2 monoPAP, 8:2 diPAP, 8:2 triPAP, 10:2 diPAP	PFBS, PFHxA, PFBA, PFPeA, PFHpA, GenX, ADONA, 4:2 FTOH, 6:2 FTOH	Evidence of binding to several nuclear receptors especially PPARα, changes in circulating hormones and hormone mediated effects in humans and animals
9—Causes immortalization	Data gap	Data gap	Data gap	Some studies on telomere length
10—Alters cell proliferation, cell death or nutrient supply	PFOA	PFOS, PFHxS, PFNA, 8:2 FTOH	PFBS, PFHxA, 6:2 FTOH	Increases in proliferation, migration and invasion in cancer cell lines, cell cycle disruption

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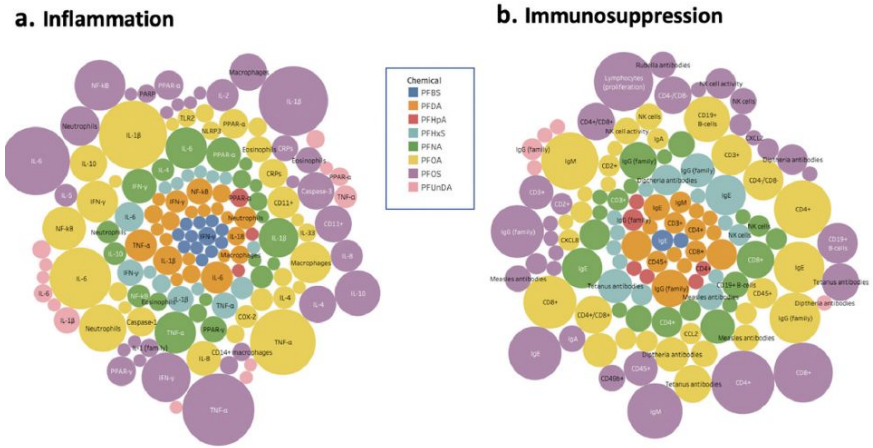
Where we are now, and next steps



1) support conclusions by Temkin et al. (2020) that PFAS exposure causes immunosuppression; 2) provide additional evidence that PFAS exposure may cause chronic inflammation; and 3) use biomarker-based evidence to propose potential underlying mechanisms as to how PFASs induce both chronic inflammation and immunosuppression

Data generated from high throughput screening assays provides more evidence and additionally assays for several key characteristics

Zhang et al (2023): A systematic evidence map of chronic inflammation and immunosuppression related to per- and polyfluoroalkyl substance (PFAS) exposure. Environ Res. 2023 Mar 1;220:115188. doi: 10.1016/j.envres.2022.115188. Epub 2022 Dec 30. PMID: 36592815; PMCID: PMC10044447.

Singh and Hsieh (2021): Exploring Potential Carcinogenic Activity of Per- and Polyfluorinated Alkyl Substances Utilizing High-Throughput Toxicity Screening Data. Int J Toxicol. 2021 Jul-Aug;40(4):355-366. doi: 10.1177/10915818211010490. Epub 2021 May 4. PMID: 33944624.

IARC classifications for PFOA and PFOS

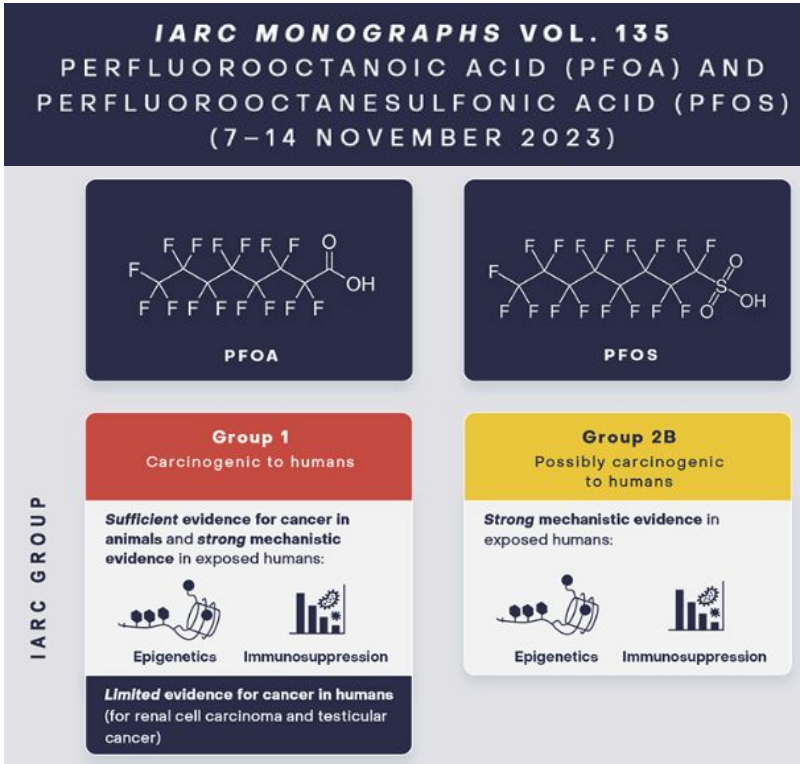
International Agency for Research on Cancer

World Health Organization

Table 1. Summary of classifications in *IARC Monographs* Volume 135

Agent	Evidence stream			Overall evaluation
	Cancer in humans	Cancer in experimental animals	Mechanistic evidence (key characteristics of carcinogens)	
Perfluorooctanoic acid (PFOA)	*Limited* (renal cell carcinoma and testicular cancer)	*Sufficient*	*Strong* in exposed humans (KCs 4, 7), human primary cells (KCs 5, 7, 8), experimental systems (KCs 4, 5, 7, 8, 10)	Group 1
Perfluorooctanesulfonic acid (PFOS)	*Inadequate*	*Limited*	*Strong* in exposed humans (KCs 4, 7), human primary cells (KCs 5, 7, 8), experimental systems (KCs 4, 5, 7, 8, 10)	Group 2B

KCs, key characteristics of carcinogens; KC4, induces epigenetic alterations; KC5, induces oxidative stress; KC7, is immunosuppressive; KC8, modulates receptor-mediated effects; KC10, alters cell proliferation, cell death, or nutrient supply.



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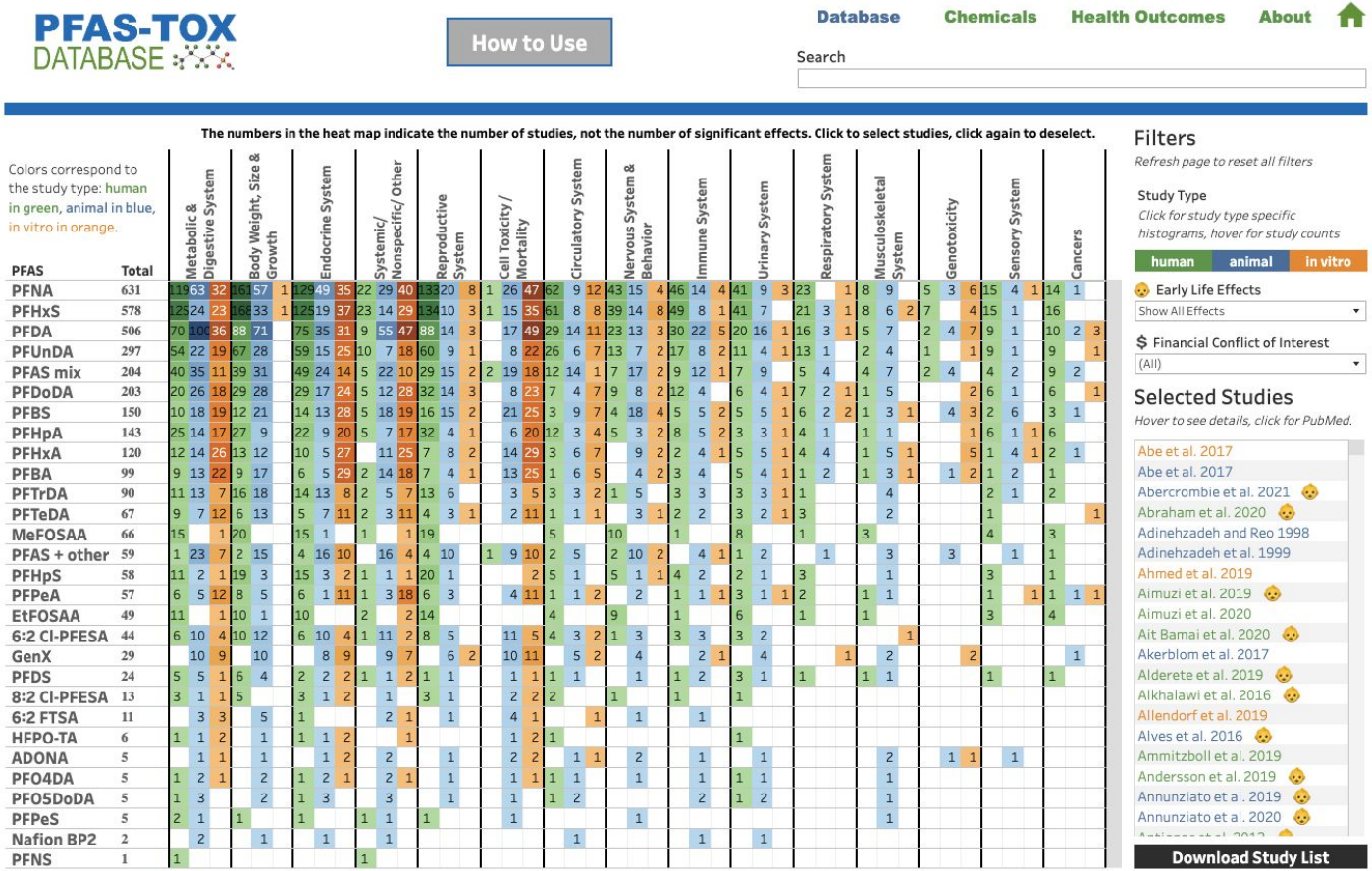
PFAS Regulatory Guidelines based on Cancer Endpoints

- Strictest drinking water standards and guidelines for PFAS are based on cancer endpoints

- EPA Proposed Maximum Contaminant Level
  - PFOA - 4 ppt, zero for MCLG
  - PFOS - 4 ppt, zero for MCLG

- California Public Health Goal
  - PFOA - 0.007 ppt
  - PFOS - 1 ppt

Addressing Data Gaps



Pelch KE, Reade A, Kwiatkowski CF, Wolffe T, Merced-Nieves FM, Cavalier H, Schultz K, Rose K, Varshavsky J. 2021.
PFAS-Tox Database available at https://pfastoxdatabase.org DOI: 10.17605/OSF.IO/F9UPX

In summary:

- Multiple PFAS exhibit several key characteristics of carcinogens
- Epigenetics and immune impacts are emerging as key mechanisms for PFAS carcinogenic properties
- Data gaps remain, and there is a need to investigate and screen poorly characterized PFAS
- Regulators and risk assessors can use this approach to effectively regulate groups and classes of PFAS

Thank you

Any questions?

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