Application of the Key Characteristics of Carcinogens to PFAS

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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
We are scientists, policy experts, advocates, and educators

EWG’s mission is simple: To empower you with breakthrough research to make informed choices and live a healthy life in a healthy environment.
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

**Developmental effects affecting the unborn child**
- Thyroid disease
- Increased cholesterol levels
- Breathing problems
- Kidney cancer
- Rheumatoid arthritis
- Liver damage
- Inflammatory bowel disease (ulcerative colitis)
- Testicular cancer
- Increased time to pregnancy
- Pregnancy-induced hypertension/pre-eclampsia (increased blood pressure)
- Delayed mammary gland development
- Reduced response to vaccines
- Lower birth weight
- Obesity
- Early puberty onset
- Increased miscarriage risk (i.e., pregnancy loss)
- Low sperm count and mobility

**Sources:** US National Toxicology Program (2016); C8 Health Project Reports (2012); WHO IARC (2017); Barry et al. (2013); Fenten et al. (2009); and White et al. (2011) apud Emerging chemical risks in Europe — “PFAS®.”

**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 |
|----------|----------------------------|-------------------------------------|-----------------------------|-----------------|-------------------|------------------------|
| PFOA®    | 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; pre-eclampsia; 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 |
| PFOS®    |                                  |                                    |                             |                 |                   |                        |
| PFNA®    |                                  |                                    |                             |                 |                   |                        |
| PFFnS®   |                                  |                                    |                             |                 |                   |                        |
| PFDA®    |                                  |                                    |                             |                 |                   |                        |
| PFDoA®   |                                  |                                    |                             |                 |                   |                        |
| PFU®     |                                  |                                    |                             |                 |                   |                        |

**Long-chain PFAS**
The Key Characteristics of Carcinogens

- Characteristics to organize and integrate evidence for chemical hazard identification
- Born out of analysis of known 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 than 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 | Perfluoroalkane sulfonic acid/sulfonamid | Polyfluoroalkyl phosphate ester |
|-------------------------------|------------------------------------------|----------------------------------|
| **Long Chain**                |                                          | **Long Chain**                   |
| PFOA, PFNA, PFDA, PFUnA, PFDoA, PFTrDA, PFTeDA | PFOS, PFHxS, PFOSA*                               | 8:2 monoPAP, 8:2 diPAP, 8:2 triPAP, 10:2 diPAP |
| **Short Chain**               |                                          | **Short Chain**                   |
| PFHxA, PFBA, PFPeA, PFHpA      | PFBS                                     | 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, PFTeDA, 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 |

**Legend:**
- **No association**
- **Somewhat suggestive evidence**
- **Suggestive evidence**
- **Strong evidence**
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.

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

Table 1. Summary of classifications in IARC Monographs Volume 135

| Agent                                           | Cancer in humans | Evidence stream | Mechanistic evidence (key characteristics of carcinogens) | Overall evaluation |
|-------------------------------------------------|------------------|-----------------|----------------------------------------------------------|-------------------|
| Perfluorooctanoic acid (PFOA)                   | Limited (renal cell carcinoma and testicular cancer) | Sufficient      | Strong in exposed humans (KC5s 4, 7), human primary cells (KC5s 5, 7, 8), experimental systems (KC5s 4, 5, 7, 8, 10) | Group 1            |
| Perfluorooctanesulfonic acid (PFOS)              | Inadequate       | Limited         | Strong in exposed humans (KC4, 7), human primary cells (KC5s 5, 7, 8), experimental systems (KC5s 4, 5, 7, 8, 10) | Group 2B           |

KC5s, 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.
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, PFDa, 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, PFDa, PFTrDA, PFTeDA, PFOSA     | PFBS, PFHxA, PFPeA                      | Evidence primarily from *in vivo* and *in vitro* assays investigating ROS levels, lipid peroxidation, antioxidant enzymes, etc. |

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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, Reede A, Kwiatkowski CF, Wolff 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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