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Via Electronic Mail (quality@epa.gov)
Information Quality Guidelines Staff
U.S. EPA Headquarters
1200 Pennsylvania Ave., NW
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Re: Request for Correction of GenX Chemicals Toxicity Assessment

Dear Sir or Madam:

We are filing this petition on behalf of The Chemours Company FC, LLC ("Chemours" or "the Company") pursuant to the Information Quality Act ("IQA")[1] requesting that the U.S. Environmental Protection Agency ("EPA" or "the Agency") withdraw and correct its October 25, 2021 GenX Chemicals Toxicity Assessment (the "Toxicity Assessment" or "HFPO-DA Assessment").[2] As discussed below, EPA's Toxicity Assessment contains substantial scientific flaws; fails to incorporate available peer-reviewed scientific literature highly relevant to the analysis; and significantly overstates the potential human risks associated with HFPO-DA. Accordingly, EPA's Toxicity Assessment does not comply with the IQA and should be corrected.

I. Introduction and Summary of Request

As one of its very first actions, the Biden Administration issued a Memorandum entitled "Restoring Trust in Government Through Scientific Integrity and Evidence-Based

[1] Section 515(a) of the Treasury and General Government Appropriations Act for Fiscal Year 2001, P.L. 106-554; 44 U.S.C. § 3516 (notes).
[2] EPA, *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3), Also Known as "GenX Chemicals"* (Oct. 2021) ("Final Toxicity Assessment").

Policymaking." That Memorandum directed agency leaders "to make evidence-based decisions guided by the best available science and data." The Administration made this commitment to prioritize the scientific integrity of agency action:

> Scientific and technological information, data, and evidence are central to the development and iterative improvement of sound policies, and to the delivery of equitable programs, across every area of government. Scientific findings should never be distorted or influenced by political considerations. When scientific or technological information is considered in policy decisions, it should be subjected to well-established scientific processes, including peer review where feasible and appropriate, with appropriate protections for privacy. Improper political interference in the work of Federal scientists or other scientists who support the work of the Federal Government and in the communication of scientific facts undermines the welfare of the Nation, contributes to systemic inequities and injustices, and violates the trust that the public places in government to best serve its collective interests.[3]

The October 2021 Toxicity Assessment for HFPO-DA is the product of a scientific process that conflicts with the Biden Administration's principles of scientific integrity. The Agency's Toxicity Assessment is flawed and contrary to EPA's scientific standards, and will, if not corrected, have very real and lasting adverse impacts on critical public interests—including undermining the confidence U.S. citizens place in the Agency's technical assessments.

[3] The White House, *Memorandum on Restoring Trust in Government Through Scientific Integrity and Evidence-Based Policymaking* (Jan. 27, 2021), https://www.whitehouse.gov/briefing-room/presidential-actions/2021/01/27/memorandum-on-restoring-trust-in-government-through-scientific-integrity-and-evidence-based-policymaking/.

Chemours therefore petitions EPA to correct information contained in its HFPO-DA Toxicity Assessment. This Request for Correction is appropriately submitted pursuant to the IQA, the Agency's own implementing guidelines,[4] as well as the guidelines of the Office of Management and Budget ("OMB")[5] because: (a) the toxicity values referenced in EPA's Toxicity Assessment constitute "information" the Agency has "disseminated" publicly[6]; (b) the Toxicity Assessment is and will continue to be (unless it is timely and publicly corrected) "influential"[7]; and (c) the Toxicity Assessment must be withdrawn and corrected to ensure that it meets, in accordance with EPA's own requirements, the Agency's most stringent data quality and scientific standards[8] (indeed, it must reflect the "best available science" and employ "sound and objective" science).[9]

Specifically, the HFPO-DA Assessment contains significant deviations from standard EPA toxicity assessment methods and is not supported by the weight of scientific evidence, and the process EPA undertook to develop the Toxicity Assessment was procedurally flawed. For example:

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[4] EPA, *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency* (Oct. 2002) (hereinafter "EPA Guidelines").
[5] *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by Federal Agencies,* 67 Fed. Reg. 8451 (Feb. 22, 2002) (hereinafter, "OMB Guidelines").
[6] EPA Guidelines at 15.
[7] By EPA's own standards, the Toxicity Assessment will have a "clear and substantial impact" on both public and private sector decisions including (as the final assessment specifically encourages) the reliance by states and localities on the assessment when articulating water quality and other standards derived from the assessment. *See* Final Toxicity Assessment at xi. Further, as discussed herein, the Toxicity Assessment has and will continue to cause impacts to Chemours, including economic harm and reputational damage, as a result of the technical and scientific deficiencies and errors in the assessment.
[8] EPA Guidelines generally and OMB Guidelines confirm: "The more important the information, the higher the quality standards to which it should be held." 67 Fed. Reg. at 8452.
[9] *See* EPA Guidelines at 22.

- The rodent liver effects underpinning the assessment are peroxisome proliferator-activated receptor alpha ("PPAR-alpha") effects that are not relevant to humans;
- The assessment did not evaluate—or even acknowledge—a critically important 2020 peer-reviewed published study by Dr. Grace A. Chappell et al. that provides compelling additional evidence that the rodent liver effects underpinning the assessment are not relevant to humans[10];
- References in the assessment to non-PPAR-alpha modes of action are not supported by scientific data and are, in some cases, directly contradicted by the very sources relied upon by EPA;
- The assessment relies on observations by the National Toxicology Program Pathology Working Group ("NTP PWG") that do not follow evaluation criteria set forth in the peer-reviewed scientific literature;
- The assessment's new toxicological endpoint—a "constellation of liver effects"—is unprecedented, and misapplies scientific criteria in determining whether observed effects are in fact adverse effects in the context of a human health risk assessment;

[10] *See* Chappell, G.A., C.M. Thompson, J.C. Wolf, J.M. Cullen, J.E. Klaunig, and L.C. Haws. 2020. Assessment of the Mode of Action Underlying the Effects of GenX in Mouse Liver and Implications for Assessing Human Health Risks. Toxicologic Pathology 48(3):494-508.

- The assessment uses inappropriate and significantly inflated uncertainty factors that are inconsistent with EPA's own guidance and practice in other toxicity assessments;
- EPA's process in developing the assessment included a significant change from EPA's prior draft toxicity assessment for HFPO-DA[11] that necessitated additional public comment (which did not occur);
- EPA failed to provide a publicly available Administrative Record and failed to undertake a proper literature review; and
- As discussed below, EPA has not taken into account available epidemiological evidence showing no increased risk of cancers or liver disease attributable to exposure to HFPO-DA.

For these and additional reasons set forth in this Request for Correction, EPA's HFPO-DA Assessment does not meet EPA's own scientific standards and does not reflect "sound and objective scientific practices," nor does the final Toxicity Assessment reflect use of the best available science. Accordingly, Chemours requests EPA to promptly grant this Request for Correction and take necessary corrective action. At a minimum, the corrective actions should include the immediate and public withdrawal of the Toxicity Assessment to correct the specific scientific errors identified in this Request for Correction and allow for additional, objective, peer review.

[11] EPA, *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3), Also Known as "GenX Chemicals"* (Nov. 2018) ("Draft Toxicity Assessment").

II. Chemours

Chemours is a global provider of performance chemicals that are key inputs in end-products and processes in a variety of industries. In producing essential products, Chemours is implementing its 2030 Corporate Responsibility Commitment goals. Included within these goals is the Company's public commitment to reduce air and water process emissions of fluorinated organic chemicals from a 2018 baseline by 99% or greater by 2030.[12]

One of Chemours's business segments is its Advanced Performance Materials ("APM") segment, which provides high-end polymers and other advanced materials that deliver unique attributes, including chemical inertness, thermal stability, and dielectric properties critical in many modern manufacturing processes. Chemours's APM business creates materials and products—including fluoropolymers—that are essential for countless industries including the medical, automotive, electronics, aerospace, energy, and semiconductor industries. Fluoropolymers are used in every car, airplane, and cellphone. They are critical to maintaining the integrity and quality of the vast majority of prescription drugs. Fluoropolymers are also used in medical equipment including catheters, saline bags, and filtration devices that supply oxygen to newborn babies that are medically compromised. The manufacturing of all computer chips requires the use of

[12] *See* Chemours, *2020 Corporate Responsibility Commitment Report Executive Summary*, https://www.chemours.com/en/-/media/files/corporate/crc/2020/corporate-responsibility-commitment-report-executive-summary.pdf?la=en&rev=70fb755d8ea5478eae655192d9e48998&hash=AC9812CAE7B78A6F47A9E040DAB830F9.

fluoropolymers, as they are essential to maintaining the highest levels of purity in the fabrication processes. Fluoropolymers are critical components of high-speed communications. Fluoropolymers also allow for light-weighting of vehicles to reduce energy consumption and reduce emissions. In industrial applications, fluoropolymers are used in the infrastructure of manufacturing processes in piping and vessels to protect employees from harsh chemicals. Fluoropolymers in ion exchange membranes are critical to the production of chlorine for applications such as water purification. Further, fluoropolymers are used in the production of hydrogen from renewable sources and are at the heart of the fuel cell for the consumption of hydrogen.

Fluoropolymers have a unique combination of properties making them durable, efficient, reliable, versatile, and ultimately fundamental to the products they enable. Their properties include fire resistance, weather resistance, temperature resistance, chemical resistance, non-wetting and non-sticking properties, and high-performance dielectric properties. While some chemistries might offer a similar performance to fluoropolymers for a particular parameter or property, it is the unique combination of properties that set fluoropolymers apart and make them vital to the sectors and industries they serve.

The responsible manufacturing of fluoropolymers in the United States is critical to furthering U.S. technology leadership, onshoring key industries (including semiconductor manufacturing), and enabling American supply chain resiliency and security. Many of Chemours's fluoropolymer products are manufactured in the United States, and there are often no domestically manufactured alternative replacement products available for these mission-critical applications.

One critical example of the importance of PFA fluoropolymers—of which Chemours is the only domestic producer—is in the manufacture of semiconductor chips. ***Put simply, semiconductor chips cannot be manufactured without fluoropolymers.*** During the pandemic, there has been a profound impact on the supply chain due to the offshoring of this industry, as everything from automobiles to consumer electronics have been affected by a chip shortage. The President has made clear that the continued erosion of the United States' leadership in semiconductor manufacturing poses significant economic and national security risks, and he has announced commitments to strengthen the domestic semiconductor industrial base. Without Chemours and its ability to make fluoropolymers onshore in a safe and reliable manner, this will not be possible.

Additionally, Chemours's chemistries are critical to achieving the United States' energy transition and decarbonization ambitions. Chemours's fluoropolymers are essential in manufacturing the lithium-ion batteries central to electrifying cars and other modes of transportation. ***Chemours is also the major domestic manufacturer of key components used in hydrogen fuel cells and water electrolysis***, which show great potential for harnessing green hydrogen as an alternative to fossil fuels.

EPA's Toxicity Assessment, unless corrected, has the potential to cause significant harm to Chemours as well as to the broader United States economy, as regulatory restrictions that are based on the assessment's flawed conclusions may inhibit critical uses of the substances for which technically feasible chemical alternatives are not available.

III. History of HFPO-DA Compounds

Integral to Chemours's manufacturing of a wide range of fluoropolymers is the use of HFPO Dimer Acid and its ammonium salt as polymerization aids. HFPO Dimer Acid and its ammonium salt are sometimes referred to collectively by the trade name "GenX" or "GenX technology" and will be collectively referred to here as "HFPO-DA".[13]

The GenX technology was originally developed by DuPont to enable the manufacture of high performance fluoropolymers without the use of perfluorooctanoic acid ("PFOA") as part of EPA's PFOA Stewardship Program. In 2006, EPA invited DuPont and other fluoropolymer and telomer manufacturers to participate in a voluntary stewardship program with goals of reducing PFOA emissions and product content by 95% by 2010 while working towards total elimination by 2015.[14] DuPont agreed to participate in the program and committed to (and then met) the goals EPA had set forth prior to its spin-off of Chemours.

To meet its PFOA Stewardship Program commitments, DuPont undertook a research and development program to find technology replacements for PFOA in the broad range of products whose manufacturing process was dependent on PFOA. From those research efforts, the GenX technology, and its use of HFPO-DA, emerged as a suitable substitute for the use of PFOA as a polymerization aid.

[13] The CAS Registry Number assigned to the substance known as HFPO Dimer Acid is 13252-13-6.

[14] *See Fact Sheet: 2010/2015 PFOA Stewardship Program*, EPA, https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/fact-sheet-20102015-pfoa-stewardship-program (last visited Jan. 4, 2022). Other participants in the program included Daikin, Asahi Glass, Arkema, 3M/Dyneon, and Solvay Solexis.

HFPO-DA is a shorter-chain molecule than PFOA (with two chains of three carbons each, as opposed to one chain of eight carbons). Based on studies showing rapid elimination in rats, mice, and primates,[15] among other studies, it is widely-accepted that HFPO-DA is rapidly eliminated from peoples' bodies.[16] This is supported by an exposure study that did not find HFPO-DA in the blood of residents of North Carolina.[17] Further, a study of volunteer Chemours workers shows an estimated elimination half-life of 82 hours.[18] HFPO-DA does not degrade into PFOA or other longer-chain compounds if released into the environment.

Because the GenX technology reflected a new technology, in accordance with Section 5 of the Toxic Substances Control Act ("TSCA"), in 2008 DuPont submitted a pre-manufacture notice ("PMN") along with initial toxicity studies and other related information to EPA seeking to authorize use of the GenX chemicals. The toxicity studies submitted were extensive and included the following:

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[15] *See, e.g.,* Gannon, S.A., W.J. Fasano, M.P. Mawn, D.L. Nabb, R.C. Buck, L.W. Buxton, G.W. Jepson, and S.R. Frame. 2016. Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. Toxicology 340(18):1–9. doi:10.1016/j.tox.2015.12.006 (laboratory studies have confirmed that HFPO-DA is eliminated within a few days, which indicates that it is not persistent in the bodies of those test animals).

[16] *See* Final Toxicity Assessment at 21–26 (acknowledging the rapid elimination of HFPO-DA and citing several sources).

[17] *See* Kotlarz, N., J. McCord, D. Collier, C. S. Lea, M. Strynar, A. B. Lindstrom, A. A. Wilkie, J. Y. Islam, K. Matney, P. Tarte, M. E. Polera, K. Burdette, J. DeWitt, K. May, R. C. Smart, D. R. U. Knappe, and J. A. Hoppin. 2020. Measurement of novel, drinking water-associated PFAS in blood from adults and children in Wilmington, North Carolina. Environmental Health Perspectives 128(7):77005 (independent researchers at North Carolina State University found no detectable levels of HFPO-DA in the blood of any participants, even for those individuals consuming drinking water with low levels of HFPO-DA).

[18] *See Ammonium 2,3,3,3-tetrafluoro-2 (heptafluoropropoxy)propanoate*, ECHA, https://echa.europa.eu/registration-dossier/-/registered-dossier/2679/7/11/6/?documentUUID=0ee876ba-9ead-4569-8f9d-09c43212acf0 (last visited Mar. 16, 2022).

> Toxicity: Acute oral toxicity, up-and-down procedure and Acute Oral Test (rats and mice); Approximate Lethal Dose (ALD) in rats and mice; Acute Dermal Toxicity in Rats; Approximate Lethal Dose (ALD) by Skin Absorption in Rabbits; Local Lymph Node Assay (LLNA) in Mice; Acute Eye Irritation in rabbits; Acute Dermal Irritation Study in Rabbits; 7-day Repeated Dose Oral Toxicity in Rats and Male Mice; 28-Day Repeated Dose Oral Toxicity Study in Rats and Mice; Corrositex in vitro test; Combined Two Week Inhalation Toxicity and Micronucleus Studies in Rats-Transformation Byproduct. In Vitro Micronucleus and Chromosome Aberration Assay in Mouse Bone Marrow Cells; In Vitro Rat Hepatocyte Screen; Bacterial Acute Mutation test; Determination of permeability coefficient (Kp) using a static in vitro diffusion cell model; In Vitro evaluation for Chromosome Aberrations in Human Lymphocytes-transformation byproduct
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> Mutagenicity Test in Salmonella Typhimurium-transformation; byproduct; Combined two week inhalation toxicity and micronucleus studies in transformation byproduct; Water solubility, vapor pressure, and octanol water partition coefficient and other p-chem properties of transformation byproduct; Thermal Transformation Byproduct
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> Ecotoxicity/Fate: Acute toxicity to fish (Rainbow trout), daphnia, and Algae; Ready Biodegradability Study; Activated Sludge Respiration Inhibition Test; and Assessment of Hydrolysis as a Function of pH[.][19]

Following its review of DuPont's PMN, and further discussions with DuPont, EPA issued in January 2009 a TSCA Section 5(e) Consent Order (the "Section 5(e) Order") which, among other requirements, permitted DuPont to manufacture HFPO-DA subject to certain restrictions, including a requirement that DuPont complete and submit additional studies

[19] EPA, *TSCA Consent Order P-08-508 & 509*, at vi (Mar. 10, 2009), https://www.regulations.gov/document/EPA-HQ-OPPT-2020-0565-0017.

specified in the Section 5(e) Order using Good Laboratory Practices and following EPA test methods.[20] For example, the Section 5(e) Order provided: "EPA believes that a 2-year Chronic Toxicity/Carcinogenicity study (OPPTS 870.3100, OECD 453) is needed" and "EPA believes that additional pharmacokinetic, reproductive, and long-term toxicological testing on the PMN substance . . . in animals is warranted."[21]

DuPont completed and submitted the required studies, the last one in 2013, and the Agency did not request—at that time or since—any additional information or follow up studies.[22]

IV. Chemours's Manufacture and Use of HFPO-DA Compounds

Chemours's manufacture of HFPO-DA and its use in manufacturing is not widespread across the country. The manufacture of HFPO-DA is limited to a single facility, Fayetteville Works in North Carolina, and Chemours's use of HFPO-DA in other manufacturing processes in the United States is limited to two facilities, Washington Works in West Virginia and Chambers Works in New Jersey. HFPO-DA is also formed or may be present as an unintended byproduct or impurity from other manufacturing processes at the Fayetteville Works facility in North Carolina and, to a lesser degree, the Chambers Works and Parlin facilities in New Jersey.

[20] *Id.*
[21] *Id.* at ix, xi.
[22] The Section 5(e) Order also requires the company to capture or recycle 99% of HFPO-DA emissions. Chemours, which has taken over from DuPont the obligations of the Section 5(e) Order, accomplishes that 99% requirement.

A. Fayetteville Works Facility, North Carolina

The Fayetteville Works facility in North Carolina is the only Chemours facility that manufactures HFPO-DA for use in the GenX technology. In addition to permit requirements, the Fayetteville Works facility is subject to a Consent Order (the "Consent Order") with the North Carolina Department of Environmental Quality ("NCDEQ") and Cape Fear River Watch, a non-governmental organization.[23] The Consent Order was intended, and has had the effect, to drastically reduce emissions and discharges of HFPO-DA and other PFAS from the facility.[24]

One of the central requirements of the Consent Order was that Chemours install a state-of-the-art thermal oxidizer by the end of 2019, less than a year from the entry of the Consent Order. Chemours completed this over $100 million project on time, and the thermal oxidizer is destroying over 99.99% of the PFAS in the vent streams that are routed to it.

In addition to addressing air emissions, the Consent Order also comprehensively addresses water discharges. First, the Consent Order prohibits any discharges of Chemours process water from the facility's outfall to the Cape Fear River unless and until a new National Pollutant Discharge Elimination System ("NPDES") permit is issued authorizing such discharges. And with respect to discharges from groundwater into the Cape Fear River, pursuant to the Consent Order, and a 2020 Addendum, Chemours is undertaking a

[23] Consent Order, *North Carolina v. The Chemours Co. FC, LLC* (N.C. Super. Ct., Feb. 25, 2019), https://deq.nc.gov/media/12453/download.
[24] HFPO-DA byproducts are covered by the 2019 State Consent Order. For example, the thermal oxidizer treats vent streams containing HFPO-DA formed as unintended byproducts.

substantial program of abatement and remediation, including the installation of a mile-long barrier wall and groundwater extraction and treatment system to capture and reduce discharges to the River.

The Consent Order also contains provisions requiring Chemours to provide alternate water supplies to residents near the facility whose private wells contain PFAS that exceed certain specified levels. These provisions relied in part on a North Carolina Department of Health and Human Services ("NCDHHS") preliminary health goal for HFPO-DA of 140 parts per trillion.[25]

Significantly here, the 140 parts per trillion threshold is subject to adjustment based on an "applicable EPA health advisory." An EPA health advisory for HFPO-DA could therefore substantially affect Chemours's obligations under the North Carolina Consent Order. For this reason, Chemours maintains a substantial interest in ensuring that the Agency's reference dose ("RfD") for HFPO-DA, and all other assessments on which any EPA health advisory will rely, are conducted according to the standards of best available science.

B. Other Facilities

Chemours has also undertaken significant abatement and remediation actions for HFPO-DA and other PFAS emissions from the Washington Works, Chambers Works, and Parlin facilities. For example:

[25] The 140 parts per trillion level is based on a provisional health goal published by NCDHHS. *See* NCDHHS, *Questions and Answers Regarding North Carolina Department of Health and Human Services Updated Risk Assessment for GenX (Perfluoro-2-propoxypropanoic acid)* (July 14, 2017), https://epi.dph.ncdhhs.gov/oee/pfas/NC%20DHHS%20Health%20Goal%20Q&A.pdf.

- At the Washington Works facility in West Virginia, Chemours operates a thermal oxidizer which destroys 99.99% of the PFAS in multiple air emission streams vented to it. Chemours also operates an extensive well pumping system so that onsite groundwater is hydraulically contained to prevent offsite migration. In addition, a robust public and private drinking water program has been in place for years to help assess and treat PFAS contamination. The Washington Works facility also has implemented a recycling process by which HFPO-DA is captured and reused, thus reducing the demand for new HFPO-DA in manufacturing.
- At the Chambers Works facility in New Jersey, after Chemours's discovery of HFPO-DA as a byproduct in the lubricant manufacturing process, Chemours installed carbon adsorption units to abate HFPO-DA emissions from this source. Chemours has also installed granular activated carbon systems in private wells for residences near the facility to provide treatment for PFAS in drinking water.
- At the Parlin facility in New Jersey, Chemours controls HFPO-DA air emissions with a thermal converter and carbon abatement.

V. Fundamental Flaws in the Development of the Toxicity Assessment

In developing the HFPO-DA Toxicity Assessment, EPA relied upon a process with fundamental flaws necessitating correction. For example, the assessment contains significant deviations from standard EPA toxicity assessment methods—including the

inexplicable omission of a highly-relevant, peer-reviewed study that is contrary to EPA's conclusions. In sum, as discussed below, EPA's Toxicity Assessment is not supported by the weight of scientific evidence. These technical issues are discussed further in Section VI below, which provides further information regarding EPA's standard and methods relied upon in developing the reference dose, and their ultimate effect in the assessment.

Furthermore, given the dramatic change in both methodology and subsequent results from the 2018 draft assessment to the final Toxicity Assessment, EPA should have provided additional opportunity for public comment before publishing the final version of the assessment. The significant departure from both the 2018 draft assessment and the use of a fundamentally new methodology disproportionately impact Chemours's processing technology. To not provide such a significant stakeholder, as described above, sufficient opportunity to comment contradicts the principles of notice and fair opportunity to be heard that are fundamental to administrative law.[26]

Moreover, EPA has not yet provided or made publicly available any Administrative Record associated with the development of the reference dose used in its assessment.[27] The lack of such a Record prevents the public and Chemours from fully evaluating and

[26] *See Chocolate Mfrs. Ass'n of the United States v. Block*, 755 F.2d 1098, 1104–05 (4th Cir. 1985) (finding the Agency should have provided an additional opportunity to comment where the final rule so differed from the proposed rule that it was no longer within the "original scheme" or a "logical outgrowth" of the proposed rule, and noting "[a]n agency . . . does not have carte blanche to establish a rule contrary to its original proposal simply because it receives suggestions to alter it during the comment period. . . . [a]n interested party must have been alerted by the notice to the possibility of the changes eventually adopted from the comments"; the court also noted that "the notice must be sufficiently descriptive to provide interested parties with a fair opportunity to comment and to participate in the rulemaking.").

[27] Five months ago, Arnold & Porter submitted a FOIA request to EPA requesting, among other documents, EPA's Administrative Record associated with its HFPO-DA Toxicity Assessment. *See* October 27, 2021 Freedom of Information Act ("FOIA") Request regarding the Toxicity Assessment (FOIA Request EPA-2022-000577). EPA has provided ***no documents*** in response to this FOIA request.

understanding the underlying process EPA used to develop the assessment. Finally, EPA's Toxicity Assessment for HFPO-DA is lacking here because the EPA failed to submit the assessment for review by EPA's Science Advisory Board ("SAB").[28]

VI. The Toxicity Assessment Significantly Deviates from Standard EPA Toxicity Assessment Methods and Weight of Scientific Evidence

As set forth in further detail in the supporting expert reports of Dr. James Klaunig, Dr. John Cullen, Dr. Damian Shea, Dr. Laurie Haws, and Dr. Chad Thompson, attached hereto as Exhibits 1-4, EPA's HFPO-DA Toxicity Assessment contains significant deviations from standard EPA toxicity assessment methods and is not supported by the weight of scientific evidence. There are multiple and significant substantive technical and scientific issues with EPA's HFPO-DA Toxicity Assessment, including:

i) the liver effects underpinning the assessment are not relevant to humans, as demonstrated by the overall weight of scientific evidence, including a critically important 2020 peer-reviewed published study that was not considered by EPA;

ii) the assessment relies on observations by the NTP PWG that do not follow evaluation criteria set forth in the peer-reviewed scientific literature;

iii) the assessment utilizes a new and unprecedented toxicological endpoint (a so-called "constellation of liver effects") and misapplies scientific criteria in

[28] Notably, EPA recently issued a memorandum setting forth new procedures to strengthen the SAB review process. In that memorandum, EPA emphasized that "[s]cientific and technical peer review is essential to assessing the quality of the science supporting EPA decisions and maintaining the integrity of the agency's regulatory and policy processes." EPA, *Science Advisory Board Engagement Process for the Review of Science Supporting EPA Decisions* (Feb. 2022).

determining whether observed effects are adverse in the context of a human health risk assessment; and

iv) the assessment uses improper and significantly inflated uncertainty factors.

These significant substantive issues are summarized below, and are addressed in technical detail in the attached expert reports.

A. The Observed Liver Effects in Rodents Are Not Relevant to Humans

As with its 2018 draft assessment, EPA's final Toxicity Assessment for HFPO-DA continues to rely on liver effects in rodents that are not relevant to humans. In the final Toxicity Assessment, EPA acknowledges that the PPAR-alpha mode of action contributes to the liver effects and "could be more relevant to rodents than humans," but incorrectly hypothesizes that other modes of action with potential human relevance could be responsible, including PPAR-gamma, cytotoxicity, and mitochondrial dysfunction.[29]

EPA made a number of significant errors in reaching this conclusion including failing to identify or evaluate a critically important 2020 peer-reviewed published study by Dr. Grace A. Chappell et al.[30] This study provides compelling evidence that the rodent liver effects underpinning EPA's Toxicity Assessment are PPAR-alpha effects and ***thus are not relevant to humans***.[31] Based on discussions with EPA, we understand that the Agency performed its scientific literature review for the final Toxicity Assessment *eighteen*

[29] *See* Final Toxicity Assessment at 29.
[30] *See* Chappell, G.A., C.M. Thompson, J.C. Wolf, J.M. Cullen, J.E. Klaunig, and L.C. Haws. 2020. Assessment of the Mode of Action Underlying the Effects of GenX in Mouse Liver and Implications for Assessing Human Health Risks. Toxicologic Pathology 48(3):494-508.
[31] *See id.*

months before the final Toxicity Assessment was published, and three days before the Chappell et al. study was published.[32] EPA failed to update its literature review during the eighteen-month period prior to its publication of the final Toxicity Assessment and failed to identify or evaluate the Chappell et al. study. This is a consequential error, material omission, and grounds alone for withdrawing and correcting the assessment.

As explained in detail in the Chappell et al. study and in the expert report prepared by Dr. James Klaunig, Dr. Laurie Haws, and Dr. Chad Thompson, attached hereto as Exhibit 1, and as summarized below, there are multiple lines of compelling and direct evidence that the rodent liver effects underpinning the EPA's Toxicity Assessment are PPAR-alpha effects and ***are not relevant to humans***.

First, multiple peer-reviewed studies previously published by other scientists from EPA, other federal agencies, academia, and industry have made clear that liver tumors occurring in rodents via the PPAR-alpha mode of action have limited to no human relevance. A leading author of certain of these studies is Dr. Christopher Corton of EPA's Office of Research and Development.[33] Dr. Corton and his colleagues have found that key events in the PPAR-alpha mode of action do not occur in humans; for example, there is no

[32] In addition, Drs. Haws and Thompson had previously shared the results of Dr. Chappell's study in 2019 with EPA, and yet these results were omitted from EPA's assessment.

[33] *See* Corton, J.C., J.M. Peters, and J.E. Klaunig. 2018. The PPARα-dependent rodent liver tumor response is not relevant to humans: addressing misconceptions. Archives of Toxicology 92(1):83–119. https://doi.org/10.1007/s00204-017-2094-7; Felter, S.P., J.E. Foreman, A. Boobis, J.C. Corton, A.M. Doi, L. Flowers, J. Goodman, L.T. Haber, A. Jacobs, J.E. Klaunig, A.M. Lynch, J. Moggs, and A. Pandiri. 2018. Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. Regulatory Toxicology and Pharmacology 92:1–7. doi:10.1016/j.yrtph.2017.11.003.

alteration of cell cycle and growth pathways in humans, nor is there evidence of increased liver weight or hypertrophy.[34]

Second, as set forth in Exhibit 1, there is compelling and extensive evidence that the rodent liver effects underpinning EPA's Toxicity Assessment are, in fact, occurring through the PPAR-alpha mode of action (and therefore are not relevant to humans). For example, it is well established in the scientific literature that there are significant differences in the biochemical response between rodents and humans following PPAR-alpha activation. As such, the non-neoplastic liver lesions that EPA used as the basis of the RfD for HFPO-DA are not relevant to humans and should not have been used.[35]

Third, EPA's effort to overcome this compelling evidence lacks scientific rigor and is unsupported by the very citations relied upon by EPA, as discussed below. In light of the weight of scientific evidence regarding the PPAR-alpha mode of action, EPA hypothesizes in its Toxicity Assessment that there may be *alternative* modes of action such as PPAR-gamma, cytotoxicity, and mitochondrial dysfunction to suggest effects potentially of greater relevance to humans. Other than a cursory discussion of these alternative modes of action, however, EPA does not provide explanation, evidence, or analysis to support its hypotheses, and in some instances the citations relied upon by EPA are directly contrary to its theory. As set forth in more detail in Exhibit 1, the data simply do not support EPA's conclusions that these alternative modes of action contribute to the rodent liver effects underpinning the Toxicity Assessment.

[34] *See id.*
[35] *See* Exhibit 1.

For example, for the PPAR-***gamma*** mode of action (as opposed to PPAR-alpha), EPA misinterprets the results of the Li et al. study (2019), which concluded that HFPO-DA has little to no PPAR-gamma binding affinity in either humans or mice and causes minimal changes in PPAR-gamma gene expression.[36] EPA similarly misinterprets the findings of the Conley et al. study (2019) with respect to the PPAR-gamma mode of action, as the Agency conflates the issues of PPAR-gamma *signaling* and PPAR-gamma *expression* and does not consider evidence demonstrating that PPAR-gamma is not highly expressed in the liver.[37] Data also do not support EPA's conclusions regarding a cytotoxicity mode of action purportedly based on single-cell necrosis and focal necrosis.[38] Data likewise conflict with EPA's conclusions regarding mitochondrial dysfunction. Multiple studies have demonstrated that the PPAR-alpha mode of action (which is not relevant to humans), and not an alternative mitochondrial dysfunction mode of action as hypothesized by EPA, mediates the expression of genes involved in mitochondrial beta-oxidation in rodent livers.[39]

In sum, the overall weight of scientific evidence—including the Chappell et al. study not considered by EPA—demonstrates that the liver effects underpinning the HFPO-

[36] *See* Li, C.H., X.M. Ren, and L.H. Guo. 2019. Adipogenic activity of oligomeric hexafluoropropylene oxide (perfluorooctanoic acid alternative) through peroxisome proliferator-activated receptor γ pathway. Environmental Science & Technology 53(6):3287-3295. doi:10.1021/acs.est.8b06978.
[37] *See* Conley, J.M., C.S. Lambright, N. Evans, M.J. Strynar, J. McCord, B.S. McIntyre, G.S. Travlos, M.C. Cardon, E. Medlock-Kakaley, P.C. Hartig, V.S. Wilson, and L.E. Gray, Jr. 2019. Adverse maternal, fetal, and postnatal effects of hexafluoropropylene oxide dimer acid (GenX) from oral gestational exposure in Sprague-Dawley rats. Environmental Health Perspectives 127(3):037008. doi:10.1289/EHP4372.
[38] As discussed in section VI.B below and in the expert report of Dr. John Cullen, Dr. Laurie Haws, and Dr. Chad Thompson, attached hereto as Exhibit 2, there are significant issues with the NTP PWG's evaluation of single-cell necrosis, and focal necrosis did not increase with dose.
[39] *See* Exhibit 1.

DA Toxicity Assessment are occurring via the PPAR-alpha mode of action that is not relevant to humans, and EPA's conclusions regarding possible non-PPAR-alpha modes of action are not supported by scientific data and studies.

B. Liver Pathology Observations Are Flawed

EPA's final Toxicity Assessment is based on liver effects observed by the NTP PWG in pathology cell blocks from a HFPO-DA reproductive/developmental study in mice.[40] The NTP PWG recorded its observations of four liver effects—cytoplasmic alteration, single-cell necrosis, focal necrosis, and apoptosis. The NTP PWG stated that its observations were based on the scientific criteria set forth in the 2016 study by Elmore et al. ("the Elmore criteria").[41] However, as set forth in detail in the attached expert report of Dr. John Cullen, Dr. Laurie Haws, and Dr. Chad Thompson (Exhibit 2), and as summarized below, the NTP PWG misapplied the Elmore criteria and other important scientific criteria.

First, the NTP PWG's observations did not properly distinguish two possible observed effects: single-cell necrosis, on the one hand, and apoptosis, on the other. This is important, because the PPAR-alpha mode of action, which is not relevant to humans, results in apoptosis. Pursuant to the Elmore criteria, necrotic cells have a pale cytoplasm, whereas apoptotic cells are hypereosinophilic (i.e., containing a high number of a certain type of white blood cells). However, contrary to these criteria, the NTP PWG characterized

[40] *See Appendix D: NTP PWG Final Report on the Pathology Peer Review of Liver Findings* (Dec. 2019) *in* Final Toxicity Assessment.

[41] *See* Elmore, S.A., D. Dixon, J.R. Hailey, T. Harada, R.A. Herbert, R.R. Maronpot, T. Nolte, J.E. Rehg, S. Rittinghausen, T.J. Rosol, H. Satoh, J.D. Vidal, C.L. Willard-Mack, and D.M. Creasy. 2016. Recommendations from the INHAND apoptosis/necrosis working group. Toxicologic Pathology 44(2):173–88. doi:10.1177/0192623315625859.

hypereosinophilic cells as necrotic, not as apoptotic. Further, while the Elmore criteria recognize that not all apoptotic cells are small or rounded, the NTP PWG only characterized small or rounded cells as apoptotic. The Elmore et al. study also noted the importance of using biochemical markers to distinguish necrosis from apoptosis. Biochemical markers—including the caspase-3 immunostaining reported in the Chappell et al. study not considered by EPA—confirm apoptosis following HFPO-DA exposure.

Additionally, there are also important discrepancies in the NTP PWG's observations of focal necrosis (i.e., necrosis involving larger groups of functional cells within the liver). The focal necrosis observed by the NTP PWG lacked a dose-response relationship—focal necrosis was present in some control animals, there was no statistically significant increase in test animals, and a 10-fold increase in HFPO-DA dose resulted in minimal or no increase in focal necrosis. Additionally, it is well established that focal necrosis is not necessarily a progression from single-cell necrosis, and it may be caused by biological processes other than direct chemical exposure.

C. EPA's "Constellation" Endpoint Is Unprecedented and Inconsistent with Standard Toxicity Assessment Protocols

EPA compounds the problems with the NTP PWG's observations by combining the four liver effects observed by the NTP PWG into a never-before-used toxicological endpoint—a so-called "constellation of liver effects."[42] In the 2018 draft assessment for

[42] Final Toxicity Assessment at 52.

HFPO-DA, EPA relied on single-cell necrosis as the toxicological endpoint[43] but inexplicably pivoted to this new endpoint in its final Toxicity Assessment.

Not only is EPA's "constellation of liver effects" unprecedented and a significant deviation from its standard toxicity assessment methods, but it is also erroneous and at odds with the science. As described in detail in the attached expert report of Dr. John Cullen, Dr. Laurie Haws, and Dr. Chad Thompson (Exhibit 2), EPA misapplies the criteria from the Hall et al. study (2012) in determining whether liver effects observed by the NTP PWG are adverse effects.[44] Had EPA properly applied these scientific criteria, the Agency would have instead correctly determined that dosing levels in treated mice did not generate effects relevant to humans.

Finally, EPA also fundamentally misinterprets and misapplies the NTP PWG's findings by using these findings for human health risk assessment in the first place. Nowhere did the NTP PWG state that their findings should be used for human health risk assessment—rather, the NTP PWG findings expressly are limited to "adversity within the confines of this study," where "[a]dversity is a term indicating 'harm' to the test animal" (i.e., mice).[45] As discussed above and in Exhibit 2, the observed effects are PPAR-alpha

[43] Draft Toxicity Assessment at viii.
[44] *See* Hall, A.P., C.R. Elcombe, J.R. Foster, T. Harada, W. Kaufmann, A. Knippel, K. Küttler, D.E. Malarkey, R.R. Maronpot, A. Nishikawa, T. Nolte, A. Schulte, V. Strauss, and M.J. York. 2012. Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd international ESTP expert workshop. Toxicologic Pathology 40(7):971–994. doi:10.1177/0192623312448935.
[45] *Appendix D: NTP PWG Final Report on the Pathology Peer Review of Liver Findings* (Dec. 2019) *in* Final Toxicity Assessment at D-22.

effects in rodents that are not relevant to humans, and thus the application of the NTP's findings to humans is both arbitrary and capricious.

D. EPA Improperly Overstates Uncertainty in Its Toxicity Assessment

As is customary, EPA increased the stringency of its toxicity value for HFPO-DA by accounting for uncertainty. However, in doing so in this case, EPA significantly deviated from past practice and sound scientific principles. Between EPA's draft and final Toxicity Assessment, the total uncertainty factors increased exponentially (from 300 to 3000), notwithstanding that the final Toxicity Assessment incorporates *additional* data and studies (and thus, in truth, there is less, not more, uncertainty). In fact, the 3000-fold uncertainty factor used in the final Toxicity Assessment is the maximum value that EPA could have used; the Agency has previously stated that any greater factor is considered too uncertain for toxicity assessment and for calculation of a reference dose.[46]

EPA's use of the 3000-fold uncertainty factor here is inconsistent with the number of toxicity studies and amount of toxicity data available for HFPO-DA as well as the Agency's toxicity assessments for other chemicals, as described further below and in the attached report prepared by Dr. Laurie Haws and Dr. Chad Thompson (Exhibit 3). Notably, based on Dr. Haws and Dr. Thompson's review of 557 toxicity assessments in

[46] *See* EPA, *A Review of the Reference Dose and Reference Concentration Processes* 4-41 (Dec. 2002), https://www.epa.gov/sites/default/files/2014-12/documents/rfd-final.pdf (hereinafter "EPA Review of Reference Dose Process") ("The Technical Panel recommends limiting the total UF applied for any particular chemical to no more than 3000 and avoiding the derivation of a reference value that involves application of the full 10-fold UF in four or more areas of extrapolation.").

EPA's IRIS database,[47] EPA has used total uncertainty factors less than 3000 in nearly 90% of its assessments, but, inexplicably, not here.

The tenfold increase in total uncertainty factors between EPA's draft and final Toxicity Assessment is purportedly due to increases in the "database uncertainty" factor and the "subchronic to chronic uncertainty" factor, each from 3 to 10. Ten is the maximum possible value EPA could have selected for each of these uncertainty factors. EPA's selections of 10 in the final Toxicity Assessment for these uncertainty factors, as discussed below, are not supported by the science. Additionally, the science does not support EPA's selection of an interspecies uncertainty factor here.

i. Database Uncertainty Factor

In the final Toxicity Assessment, EPA claims that new data and studies have made the database of toxicity studies for HFPO-DA more uncertain with respect to potential reproductive or developmental effects. That additional data and studies could result in *more* uncertainty is plainly counterintuitive, and EPA has not reasonably explained how this could be the case here. Rather, the Agency's substantive explanations regarding the database uncertainty factor are very similar in both the draft and final Toxicity Assessments, and there is no scientific basis for increasing that factor in the final assessment.[48]

Moreover, the new studies relied upon by EPA actually reduce (and do not increase) uncertainty. This is because observed effects in all of these studies do not occur until levels

[47] *IRIS Advanced Search*, EPA, https://cfpub.epa.gov/ncea/iris/search/index.cfm (last visited Jan. 6, 2022).
[48] *Compare* Draft Toxicity Assessment at 56–57 *with* Final Toxicity Assessment at 96.

of exposure that are significantly higher than the point of departure used (incorrectly) by EPA in its Toxicity Assessment. Thus, as Dr. Damian Shea explains in his supporting expert report (Exhibit 4), "[t]here is no scientifically defensible way to justify increasing the database uncertainty factor based on [the newer] studies." Rather, as Dr. Shea concludes, "this new information greatly *reduced* uncertainty regarding HFPO-DA toxicity."

If EPA had concerns with the new data and studies, it would have been more appropriate and scientifically supportable for EPA to have used those new studies as the basis for calculating its reference dose. Instead, in the final Toxicity Assessment, EPA uses the most sensitive potential liver effects and then increases the uncertainty factor based on *less sensitive* potential reproductive or developmental effects in the newer studies, thereby double counting and compounding uncertainty without scientific basis. As shown in Exhibit 3, the reference doses derived from the newer studies are significantly *higher* than the reference dose in EPA's final Toxicity Assessment, yet EPA cites those studies as its purported reason for increasing the database uncertainty factor and correspondingly lowering the reference dose.

Further, the database of toxicity studies for HFPO-DA includes multiple studies of varying durations in rats and mice and multiple toxicity endpoints. As demonstrated in Exhibit 3, it is inconsistent with EPA's practice in other toxicity assessments, including

recent toxicity assessments for the PFAS compounds PFBA and PFHxA, as well as EPA's own guidance,[49] to assign a database uncertainty factor of 10 to such a robust database.

ii. Subchronic to Chronic Uncertainty Factor

As with its 2018 draft assessment, EPA's final Toxicity Assessment for HFPO-DA continues to rely on a reproductive/developmental study in mice as the critical study for calculating its chronic reference dose. In the draft assessment, EPA relied on critical effects in male mice from that study, and then applied an uncertainty factor of 3 in calculating the chronic reference dose for scaling from subchronic to chronic exposure. In the final Toxicity Assessment, EPA relies on critical effects in female mice from the same study, and then applies an uncertainty factor of 10 in calculating the chronic reference dose for scaling from subchronic to chronic exposure.

As explained in Exhibit 3, EPA should not have applied a subchronic to chronic uncertainty factor here at all, and EPA also had *no basis* to increase that factor from 3 to 10 from the 2018 draft assessment to the 2021 final assessment. First, there is no strong indication of a progression of rodent liver lesions with longer exposure duration. Second, the Agency's explanations regarding the subchronic to chronic uncertainty factor are very similar in the draft and final assessments and thus do not provide a scientific basis for

[49] *See* EPA Review of Reference Dose Process at 4-45 ("If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing."). There is a prenatal toxicity study (performed pursuant to OECD Guideline 414) for HFPO-DA, yet EPA applied a database uncertainty factor of 10 here. Additionally, there is also a one-generation reproduction study for HFPO-DA that provides relevant data for that endpoint. EPA required this one-generation reproduction study, rather than a two-generation reproduction study, in the 2009 TSCA Section 5(e) Order.

increasing that factor.[50] Third, EPA guidelines indicate that there should be no subchronic to chronic uncertainty factor here at all, as the critical effects in female mice underpinning the final Toxicity Assessment are from a maternal rodent toxicity study for which "an uncertainty factor is not [to be] applied to account for duration of exposure."[51]

iii. Interspecies Uncertainty Factor

EPA incorrectly applied an interspecies uncertainty factor of 3 in the final Toxicity Assessment for HFPO-DA. The assessment is based on rodent liver effects that have no relevance to humans, as discussed above. Therefore, and as set forth in Exhibit 3, EPA should not have applied an interspecies uncertainty factor for potential human sensitivity at all—humans are not susceptible to these liver effects, and humans are certainly not *more* susceptible to the effects than are rodents.

***

As a cumulative result of the multiple substantive technical errors summarized above and described in Exhibits 1-4, EPA's chronic reference dose in the final HFPO-DA Toxicity Assessment is fundamentally flawed and overly conservative by orders of magnitude. EPA's final chronic reference dose is *26 times lower* than its chronic reference dose in its 2018 draft assessment, which itself was overly conservative. EPA's final chronic reference dose is *3,333 times lower* than the already conservative chronic reference

[50] *Compare* Draft Toxicity Assessment at 55–56 *with* Final Toxicity Assessment at 93.
[51] EPA, *Guidelines for Developmental Toxicity Risk Assessment* 42 (Dec. 1991), https://ofmpub.epa.gov/eims/eimscomm.getfile?p_download_id=4560.

dose for HFPO-DA published by Thompson et al. in the Journal of Applied Toxicology in June 2019.[52]

VII. Epidemiological Analysis and North Carolina Cancer and Liver Disease Rates

The flaws in EPA's HFPO-DA Toxicity Assessment are further corroborated by real-world epidemiological data. In short, there is no epidemiologic evidence showing an increased risk of cancers or liver disease attributable to exposure to HFPO-DA, including in the counties surrounding the Fayetteville Works facility.

In 2017, NCDHHS analyzed data from the North Carolina Central Cancer Registry and found no trends of increased cancer risk in the counties with water allegedly impacted by HFPO-DA originating from the Fayetteville Works facility. In fact, NCDHHS concluded that rates of liver and other cancers are *generally lower* in North Carolina counties with exposures to HFPO-DA than the rates reported in the U.S. general population, in the state of North Carolina, and in North Carolina counties without alleged exposure to HFPO-DA. According to NCDHHS, "the results do not point to any consistent trends in counties that get their water from the lower Cape Fear. 'Overall the results are what we would expect to see looking at multiple types of cancer in multiple counties, with some rates below and above the state rate.'"[53] NCDHHS's analysis further revealed that, "[o]verall, cancer rates in the four counties were similar to state rates," and that during the

[52] Thompson, C.M., S.E. Fitch, C. Ring, W. Rish, J.M. Cullen, and L.C. Haws. 2019. Development of an oral reference dose for the perfluorinated compound GenX. Journal of Applied Toxicology 39(9):1267-1282. doi:10.1002/jat.3812.

[53] Press Release, NCDHHS, *N.C. DHHS Releases Summary of Selected Cancer Rates for Counties in Cape Fear Region* (June 29, 2017), https://www.ncdhhs.gov/news/press-releases/2017/06/29/nc-dhhs-releases-summary-selected-cancer-rates-counties-cape-fear-region.

most recent five-year interval (2011–2015), no county-specific cancer rates examined were significantly higher than state rates.[54]

Data from the National Cancer Institute's Surveillance, Epidemiology, and End Results ("SEER") Program database—and a different study period—show the same conclusion as NCDHHS: there is no increased cancer risk in the counties allegedly impacted by HFPO-DA when compared to the United States or the rest of North Carolina.[55] As set forth in the attached expert report of Dr. Ellen Chang, a nationally-recognized, leading epidemiologist, the counties of Bladen, Brunswick, Cumberland, New Hanover, and Pender "do not indicate a pattern of increased cancer incidence" when compared to adjacent counties, as well as with matched counties with similar socioeconomic status and population size.[56] Comparisons to the United States and North Carolina as a whole similarly do not show increased cancer risk in the affected counties.[57]

Dr. Chang also conducted an analysis of age-adjusted liver disease mortality rates using the CDC WONDER database and found that available epidemiological data "do not support an effect of HFPO-DA on liver disease in humans."[58]

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[54] *Summary of Selected Cancer Rates for Bladen, Brunswick, New Hanover and Pender Counties, 1996–2015, and Comparison to Statewide Rates*, https://epi.dph.ncdhhs.gov/oee/pfas/Summary%20of%20Selected%20Cancer%20Rates_all%20counties_7Nov2018.pdf.

[55] Expert report of Dr. Ellen Chang, *Epidemiology of Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt*, attached as Exhibit 5.

[56] *Id.* at 1.

[57] *Id.* at Table 1.

[58] *Id.* at 13.

VIII. Needed Corrections and Next Steps

As the foregoing summary, and the attached expert reports make clear, EPA's HFPO-DA Toxicity Assessment was developed through a flawed process and contains numerous fundamental scientific errors. EPA should promptly withdraw the Toxicity Assessment to address and correct its scientific deficiencies and develop a revised assessment that is scientifically sound, objective, and supported by the evidence.

To correct substantive scientific and technical deficiencies, EPA should develop a revised assessment based on statistically significant adverse toxicological effects that may be relevant to human health, not based on PPAR-alpha effects in rodents that are not relevant to human health. Further, for the revised assessment, EPA's selection of each uncertainty factor should be consistent with EPA's standard toxicity assessment methods, objective and reasonable, and supported by the weight of scientific evidence.

Before issuing a final assessment, EPA should convene another scientific peer review panel after soliciting input on its membership. This peer review panel should be comprised of leading scientists from government, academia, and industry, and should be reflective of the entire body of the peer-reviewed scientific literature on relevant toxicology matters. Having a scientifically representative and balanced group of peer reviewers and a robust peer review process is essential for developing a revised assessment that is objective and science-based.

As set forth in further detail in Exhibit 6, while there are already several toxicity studies and significant amounts of toxicity data available for HFPO-DA, in order to address EPA's concerns regarding uncertainty, Chemours is sponsoring a state-of-the-science *in*

vitro study that will be made available to the Agency as soon as it can be completed.[59] An *in vitro* study involves the use of cell cultures. Chemours's *in vitro* study will test HFPO-DA and control compounds on human and rodent liver cell cultures. The study design is based on the 2020 publication by Dr. Patrick McMullen et al.[60] *See* Exhibit 6 (describing the study design for this *in vitro* study).

Chemours is also sponsoring ongoing research related to liver pathology, including cellular staining to distinguish necrosis from apoptosis and transcriptomics (gene sequencing). The *in vitro* study and the liver pathology research should be reviewed and taken into account by EPA scientists, and its peer reviewers, before the HFPO-DA Toxicity Assessment is revised and released.

EPA has stated that it plans to publish a drinking water health advisory level for HFPO-DA in Spring 2022.[61] EPA's health advisory should be based upon a revised assessment for HFPO-DA that addresses and corrects the procedural and scientific

[59] Chemours has previously invited the Agency to participate in the design of this study and reiterates its desire to work together with the EPA in the implementation of this work.

[60] McMullen, P.D., S. Bhattacharya, C.G. Woods, S.N. Pendse, M.T. McBride, V.Y. Soldatow, C. Deisenroth, E.L. LeCluyse, R.A. Clewell, M.E. Andersen. Identifying qualitative differences in PPARα signaling networks in human and rat hepatocytes and their significance for next generation chemical risk assessment methods. Toxicology in Vitro, Vol. 64, 2020, 104463. ISSN 0887-2333. https://doi.org/10.1016/j.tiv.2019.02.017.

[61] A health advisory under the Safe Drinking Water Act ("SDWA") would constitute a final agency action. *See* SDWA § 1412(b)(1)(F). Health advisories have direct and appreciable legal consequences, including through incorporation by state law, substantial impacts to consent order obligations, and effects on environmental permitting standards. *See Appalachian Power Co. v. E.P.A.*, 208 F.3d 1015, 1024 (D.C. Cir. 2000) (holding that the effect of an agency action, rather than its label, is determinative of finality); *Dow AgroSciences LLC v. Nat'l Marine Fisheries Serv.*, 637 F.3d 259, 267 (4th Cir. 2011) (holding that a National Marine Fisheries Service biological opinion is final, despite not having independent legal effect, because other agencies rely on the opinion in taking actions with legal consequences); *Chlorine Chemistry Council v. E.P.A.*, 206 F.3d 1286, 1290 (D.C. Cir. 2000) (holding that promulgation of a maximum contaminant level goal under the SDWA is final agency action despite being aspirational and not independently enforceable).

deficiencies noted herein and incorporates the results of the *in vitro* study and liver pathology research. The health advisory should not be undertaken until after a revised assessment can be peer reviewed.

Finally, EPA's eventual HFPO-DA drinking water health advisory level should be based on realistic assumptions regarding potential exposures, including assumptions related to body weight, age, drinking water consumption rate, and relative source contributions from drinking water.[62] Chemours is prepared to provide the EPA with extensive data and evidence related to these health advisory inputs.

IX. Conclusion

For the foregoing reasons, and those set forth in the attached expert reports, Chemours requests that EPA withdraw and correct its October 25, 2021 GenX Chemicals Toxicity Assessment.

Sincerely,

Brian D. Israel

cc: Todd A. Coomes, Associate General Counsel, The Chemours Company

[62] Extensive data indicate that drinking water is the primary potential pathway for HFPO-DA exposure and thus support a relative source contribution from drinking water of at least 80%.

EXHIBITS TO THE CHEMOURS COMPANY'S MARCH 18, 2022 REQUEST FOR CORRECTION OF GENX CHEMICALS TOXICITY ASSESSMENT

Exhibit	***Description***
1	Haws, L.C., J.E. Klaunig, and C.M. Thompson, *Issues with the Proposed Mode of Actions (MOAs) for HFPO-DA Induced Liver Effects Hypothesized in USEPA Toxicity Assessment (2021)*
2	Cullen, J.M., L.C. Haws, and C.M. Thompson, *Issues with the NTP PWG Report and USEPA's Use of that Report for Their HFPO-DA Toxicity Assessment (2021)*
3	Haws, L.C., and C.M. Thompson, *Issues with the Uncertainty Factors in USEPA Toxicity Assessment (2021)*
4	Shea, D., *Inappropriate Use of the Database Uncertainty Factor in the US EPA Human Health Toxicity Values for "GenX Chemicals"*
5	Chang, E.T., *Epidemiology of Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt*
6	ToxStrategies, In Vitro *Human and Rodent Hepatocyte Study Protocol*

EXHIBIT 1

Issues with the Proposed Mode of Actions (MOAs) for HFPO-DA Induced Liver Effects Hypothesized in USEPA Toxicity Assessment (2021)

MARCH 16, 2022

ToxStrategies

Innovative solutions
Sound science

Issues with the Proposed Mode of Actions (MOAs) for HFPO-DA Induced Liver Effects Hypothesized in USEPA Toxicity Assessment (2021)

MARCH 16, 2022

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Executive Summary

ToxStrategies, Inc. and Dr. James Klaunig reviewed the 2021 United States Environmental Protection Agency (USEPA) Toxicity Assessment for GenX Chemicals (referred to herein as "HFPO-DA"). Within their assessment, USEPA suggests that alternative Modes of Action (MOAs) other than the peroxisome proliferator-activated receptor alpha (PPARα) MOA are associated with the observed liver toxicity caused by HFPO-DA in rodents. However, the overall weight of the evidence for HFPO-DA clearly demonstrates that liver effects in mice are occurring via the PPARα MOA and not by alternative MOAs. This evidence is based on data supporting the key events that occur early in the PPARα MOA. The PPARα MOA for liver tumors in rodents is not relevant to humans. The available evidence for alternate MOAs suggested by USEPA is weak and, in some cases, taken out of context or inaccurately cited.

1 Introduction

On page 82 of the USEPA Toxicity Assessment for GenX Chemicals (2021; referred to herein as "HFPO-DA"), the USEPA states, "the available data indicate that multiple MOAs **could be** [emphasis added] involved in the liver effects observed after GenX chemical exposure. The available studies provide support for a role for PPARα, cytotoxicity, mitochondrial dysfunction, and PPARγ." We disagree with USEPA's contention that MOAs other than PPARα are associated with the observed liver effects. Evidence presented by the USEPA for these alternate MOAs is not supported by the data and relies on very limited empirical data, which in some cases, has been taken out of context or inappropriately cited.

Each hypothesized MOA suggested by USEPA is described below. As will be shown, there is overwhelming evidence that HFPO-DA causes liver lesions in mice via a well-established MOA involving PPARα activation that is not relevant to humans. In contrast, the experimental evidence supporting alternative MOAs for HFPO-DA-induced liver toxicity suggested in USEPA (2021) is weak and, in some cases, overstated or misinterpreted.

2 The Overall Weight of the Evidence Demonstrates that Liver Effects Are Occurring Via a PPARα MOA

2.1 Overview of the PPARα MOA (as reviewed in Corton et al., 2014 and 2018)

The mode of action (MOA) for liver tumors resulting from exposure to PPARα activators is well-established in the scientific literature (Corton et al., 2014, 2018; **Figure 1**). Although HFPO-DA has not been shown to induce liver tumors in mice in short-term studies, several of the early (upstream) key events in the MOA for PPARα-related liver

tumors have been observed. Given the interest in the early/upstream responses following HFPO-DA exposure as opposed to the apical endpoint (i.e., liver tumors), it is important to characterize the evidence base for the first three key events of the PPARα MOA in **Figure 1**. Broadly, evidence streams for PPARα activation (Key Event (KE) 1) include PPARα receptor binding and/or activation, increased expression of genes/proteins involved in fatty acid β-oxidation, increased palmitoyl-CoA oxidase activity, and morphological evidence of peroxisome proliferation (Corton et al., 2018). In addition, analysis of mRNA or transcriptomic responses to PPARα activation, or the loss of any of the aforementioned effects in knockout studies, also provide evidence of PPARα activation.

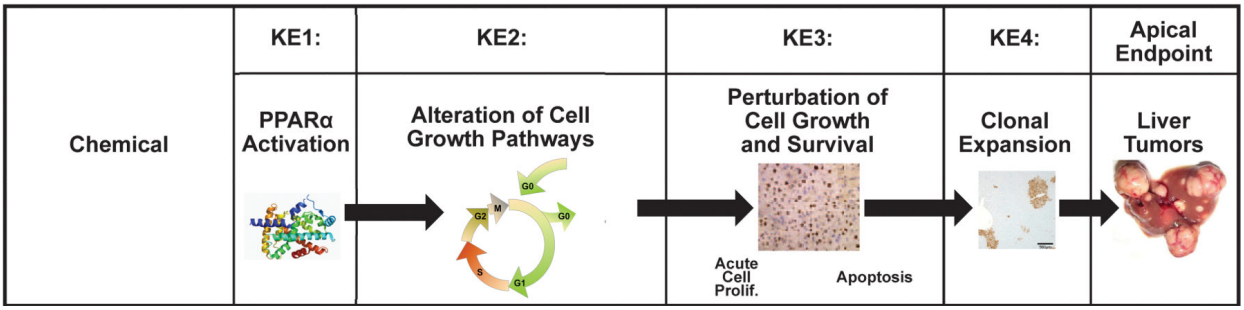

Figure 1. MOA for PPARα-induced liver tumors in mice and rats (Corton et al., 2018).

Evidence for altered cell growth pathways (KE 2) may include involvement of activation of non-parenchymal cells (e.g., Kupffer cells) that, once activated, secrete cytokines such as tumor necrosis factor α (TNFα), interleukin-1α (IL-1α), and interleukin-1β (IL-1β) (Corton et al., 2018). In addition, the up-regulation of genes leading to increased cell proliferation including c-myc, cyclin D1 (Cd1), cyclin-dependent kinase 1 (Cdk1) and cyclin-dependent kinase 4 (Cdk4) expression has been observed in rodent liver following exposure to PPARα activators (Morimura et al., 2006; Corton et al., 2018; Chappell et al., 2020).

Evidence for perturbation of cell growth and survival (KE 3) includes hepatocyte proliferation (increased cell number) and/or decreased apoptosis, resulting in hepatocyte hypertrophy and subsequently liver enlargement. At higher doses, there is evidence of sustained increase in cell proliferation. Although Corton et al. (2018) do not specifically use the term "hypertrophy" in describing KE 3, elsewhere they state, "In addition to the increased occurrence of hepatic tumors, chronic exposure of rats and mice to peroxisome proliferators is linked to several hepatic adaptive responses, including hepatocellular hypertrophy and hyperplasia, changes in apoptosis rates…" Our interpretation of the above indicates that KE 3 is strongly associated with hypertrophy.

2.2 Lack of Human Relevance of PPARα MOA

It is widely accepted that rodent liver tumors resulting from exposure to PPARα activators are not relevant to humans (Corton et al., 2018). While PPARα is expressed in many species and plays a role in lipid metabolism across species, the downstream cell proliferation signaling occurs specifically in rodents including mice and rats. Increased

cell proliferation is a key and required event in the formation of hepatic tumors. As such, PPARα induced liver tumors in mice and rats are of little relevance to humans. A critical question, however, is whether the non-neoplastic changes in the liver (i.e., KE1-3) seen with PPARα activators like HFPO-DA are unique to mice and rats.

The human relevance of the Key Events underlying the PPARα MOA is summarized in **Figure 2**. Only KE 1, PPARα activation, is shared across species (Corton et al., 2018). Upon activation, PPARα-mediated gene expression in humans and primates produces only a subset (i.e., lipid modulating effects) of the responses observed in mice and rats (Rakhshandehroo et al., 2009; McMullen et al., 2020; Bjork et al., 2011). In addition, the absence of a hyperplastic response in human hepatocytes exposed to PPARα activators directly addresses the question of whether the non-neoplastic lesions induced by PPARα activators such as HFPO-DA are relevant to humans (Elcombe et al., 1996; Goll et al., 1999; Perrone et al., 1998; Corton et al., 2014). While *in vivo* data in humans is limited, the overall weight of evidence for patients treated with fenofibrate or hypolipidemic drugs demonstrates that patients did not have increased liver weights (Gariot et al., 1987) or induction of peroxisome proliferation (Bentley et al., 1993), respectively.

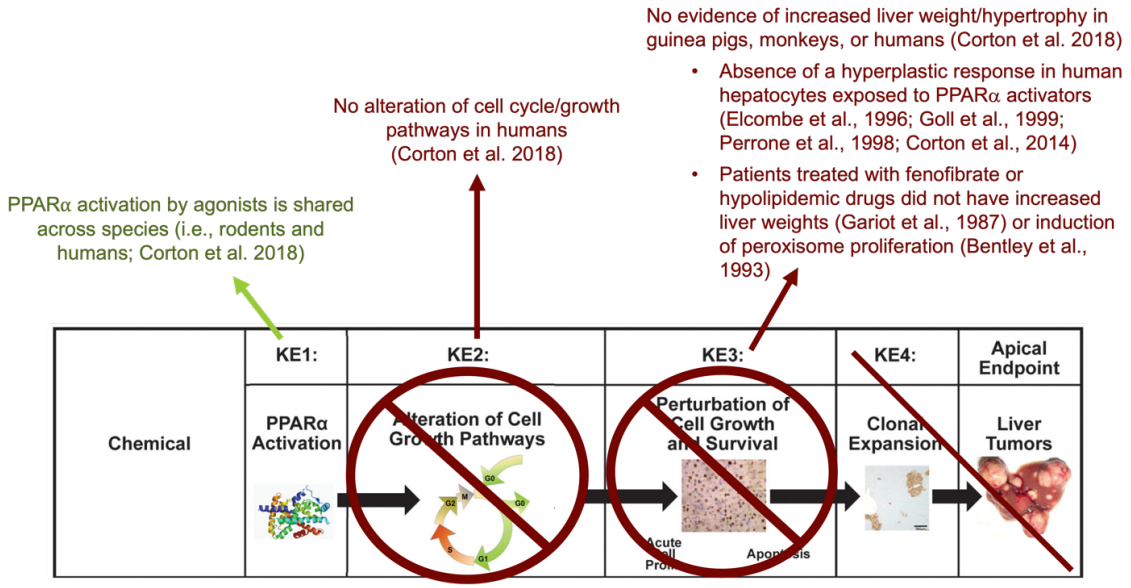

Figure 2. Human relevance of PPARα MOA in liver (modified from Corton et al. 2018).

2.3 Evidence Supporting the PPARα MOA for HFPO-DA-Induced Liver Effects

As demonstrated by the empirical data available for HFPO-DA for KEs 1 - 3 of the PPARα MOA, described in more detail in the sections below, the evidence overwhelmingly supports the conclusions that the observed liver effects are occurring through the PPARα MOA and thus are not relevant to humans.

Most of the data gaps concerning the PPARα MOA identified by the USEPA were addressed in Chappell et al. (2020). Notably, this critical HFPO-DA transcriptomic study was *not* cited in the USEPA (2021) assessment. In fact, the transcript data published in Chappell et al. (2020) were discussed with USEPA prior to the publication during a meeting with the USEPA on March 28, 2019.

KE 1, PPARα activation, is supported by several lines of evidence, including the activation of both rat and mouse PPARα receptors by HFPO-DA in *in vitro* reporter assays (Chappell et al., 2020; **Figure 3**).

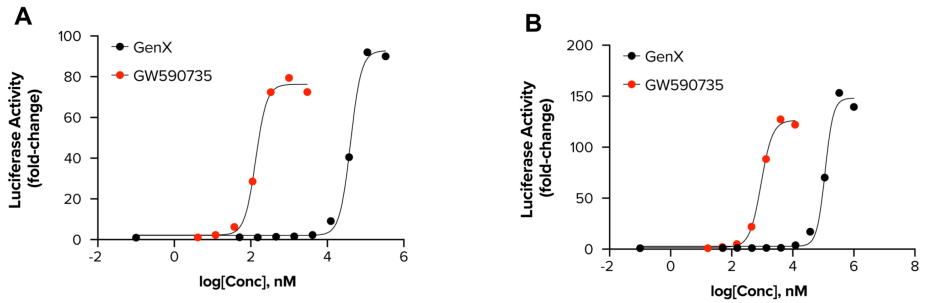

Figure 3. Activation of mouse (A) and rat (B) PPARα receptors by HFPO-DA in cell reporter assays (Chappell et al., 2020).

Exposure of HFPO-DA for 28 days also increased hepatic peroxisome β-oxidation activity in both mice and rats (**Figure 4**). In addition, hepatic transcriptomic results in male and female mice from Chappell et al. (2020; 90-day study) demonstrated significant enrichment of both the KEGG peroxisome and REACTOME peroxisomal lipid metabolism gene sets at 0.5 and 5 mg/kg HFPO-DA (**Table 1**), providing further evidence of increased hepatic peroxisome β-oxidation and support for KE 1.

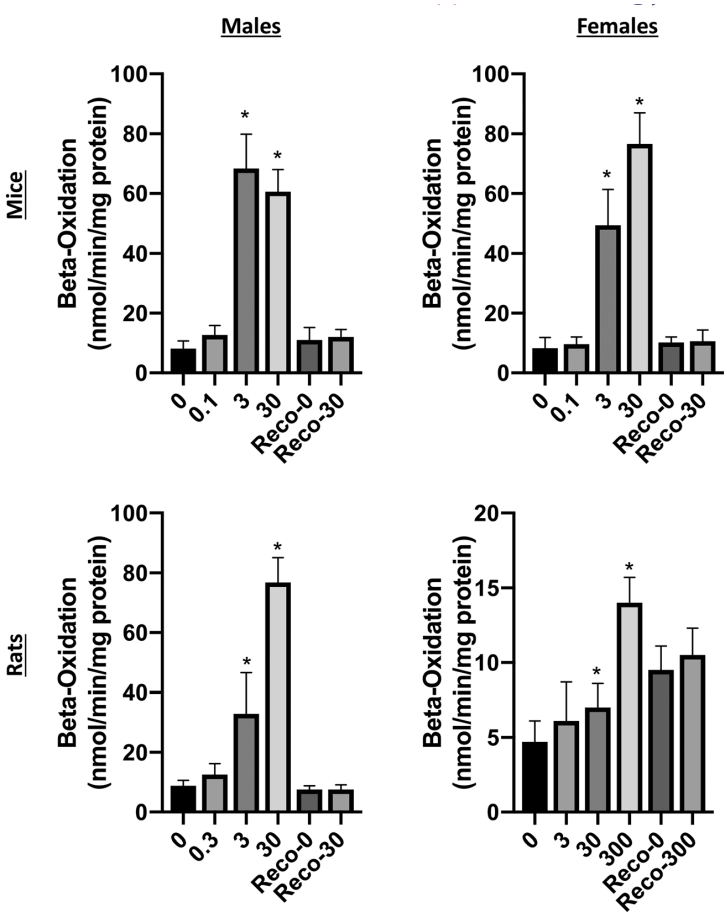

Figure 4. β-oxidation activity in mice and rats after 28-day exposure to HFPO-DA (Thompson et al., 2019).

Table 1. Transcriptomic analysis of PPAR pathways in mice following 90-day exposure to HFPO-DA (reanalysis of data published in Chappell et al. 2020).

Gene set (MSigDB Canonical Pathways version 7.4)		Sex	mg/kg bw/day	Adjusted p-value up*	Adjusted p-value down*
General PPAR / Peroxisomal Signaling	KEGG PEROXISOME	female	0.1	1	1
			0.5	**4.72E-10**	1
			5	**5.79E-20**	1
		male	0.1	1	1
			0.5	**3.09E-11**	1
			5	**4.61E-10**	0.16864269
	KEGG PPAR SIGNALING PATHWAY	female	0.1	1	1
			0.5	**1.35E-10**	1
			5	**1.35E-17**	0.10113537
		male	0.1	1	1
			0.5	**1.02E-07**	1
			5	**1.73E-16**	**0.00978691**
	REACTOME PEROXISOMAL LIPID METABOLISM	female	0.1	1	1
			0.5	**6.94E-09**	1
			5	**1.28E-08**	1
		male	0.1	1	1
			0.5	**4.47E-07**	1
			5	**6.66E-07**	0.16788364
	WP PPAR SIGNALING PATHWAY	female	0.1	1	1
			0.5	**2.68E-09**	1
			5	**2.10E-16**	0.09804043
		male	0.1	1	1
			0.5	**8.43E-07**	1
			5	**1.53E-14**	**0.00892224**
PPAR-alpha signaling	BIOCARTA PPARA PATHWAY	female	0.1	1	1
			0.5	**0.00474489**	1
			5	**0.00784184**	0.47980917
		male	0.1	1	1
			0.5	**0.01359159**	1
			5	**0.01349897**	0.23989665
	REACTOME REGULATION OF LIPID METABOLISM BY PPARALPHA	female	0.1	1	1
			0.5	0.08989324	1
			5	**0.00066739**	0.98474294
		male	0.1	1	1
			0.5	**0.01525821**	1
			5	**0.00068469**	0.90318771
	WP PPAR ALPHA PATHWAY	female	0.1	1	1
			0.5	**1.79E-07**	1
			5	**9.66E-08**	0.14459302
		male	0.1	1	1
			0.5	**0.00074672**	1
			5	**0.00174326**	**0.00481777**
PPAR-gamma signaling	BIOCARTA PPARG PATHWAY	female	0.1	1	1
			0.5	1	1
			5	1	0.73021685
		male	0.1	1	1
			0.5	1	1
			5	1	0.95320462
	REACTOME ACTIVATION OF PPARGC1A PGC 1ALPHA BY PHOSPHORYLATION	female	0.1	1	1
			0.5	1	1
			5	1	1
		male	0.1	1	1
			0.5	1	0.96305474
			5	1	0.33445311
	WP HIF1A AND PPARG REGULATION OF GLYCOLYSIS	female	0.1	1	1
			0.5	0.86244879	1
			5	0.09654073	1
		male	0.1	1	1
			0.5	0.12756229	1
			5	0.7750924	1

***Bold p-values are statistically significant.**

Evidence for KE 2, alteration of cell growth pathways, is also supported by results from Chappell et al. (2020). At low doses, genes associated with peroxisomal lipid metabolism are induced (**Table 1**), followed by increases in mitotic and apoptotic signaling at higher doses (**Figure 5**). Induction of pro-apoptotic gene expression at higher doses was consistent with evidence for hepatocyte apoptotic cell death via H&E staining as well as caspase-3 immunostaining (Chappell et al., 2020). While PPARα activators are reported to suppress apoptosis under acute exposure scenarios, PPARα activators have also been reported to increase apoptosis in mouse liver undergoing cell proliferation in repeat dose studies (Corton et al., 2018), indicating that apoptosis is likely increased in the liver under longer-term exposure scenarios. This increase could be a homeostatic response to prevent the liver from severe overgrowth. Other PPARα activators have also been shown to induce pro-apoptotic gene expression pathways in wild type but not PPARα-null mice (Xiao et al., 2006).

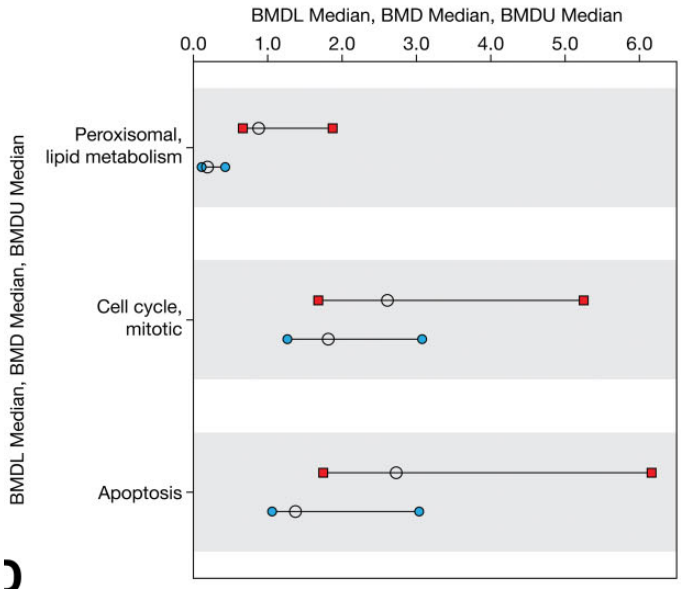

Figure 5. BMDL, BMD, and BMDU values for pathways related to peroxisomal lipid metabolism, cell proliferation, and apoptosis in mouse liver following 90 days of exposure to HFPO-DA (Chappell et al., 2020).

Support for KE 3, perturbation of cell growth and survival, is well established in studies conducted by DuPont, as exposure of mice to HFPO-DA has been shown to induce hepatocellular hypertrophy (**Table 2**).

Table 2. Increased liver weight in HFPO-DA-exposed F_0 female mice in reproduction/developmental toxicity study (DuPont-18405-1037, 2010).

Dose, mg/kg	Abs Liver Weight	Rel Liver Weight	% RLW
0	2.1 ± 0.27	6.0 ± 0.55	
0.1	2.3 ± 0.21	6.5 ± 0.41	109
0.5	2.6 ± 0.39*	7.1 ± 0.80*	118
5	4.3 ± 0.49*	10.8 ± 1.1*	181

* indicates statistical significance ($p < 0.05$) compared to control group.

As summarized in Figure 6, the empirical data for HFPO-DA provide direct evidence that the HFPO-DA-induced liver effects are occurring via a PPARα MOA.

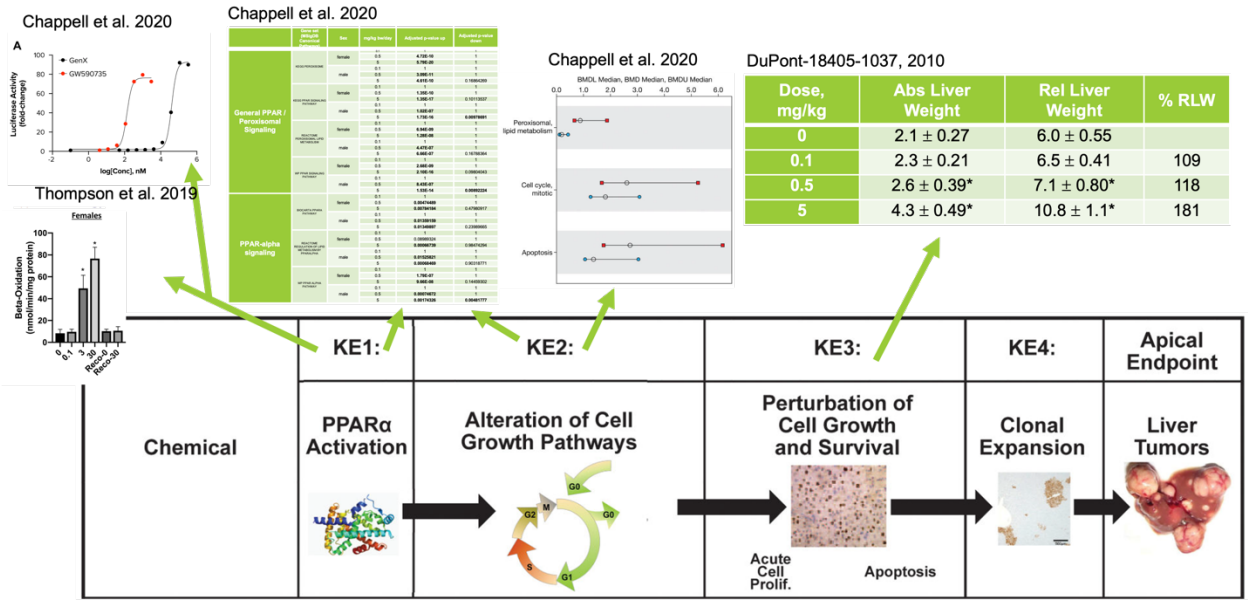

Figure 6. Empirical data supporting the PPARα MOA (from Corton et al. 2018) for HFPO-DA-induced liver effects in rodents.

3 Available evidence for alternate MOAs suggested by USEPA is weak

As noted above, alternate MOAs suggested by USEPA included PPARγ, cytotoxicity, and mitochondrial dysfunction. Each of these are addressed in the sections that follow.

3.1 PPARγ Activation

On page 85 of the assessment, USEPA cites one *in vitro* and one *in vivo* study as evidence of PPARγ activation by HFPO-DA. Specifically, USEPA states that Li et al. (2019) found evidence for "activation of genes associated with the PPARγ signaling pathway" in HEK293 embryonal kidney cells. This statement made by the USEPA about findings made

by Li et al. (2019) is not accurate, as Li et al. (2019) used HEK293 cells in a luciferase reporter gene assay to measure PPARγ transcriptional activation, but not the activation of downstream genes associated with PPARγ. Li et al. (2019) tested HFPO-DA in addition to PFOA and HFPO-TA in all their experiments, and determined that HFPO-TA, followed by PFOA, had the highest PPARγ agonistic activity, whereas HFPO-DA weakly activated both mouse and human PPARγ, with only a 1.2-fold increase in activity at the highest concentration tested (50 μM). Further, these authors also compared these 3 compounds in binding assays to the PPARγ ligand binding domain (LBD) and showed that HFPO-DA had little to no binding affinity to either human or mouse PPARγ LBD (IC_{50} values beyond detection). The effects of PFOA, HFPO-DA, and HFPO-TA on expression of aP2, Cebpα, Adip, Lep, LPL, and PPARγ genes in murine 3T3-L1 cells and human primary adipocytes were also measured. HFPO-DA caused minimal changes in gene expression in both cell lines in comparison to HFPO-TA and PFOA (Li et al., 2019).

The *in vivo* study cited by USEPA as purported supporting evidence for *in vivo* PPARγ activation by HFPO-DA, was a study by Conley et al. (2019). Specifically, USEPA states that Conley et al. (2019) reported "upregulation of genes in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18, which are associated with PPARγ signaling, including Pck1, Aqp7, and Gk." While these three genes are associated with PPARγ signaling, these gluconeogenesis genes are also regulated by PPARα in the liver (Zhang et al. 2019; Kersten, 2014). Therefore, it is likely that these genes were induced by PPARα rather than PPARγ based on increased expression of numerous other PPARα-regulated genes in maternal, fetal and neonatal livers (Conley et al., 2019, 2021). In addition, PPARγ is predominately expressed in adipose tissues (Chawla et al., 1994), whereas PPARα is predominantly expressed in the liver (Corrales et al., 2018). It is also important to note that Pck1, Aqp7, and Gk were *not* significantly upregulated in maternal livers, only in fetal livers (Conley et al., 2019). Moreover, transcriptomic results between studies with HFPO-DA exposure in rodents are conflicting. The key gene in the regulation of gluconeogenesis, Pck1, was downregulated in the livers of female mice and rats when measured by RNA sequencing (Chappell et al., 2020; Heintz et al., 2022). Review of the findings reported in Li et al. (2019) and Conley et al. (2019) demonstrates that the weight of evidence presented by USEPA for HFPO-DA activation of PPARγ is poorly supported.

USEPA also used data for PFOA and other PFAS (not including HFPO-DA) as purported evidence for PPARγ activation; specifically, USEPA cited a study by Rosen et al. (2017) stating that findings demonstrated "that 11%-24% of the PFAS-induced increase in transcriptional activity is PPARα independent, depending on the PFAS." Despite these claims by USEPA, the study authors main conclusion from this comparative study was that greater than ~75% of all genes regulated by PFAS in wild-type mice are in fact PPARα *dependent* (Rosen et al., 2017).

Using data from Chappell et al. (2020), we have further examined the potential involvement of other PPAR isoforms, such as PPARγ and PPARδ, following exposure to HFPO-DA. Pathway enrichment analysis of hepatic transcriptomic data from the 90-day study in mice exposed to HFPO-DA has been updated to include a new assessment of other

PPAR pathways, especially PPARγ. As shown in **Table 1**, there is significant evidence for PPARα activation and little evidence for PPARγ activation. While this specific analysis was not included in Chappell et al. (2020), raw sequencing data from this study are publicly available at NCBI's Gene Expression Omnibus (GEO) series accession number GSE135943.

3.2 Cytotoxicity

On pages 84-85 of the assessment, USEPA acknowledges that there is evidence for a PPARα MOA but then suggests that it may be operational only at high doses. USEPA then suggests that there is also evidence for a cytotoxic MOA, stating "liver necrosis was consistently observed in rodent toxicity studies with HFPO dimer acid ammonium salt and was reaffirmed by the NTP PWG's review of the 90-day subchronic study in mice and the reproductive and developmental toxicity study in mice, which suggests that cytotoxicity is also a possible MOA." This hypothesis appears to be based, in part, on evidence for necrosis at intermediate doses.

Importantly, the USEPA assessment (2021) implies that there is a connection between single cell necrosis and focal necrosis, with the latter being a more severe manifestation of the former. However, across four datasets involving mice exposed to HFPO-DA, focal necrosis was not significantly increased (**Table 3**). Notably, a 10-fold increase in dose from 0.5 to 5 mg/kg was, in one dataset, associated with a *decreased* incidence. These findings do not support a cytotoxic MOA for the liver effects observed in mice. Additional issues regarding focal necrosis and single cell necrosis are described in a separate expert report submitted to USEPA as part of the Request for Correction.

Table 3. Incidence of focal necrosis in four HFPO-DA repeat dose studies.

Dose, mg/kg	90-day males	90-day females	Repro males	Repro females
0	0/10	1/10	0/25	2/25
0.1	0/10	0/10	0/25	2/25
0.5	0/10	2/10	4/25	4/25
5.0	1/10	4/10	3/25	5/25

Furthermore, the amount and apparent temporal nature of the so-called cytotoxicity (single cell necrosis) observed in mouse livers following HFPO-DA exposure does not align with the cytotoxicity MOA. In the latter, exemplified by chloroform in the liver, there is consistent necrosis with a resulting increase in compensatory hyperplasia. Both the necrosis (centrilobular in nature) and the cell proliferation seen in the cytotoxicity mode of action are significantly greater and prolonged than the single cell necrosis and focal necrosis seen with the PPAR alpha activators.

3.3 Mitochondrial Dysfunction

On page 85 of the assessment, USEPA (2021) cites Blake et al. (2020) and Conley et al. (2019) as purported evidence that HFPO-DA induces an increase in mitochondria that USEPA then states is atypical of PPARα activators: "Blake et al. (2020) reports an increase in subcellular organelles consistent with peroxisomes and mitochondria in pregnant dam livers exposed to 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E11.5 or E17.5 using TEM. This increase in mitochondria is not typical of PPARα activation and suggests an alternate MOA … Further supporting this alternate MOA, a number of genes upregulated in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18 are specific to mitochondrial beta oxidation (*Cpt1a*, *Cpt1b*, *Cpt2*, *Acaa2*, *Acadl*, *Acadm*), mitochondrial ketogenesis (*Hmgcs2*), and mitochondrial electron transfer (*Etfdh*) (Conley et al., 2019)."

However, in contrast to this statement by USEPA, Aoyama et al. (1998) demonstrated that PPARα modulates the expression of genes involved in mitochondrial β-oxidation, as both peroxisomal and mitochondrial enzymes were induced following treatment with WY-14,643 in wild type but not PPARα-null mice (Aoyama et al., 1998). Similar findings have also been observed in mice treated with other PPARα agonists such as ciprofibrate (Cook et al., 2000). Collectively, these data indicate increased mitochondrial fatty acid metabolism occurs as a result of PPARα activation.

In addition, evidence in the scientific literature indicates increased peroxisome and mitochondrial number are directly linked via PGC-1α activation (Austin and Pierre, 2012; Bagattin et al., 2010; Fransen et al., 2017). PGC-1α binds to PPARs to coactivate expression of target genes involved in mitochondrial function and biogenesis (Wenz, 2009). PGC-1α also has been shown to regulate peroxisome biogenesis in various tissues including the liver (Bagattin et al., 2010). Furthermore, to properly show an increase in mitochondria relative number and area, further analysis using stereology and morphometric techniques in treated and untreated liver is required. The only way to assess an increase in organelle compartments in a cell with electron microscopy is to perform morphometry and stereology on the transmission electron microscopy (TEM) samples. Precise and accurate quantification of cellular changes in electron micrographs has traditionally used morphometric tools to measure numbers of organelles as well as the surfaces, lengths, and volumes (Cheville and Stasko, 2014). According to the available information, Blake et al. (2020) did not perform such analyses for their histopathological assessment.

Findings by Aoyama et al. (1998) and Cook et al. (2000), when considered collectively with the entire body of evidence in the scientific literature, demonstrate that the liver effects occurring in rodents exposed to HFPO-DA are occurring via a PPARα MOA rather than via some alternate MOA. Overall, USEPA (2021) failed to consider the weight of evidence that does not support the Agency's proposed alternative MOAs. USEPA downplayed the preponderance of compelling evidence supporting the PPARα MOA.

4 Conclusion

Within the assessment, alternative MOAs other than the PPARα MOA are suggested by the USEPA to be associated with the observed liver toxicity caused by HFPO-DA in rodents. However, the available evidence for these alternate MOAs is weakly supported, and in some instances, taken out of context or incorrectly referenced. The overall weight of the evidence demonstrates that the observed liver effects in mice exposed to HFPO-DA occur via the PPARα MOA. The PPARα MOA for liver tumors in rodents is not relevant to humans.

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EXHIBIT 2

Issues with the NTP PWG Report and USEPA's Use of that Report for Their HFPO-DA Toxicity Assessment (2021)

MARCH 16, 2022

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Issues with the NTP PWG Report and USEPA's Use of that Report for Their HFPO-DA Toxicity Assessment (2021)

MARCH 16, 2022

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Executive Summary

ToxStrategies, Inc. and Dr. John Cullen reviewed the National Toxicology Program (NTP) pathology working group (PWG) report titled *NTP PWG Final Report on the Pathology Peer Review of Liver Findings*, in Appendix D of the USEPA (2021) toxicity assessment for HFPO-DA. Several issues were identified that impact the RfD for HFPO-DA. These issues include USEPA's misapplication of the terms "adverse" and "constellation" of liver lesions. The NTP indicated that the liver changes in mice were adverse to mice, but did not consider the issue of whether these changes were relevant to humans. Although the NTP considered a collection of lesions as a "constellation" of liver lesions, they did not themselves combine the various lesions into a single category, which USEPA later did for quantitative dose-response modeling. Notably, one of the changes in the constellation (*viz*., focal necrosis) did not increase significantly with dose; moreover, focal necrosis can be secondary to other changes in the constellation. As such, there are fundamental uncertainties in using the constellation of liver lesions as an endpoint for quantitative dose-response modeling. In addition to these issues, some of the changes comprising the constellation of liver lesions are known PPARα rodent specific responses that have no relevance to human health risk assessment. Finally, there are diagnostic criteria discrepancies and/or shortcomings in the distinguishing of single cell necrosis and apoptosis. Classic forms of cellular necrosis were not observed, whereas the predominant indication of single cell necrosis identified by the NTP PWG were clusters of inflammatory cells that were similar to inflammatory foci that may be unrelated to HFPO-DA-induced hepatocyte death. Importantly, molecular staining for markers of apoptosis indicated the presence of apoptotic cells associated with some of these foci. Taken together, many of the foci considered indicative of single cell necrosis by the NTP PWG are unlikely to be hepatocytes undergoing HFPO-DA-mediated necrotic cell death. As single cell necrosis was part of the constellation of lesions, these issues further confound the use of the constellation of liver lesions as an endpoint for quantitative dose-response modeling.

1 Introduction

Appendix D of the USEPA (2021) toxicity assessment for HFPO-DA contains a report on a liver cell slide review conducted by a National Toxicology Program (NTP) pathology working group (PWG). The report, *NTP PWG Final Report on the Pathology Peer Review of Liver Findings*, is a reevaluation of H&E liver slides from two DuPont studies: a 90-day toxicity study in mice (18405-1307) and a reproduction/developmental toxicity study in mice (18405-1037). Both the NTP PWG report and USEPA's interpretation of the report have several significant issues. These issues are enumerated below.

2 The NTP PWG's definition of adversity and misinterpretation by USEPA.

The USEPA (2021) toxicity assessment relies on the NTP PWG for the selection of the critical effect in mice that USEPA used to derive its chronic reference dose (RfD) for

estimating safe levels of exposure in humans. The NTP PWG report defines adversity as follows:

> *"Adversity is a term indicating "harm" to the test animal within the constraints of a given study design (dose, duration, etc.). Assessment of adversity should represent empirical measurements (i.e., objective data) integrated with well-informed subjective judgements to determine whether or not a response is considered harmful to an organism (Kerlin et al., 2016)."*

The terms "within the constraints of a given study design" and "harmful to an organism" indicate limits of applicability. In fact, Kerlin et al. (2016) make ten recommendations that a toxicologist or toxicologic pathologist should consider when interpreting toxicity study data. Here we highlight a few of the recommendations from Kerlin et al. (2016) that are pertinent to the NTP PWG report. The original numbering and underlining from Kerlin et al. (2016) has been retained:

1. Adversity is a term indicating <u>harm to the test animal</u>.

3. Adversity as identified in a nonclinical study report <u>should be applied only to the test species and under conditions of the study</u>.

   a. When toxicity in a test animal is interpreted as being specific to that species and lacking relevance to humans, the test article effect may still be an adverse response for the species being tested.

5. Communication of what is considered adverse and assignment of the NOAEL in the overall study report should be consistent with, and supported by, the information provided in the study subreports.

   b. Test article–related adverse findings considered to be part of a constellation of related effects should be discussed together.

9. Nonclinical scientists, including toxicologists, pathologists, and other contributing subject matter experts who interpret data from nonclinical studies, should be active participants in assessing and communicating human risk.

10. All available data from all nonclinical studies must be evaluated together to define any potential toxicities and to predict human risk.

Numbers 1 and 3 (above) clearly indicate that adversity in a study applies to the species being investigated. As such, the NTP is correct to consider the lesions in mice adverse to mice, as the likely role of PPARα activation induces a rodent-specific response that can, under chronic exposure scenarios, lead to tumors in rodents. Number 5 (above) will be discussed later in the following section. Numbers 9 and 10 (above) address human relevance of the lesions observed in toxicity studies. Nowhere does the NTP PWG report state that the lesions observed in mice are relevant to humans or should be considered for use in human health risk assessment. If the NTP PWG did consult with USEPA risk assessors and the group agreed that these lesions are suitable for human health risk assessment, then this should be stated explicitly.

3 USEPA misused the NTP PWG's term "constellation of lesions" and grouping lesions into a single category for modeling was flawed

3.1 The term constellation does not imply that lesions should be grouped into a single category

As indicated above, Kerlin et al. (2016) recommends adverse findings considered to be part of a constellation of related effects should be discussed together. Indeed, after defining adversity, the NTP PWG concluded:

> *After discussion, the PWG members agreed that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) rather than one lesion by itself,* ***represents adversity within the confines of this study*** *[emphasis added]…"*

Kerlin et al. (2016) are simply stating that multiple related lesions should be ***discussed*** together when appropriate. Kerlin et al. (2016) provide an example where clinical chemistry indicative of liver damage should be viewed in the context of changes in liver weight and morphology. Kerlin et al. (2016) also state that in addition to single test article-related changes, a spectrum of changes might be "used collectively to establish a NOAEL even though each finding might be viewed as inconsequential if each occurred in isolation." It should be noted that Kerlin et al. (2016) do not elaborate on how this should be done. For example, Kerlin et al. (2016) do not state whether this is a statistically based NOAEL or one based on expert judgement. Notably, the NTP PWG did not specifically score each animal for the presence or absence of the "constellation," but rather scored several individual lesions that they considered to collectively represent a constellation of liver lesions. It was USEPA that later tallied the individual lesions and assigned each animal as either exhibiting or not exhibiting the constellation of liver lesions for subsequent dose-response modeling. Ultimately, it was USEPA (not the NTP PWG) that modelled the constellation of lesions. We are unaware of any USEPA risk assessment guidance documents that describe methods and circumstances for modeling constellations of lesions.

There are many toxicity values in USEPA's Integrated Risk Information System (IRIS) database that are based on liver lesions, yet none appear to be based on a constellation of lesions or appear to be based on something akin to a constellation of lesions. Furthermore, two recent USEPA toxicity assessments describe multiple individual liver lesions in response to PFAS exposure as a constellation of liver lesions but did not combine these lesions into a single incidence category to develop an RfD and instead modeled the individual lesions separately (USEPA, 2021b; USEPA, 2022).

3.2 Grouping lesions into a single category for modeling was flawed because some individual lesions within the "constellation of lesions" did not increase as a function of dose

Another problem with the constellation modeled in the USEPA (2021a) toxicity assessment for HFPO-DA is that one of the lesions in the constellation did not increase

with dose. **Table 1** shows the NTP PWG scoring for the four lesions in female mice in the DuPont reproductive/developmental toxicity study. Three of the four lesions increased with treatment, particularly as the dose increased 10-fold from 0.5 to 5 mg/kg. One lesion, focal necrosis, was present in control mice and not significantly increased with treatment. Similar findings and additional issues regarding focal necrosis are observed in other HFPO-DA toxicity studies (discussed in section 5, below). The main point here is that combining lesions that do and do not clearly respond to treatment dose is not scientifically justified.

Table 1. The Four Individual Lesions Comprising the "Constellation of Lesions"

Dose, mg/kg	N	Cytoplasmic alteration	Apoptosis	Single cell necrosis	Focal necrosis	Constellation
0	25	0	0	0	2	2
0.1	25	1	0	2	2	3
0.5	25	**16**	0	3	4	**17**
5.0	25	**25**	**10**	**19**	5	**24**

Bolded numbers differ significantly from control group; note: for reasons beyond the scope of this report, the constellation of lesions will not necessarily be the sum of the individual lesions

4 Some of the individual lesions comprising the "constellation of lesions" are not relevant to humans.

Consistent with Kerlin et al. (2016) recommendation 3a (see section 2, above), the constellation of liver lesions applies to the species tested, i.e., mice. The relevance of these lesions to other species must be considered. The most sensitive and highest incidence lesion, cytoplasmic alteration (i.e., hypertrophy), is highly associated with PPARα activation and these lesions are not observed in humans exposed to PPARα activators and therefore are not relevant for purposes of human health risk assessment (Hall et al., 2012; Corton et al., 2018). Similarly, elevated apoptosis in rodent liver is also seen in response to PPARα activation (Xiao et al., 2006; Corton et al., 2018); therefore, these lesions are also not relevant for human health risk assessment. Critically, Chappell et al. (2020), which was not cited in the USEPA toxicity assessment (2021a), unequivocally demonstrated transcriptomic evidence for PPARα activation in mouse liver samples from 90-day toxicity studies in mice exposed to HFPO-DA. Evidence related to the key events underlying the PPARα mode of action (MOA) is described in more detail in a separate expert report submitted as part of the Request for Correction.

5 The NTP PWG did not fully characterize focal necrosis and the USEPA overestimated the link between focal necrosis and single cell necrosis.

USEPA (2021) hypothesized that HFPO-DA might cause cytotoxicity in the liver as evidenced by the NTP PWG's scoring of single cell necrosis and focal necrosis. However,

one example of focal necrosis shown in the NTP PWG report is an example of subcapsular focal necrosis (**Figure 1**). The blood supply to the liver is limited just below the capsule and expansion from hypertrophy or pressure from adjacent organs can lead to focal hypoxia and cell death. (Thoolen et al., 2010). This form of focal necrosis in mice treated with HFPO-DA is likely secondary to observed hypertrophy (also called cytoplasmic alteration) in the NTP PWG report as opposed to a cluster of cells undergoing direct HFPO-DA induced necrotic cell death. As such, there is a need to distinguish between direct and indirect forms of focal necrosis, which may impact the interpretation of any potential treatment-related increase in focal necrosis. The NTP PWG did not further diagnose/distinguish "subcapsular" focal necrosis from generic focal necrosis, whereas Dr. Cullen noted that focal necrosis was often found in a subcapsular location. This is a critical distinction that needs to be addressed.

It is also notable that mice can have scattered foci of hepatocyte necrosis and inflammation as a spontaneous finding (e.g., reduce the incidence of focal necrosis in treated mice) which is typically attributed to bacterial or inflammatory mediators that enter the portal circulation from the gastrointestinal mucosa. This is exemplified by the focal necrosis diagnosed in the control mice of the reproduction study (**Table 2**). Oral administration of xenobiotics has the potential to irritate or injure the GI tract mucosa leading to increased mucosal permeability and a dose-related increase in such foci, independent of direct hepatic toxicity. This potential, when considered collectively with other evidence described herein, raises questions as to the relationship between this finding and liver injury and whether this finding should be included in the "constellation of lesions." As previously stated, compression-related focal necrosis can represent adversity in mice, but if it is the result of PPARα-related hypertrophy, then it has no relevance to human health risk assessment. Moreover, USEPA suggests that there is a relationship of so-called single cell necrosis progressing to focal necrosis. That focal necrosis in HFPO-DA treated mice is related to individual hepatocyte necrosis is unlikely given that focal necrosis was diagnosed in some mice that did not exhibit any potential individual hepatocyte necrosis (**Table 2**). As described in the following section, there are also significant issues and/or errors regarding the diagnosis criteria for single cell necrosis.

Table 2. Incidence of Focal Necrosis in Four HFPO-DA Repeat Dose Studies

Dose, mg/kg	90-day males	90-day females	Repro males	Repro females
0	0/10	1/10	0/25	2/25
0.1	0/10	0/10	0/25	2/25
0.5	0/10	2/10 (0)	4/25 (0)	4/25 (1)
5.0	1/10 (1)*	4/10 (1)	3/25 (2)	5/25 (4)

*Numbers in parentheses are the number of animals with focal necrosis that the NTP PWG also diagnosed as having single cell necrosis

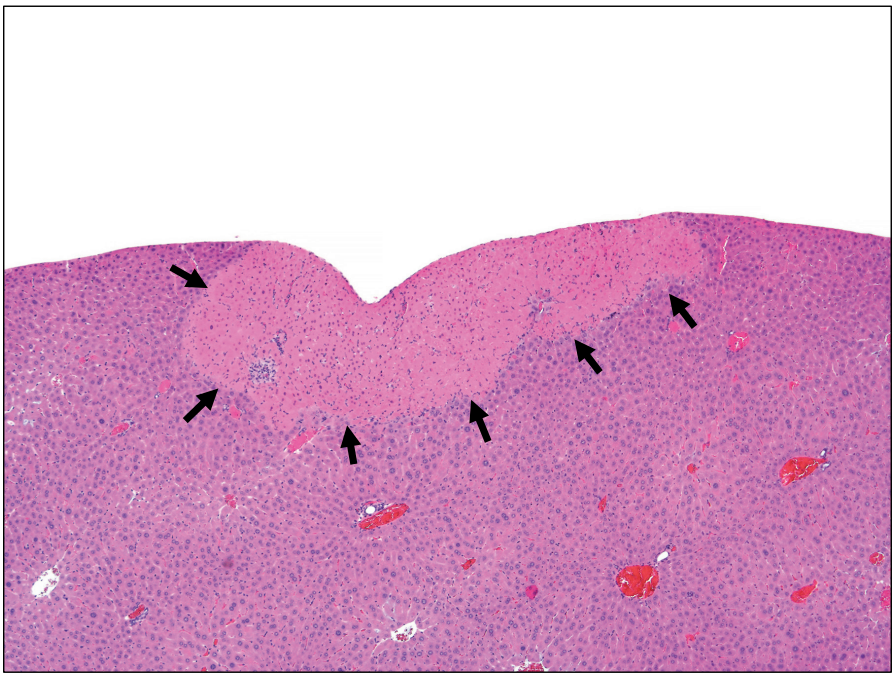


Figure 1. Focal necrosis (area delineated by arrows) in the liver of a Group 4 male mouse (animal 456) from the 18405-1307 subchronic study (as presented in USEPA (2021) Appendix D Figure 6).

6 Some of the NTP PWG diagnostic criteria are inconsistent with those described in Elmore et al. (2016).

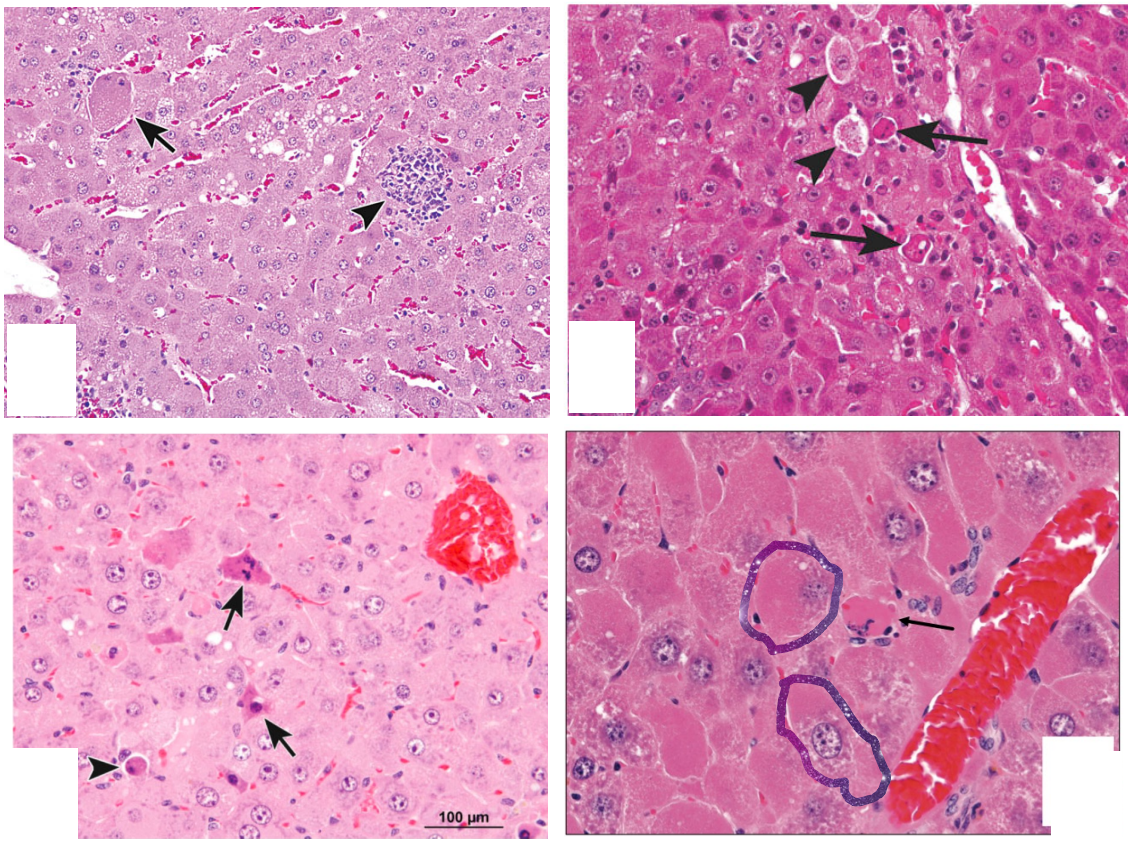
6.1 H&E staining intensity

The NTP PWG attempts to score both apoptosis and single cell necrosis. The rationale for distinguishing these two types of cell death relates to potential insights into the mode of action (MOA) for treatment-induced changes in liver histopathology. Here, the implications for MOA are not discussed, but rather we focus on the criteria for distinguishing apoptotic and necrotic cell death. The NTP PWG report indicates that Elmore et al. (2016) was used to distinguish "cell death/necrosis/apoptosis." Elmore et al. (2016) defines necrotic cells as follows:

> "In general, ''necrosis, single cell'' describes single, noncontiguous cells in a tissue that are characterized by cell and nuclear swelling and pale cytoplasm...This is in contrast to the smaller, shrunken hypereosinophilic apoptotic cell…"

One example of a necrotic hepatocyte is shown in **Figure 2A (arrow).** An example of a liver section with both apoptotic and necrotic hepatocytes is shown in **Figure 2B**. The main distinguishing features are that necrotic hepatocytes are often pale and swollen, whereas apoptotic hepatocytes are often small and hypereosinophilic. However, **Figure 2C** contains apoptotic hepatocytes that are not small and rounded. **Figure 2D** is an example from the

NTP PWG report showing an example of single cell necrosis that is described as swollen with a brightly eosinophilic cytoplasm and a karyorrhectic nucleus. This characterization of necrotic cells as brightly eosinophilic is in contrast to the description above from Elmore et al. (2016).



roup (a 405)
subchronic study. The necrotic cell (arrow) is swollen, and has brightly eosinophilic cytoplasm and a karyorrhectic nucleus. Two nearby hepatocytes are outlined in blue (this was superimposed on the original image by the authors of this report). Source: A-C = Elmore et al. (2016); D = USEPA (2021, Appendix D)

6.2 Cell size

Although many examples of apoptotic hepatocytes appear as small, rounded cells, **Figure 2C** provides examples of apoptotic cells that are similar in size to the surrounding hepatocytes. The necrotic cell described as swollen in **Figure 2D** appears to be smaller than nearby hepatocytes. Therefore, the "swollen" and "brightly eosinophilic" cells the NTP PWG identified as necrotic (**Figure 2D**) may, in fact, be apoptotic cells. In support of this notion, slides previously stained for activated caspase-3, a key enzyme in the apoptosis pathway, originally described in Chappell et al. (2020), were reexamined to

determine the presence of and cytologic features of caspase-3 positive cells. Examples of caspase-3 positive cells that have irregular outlines and/or are equal to or larger than surrounding hepatocytes are shown in **Figure 3A-B**. Notably, **Figure 3B** shows a caspase-3 positive irregularly shaped cell similar in shape to the cell that the NTP PWG highlighted as an example of a necrotic cell in H&E stained sections (**Figure 3C**). These findings indicate significant issues and/or errors with the single cell necrosis criteria the NTP PWG employed.

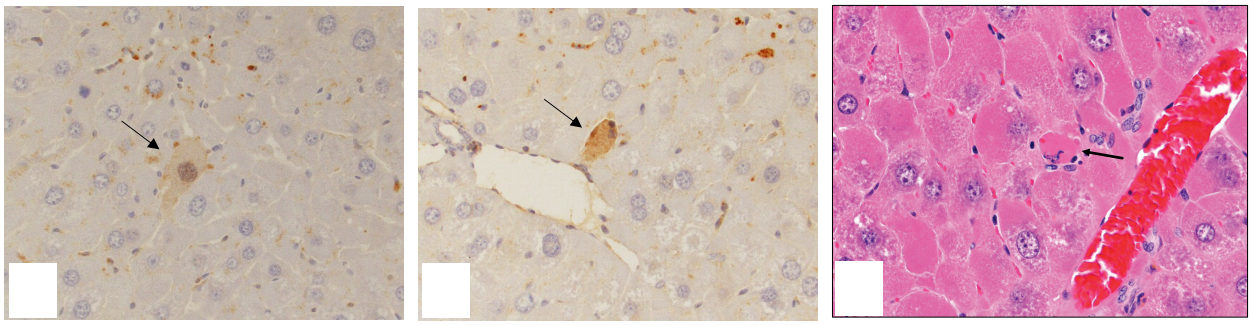


Figure 3. Examples of non-shrunken apoptotic hepatocytes. (A) Example of a swollen hepatocyte (arrow) with an irregular outline and with pale Caspase-3 staining. (B) Example of a caspase-3 stained hepatocyte (arrow) that has an oblong outline. (C) Example of a hepatocyte categorized as an individual necrotic cell (arrow) in H&E stained liver from the NTP PWG report with a similar profile to the Caspase-3 stained cells in panels A and B. Source: A-B = male mouse 404 from DuPont 90-day study 18405-1307 that was stained for the publication Chappell et al. (2020). C = male mouse 405 from DuPont 90-day study 18405-1307 as presented in USEPA (2021) Appendix D.

6.3 Inflammatory cells

Figure 4A (reproduced from Elmore et al. 2016) contains two proposed examples of individual necrotic hepatocytes[1]. The arrow in **Figure 4A** points to a swollen necrotic cell, whereas the arrowhead points to a small focus of inflammatory cells and a presumed necrotic hepatocyte; however, a necrotic hepatocyte is not evident. Elmore et al. (2016) suggest that the focus (arrowhead) represents a later phase of the swollen necrotic cell (arrow); note that both forms of necrotic cell are present in the same liver section. Similar aggregates were termed "mixed cell aggregates" in the PWG report (**Figure 4B**) and were seen in many animals with no indication of a treatment-related response (**Table 3**). Note the strong resemblance of **Figure 4B** to the "focus of inflammation" (arrowhead) in **Figure 4A** that Elmore et al. (2016) considered "most likely secondary to cell rupture" despite the absence of a necrotic hepatocyte. The NTP PWG may have considered some of the mixed infiltrates as necrosis; however, these foci should not be linked to dead hepatocytes. Mixed cell infiltrates can arise from multiple causes and should not be used to connote individual cell necrosis. Gastrointestinal organisms and inflammatory mediators are one potential cause and this is likely given the presence of mixed cell infiltrates in male and female

[1] Figure 3C is the same as Figure 2D and is provided here to make different comparisons.

control mice in the studies listed in **Table 3.** This process may also explain the presence of individual hepatocytes undergoing necrosis with adjacent neutrophils.

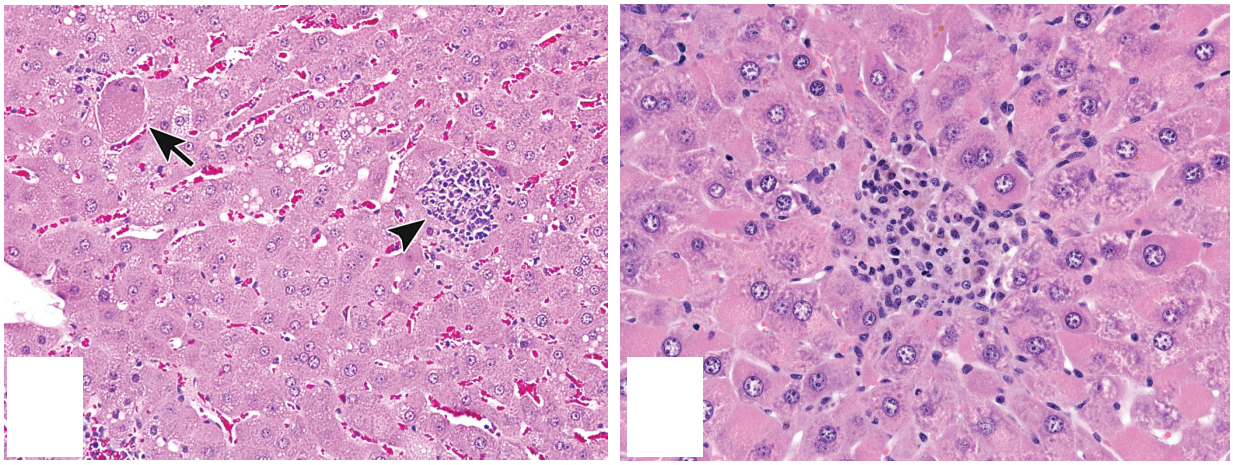


Figure 4. Examples of necrotic hepatocytes and mixed cell infiltrates. (A) "Examples of single cell necrosis in the liver. There is marked cell swelling and karyorrhexis in a necrotic hepatocyte (arrow) and a nearby small focus of inflammation (arrowhead), most likely secondary to cell rupture", *although no necrotic hepatocyte is present*. (B) An example of "mixed cell infiltrates" in a male mouse exposed to 5 mg/kg HFPO-DA that bears marked si xed cell infiltrates can arise from multiple ca dual cell necrosis (see text). Source: A = El from DuPont reproductive/developmental toxi A (2021) Appendix D.

T our HFPO-DA Repeat Dose Studies

		s	**Repro males**	**Repro females**
			6/25	12/25
			3/25	7/25
			11/25	17/25
			8/25	15/25

No treatment doses differed significantly from respective control groups

As it would be somewhat unexpected to find only later stages of individual cell necrosis without hepatocytes with swollen nuclei and hepatocyte cytoplasm, these mixed cell aggregates were previously disregarded in analyses by Dr. Cullen, as were hepatocytes with only modest changes in the hepatocyte outline due to the absence of the combination of swollen hepatocytes with swollen nuclei. Notably, slides previously stained for activated caspase-3 originally described in Chappell et al. (2020) were re-examined to determine if any damaged hepatocytes were associated with caspase-3 immunoreactivity. An example of an inflammatory focus surrounding a caspase-3 positive cell is shown in **Figure 5A**. This indicates that at least some of the necrotic foci scored by the NTP PWG (e.g., **Figure 5B**) may instead represent apoptosis. Elmore et al. (2016) have previously noted that

inflammatory cells are typically associated with necrotic cells as opposed to apoptotic cells; however, the examples in **Figure 5** suggest that inflammatory cells might also associate with caspase-3 positive cells. Given the apparent absence of swollen necrotic cells with swollen nuclei, the inflammatory foci or infiltrates affecting a single hepatocyte may have other etiologies including bacterial and inflammatory mediators derived from the gastrointestinal tract. Indeed, inflammatory cells, recruited by inflammatory mediators or bacteria from the portal blood, can kill adjacent healthy hepatocytes so the presence of inflammatory cells does not necessarily mean that they were attracted by the release of dying cell constituents, but rather be the instigators of hepatocyte death.

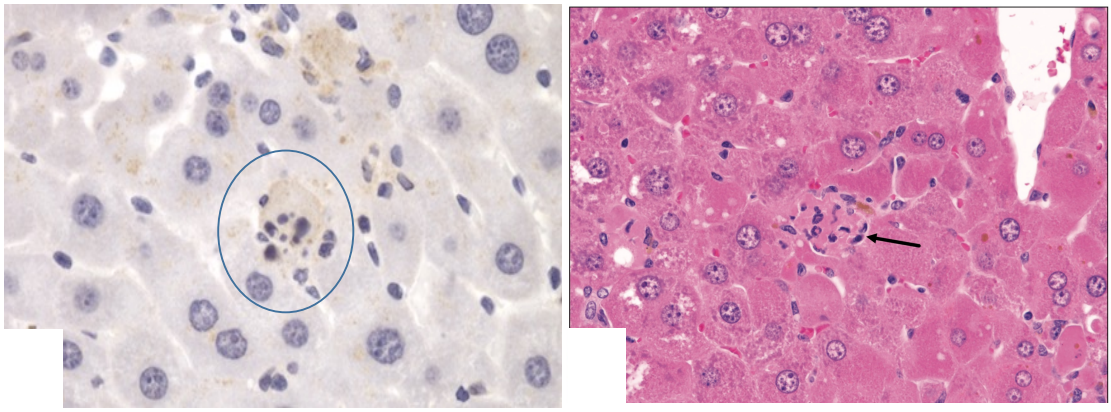


Figure 5. Examples of inflammation associated with an individual hepatocyte. (A) An example of a caspase-3 stained hepatocyte, indicative of apoptosis, with inflammatory cells adjacent (circled). Example of inflammatory cells in contact a with caspase-3 positive hepatocytes from a liver section of a mouse exposed to 5 mg/kg HFPO-DA. (B) Example of a potentially necrotic cell surrounded by inflammatory cells (arrow) in a H&E stained liver section from a female mouse exposed to 5 mg/kg HFPO-DA. Source: A = new unpublished caspase-3 stained section from female mouse 5020 from DuPont reproductive/developmental toxicity study 18405-1037. B = male mouse 410 from DuPont 90-day study 18405-1307 as presented in USEPA (2021) Appendix D (NTP PWG Report).

6.4 Caspase-3 staining

Elmore et al. (2016) acknowledges that it is sometimes difficult to distinguish apoptosis from necrosis based on morphology in H&E stained slides. In such cases, Elmore et al. (2016) recommend follow-up tests to confirm apoptosis. Indeed, the presence of apoptosis was confirmed with immunochemical staining with anti-Caspase-3 antibodies in Chappell et al. (2020). While such staining does not preclude the possibility that there might be necrotic cells present in the same tissue section as apoptotic cells, it unequivocally establishes the presence of apoptotic cells whereas diagnosis of necrotic cells is more subjective. As demonstrated above, there are multiple examples of cells that the NTP PWG considered necrotic that, based on caspase-3 staining, may be apoptotic. Notably, the NTP PWG report was finalized December 4, 2019, before Chappell et al. (2020) was published online.

In summary, the NTP PWG's characterization of single cell necrosis as hypereosinophilic contrasts with the general characterization of single cell necrosis as having pale cytoplasm. In addition, at least one example the NTP PWG considered as swollen actually appears to be smaller than surrounding cells. Caspase-3 positive cells have been observed of varying shape, size and, in some cases, surrounded by inflammatory cells. All these observations indicate significant issues with the conclusions of the NTP PWG.

7 USEPA misapplied the Hall Criteria

The Hall Criteria are based on a publication by Hall et al. (2012) that attempts to determine when increased relative liver weight (RLW) in a short-term rodent bioassay is an adaptive or adverse effect. Like the discussion in section 2 (above), adverse refers to the test animal, not necessarily whether the adversity is relevant to humans. According to Hall et al. (2012), when increased RLW occurs in the presence of large increases in serum liver enzymes or histological evidence of "structural degenerative or necrotic" changes, the RLW is considered adverse. In the absence of increased serum liver enzymes or histological changes, the increased RLW is considered non-adverse (or adaptive) if there is evidence of nuclear receptor activation such as PPARα. The adversity being referred to is in the context of setting dose levels for longer-term toxicity studies. As such, a test dose in a subchronic study that increases RLW as well as serum liver enzymes or structural changes should not be included in a chronic bioassay. If a given test dose results in a significant increase in RLW in the absence of serum liver enzymes or structural changes *and* the test article activates PPARα or certain other nuclear receptors (e.g., CAR), then the RLW is non-adverse and could be included in a chronic bioassay. Hall et al. (2012) also suggest that doses of a PPARα activator (or similar) that increase RLW more that 150% of control animals might be considered a maximum tolerated dose (MTD). The rationale is that data suggest that nuclear receptor activators (e.g., PPARα activators) that increase the RLW by 150% or more in short-term studies will likely result in liver tumors in chronic bioassays. By avoiding the use of adverse doses and MTDs when designing chronic studies, any adverse effects observed in longer-term studies in rodents might be relevant to humans.

The above interpretation is supported by quotes from Hall et al. (2012):

> "In addition, while the initial effects of chemicals that induce hepatic metabolism may be regarded as adaptive and noninjurious, i.e., non-adverse (Greaves 2007; Schulte-Hermann 1974), it is clear that at higher dose levels, or following prolonged exposure, these adaptive responses can fail leading to degenerative hepatocellular changes including necrosis with additional involvement of the biliary systems as compensatory metabolic systems are overcome or where novel cytotoxic metabolites are generated (Klaunig et al. 1998; Williams and Iatropoulos 2002). In extreme cases, hepatocyte hypertrophy may lead to compression of the sinusoidal blood circulation and anoxic necrosis (Farber 1980). In these circumstances, the use of the term non-adverse is only valid for the dose and duration of exposure of that chemical as defined by the study in question."

Indeed, the above quote describes a scenario similar to that described for HFPO-DA in section 5 (above), where some of the observed focal necrosis is secondary to adaptive hypertrophic effects. Hall et al. (2012) continues:

> "Furthermore, the use of humanized mice has now shown that the rodent liver is primed toward proliferation in response to CAR/PXR/PPARa activation whereas the human liver shows considerable resistance to this mechanism of hepatocarcinogenesis. Therefore, the induction of a proliferative or even neoplastic response in the rodent liver through enzyme induction would be considered to have little relevance to man in the context of estimating the risk of human hepatocarcinogenesis."

Since there is overwhelming evidence for PPARα signaling in the mouse liver (see accompanying expert report submitted as part of the Request for Correction), not only are proliferative or neoplastic responses in the rodent liver irrelevant to humans but so is the "constellation" of liver lesions described by the NTP PWG.

Table 4 includes data from male mice exposed to HFPO-DA for 90 days. At the intermediate dose, the relative liver weight (RLW) is increased significantly 111%, there is no evidence of single cell necrosis (using the original study terminology that did not distinguish apoptosis and necrosis), and minimal increases in serum liver enzymes. As such, the changes at the intermediate dose (and low dose) are not considered adverse. At 5 mg/kg, the RLW is 229%, well above the 150% level that Hall et al. (2012) consider to be an MTD. The single cell necrosis and increased liver enzymes would indicate adversity; however, these are occurring at doses that are adverse to the mice. Because these changes occur as a result of PPARα activation, this adversity is not relevant to humans. Similar results were observed in female mice from the reproductive/developmental toxicity study (**Table 5**).

Table 4. Liver Changes in Male Mice Exposed to HFPO-DA for 90 Days

Dose, mg/kg	N	CA	MI	SCN	AST % cont	ALT % cont	RLW % cont	Adversity
0	10	0	0	0	--	--	--	
0.1	10	0	0	0	108	127	99	Non-adverse
0.5	10	**8**	0	0	135	135	**111**	Non-adverse
5	10	**10**	**9**	**10**	**206**	**520**	**229**	Exceeds MTD

CA = cytoplasmic alteration (hypertrophy); MI = mitosis; SCN = single cell necrosis (older definition); RLW = relative liver weight; AST and ALT = serum liver enzymes; MTD, maximum tolerated dose; bold items are statistically different from control group

Table 5. Liver Changes in Female Mice Exposed to HFPO-DA for 53-65 Days

Dose, mg/kg	N	CA	MI	SCN	AST % cont	ALT % cont	RLW % cont	Adversity
0	25	0	0	1	ND	ND	--	
0.1	25	1	0	3	ND	ND	109	Non-adverse
0.5	25	**14**	0	2	ND	ND	**118**	Non-adverse
5	25	**24**	**5**	**21**	ND	ND	**181**	Exceeds MTD

CA = cytoplasmic alteration (hypertrophy); MI = mitosis; SCN = single cell necrosis (older definition); RLW = relative liver weight; AST and ALT = serum liver enzymes; MTD, maximum tolerated dose; ND = not done; bold items are statistically different from control group

8 Conclusion

Several issues were identified that impact the RfD for HFPO-DA. The diagnostic criteria for single cell necrosis and apoptosis were likely erroneous, especially since the NTP did not conduct any molecular analyses (e.g., staining for caspase-3). Combining of liver changes into a single category, where some lesions did not increase with dose and some lesions were misdiagnosed, for dose-response modeling impacts the derivation of the current RfD. More importantly, the relevance of the adverse effects in mice to human health risk assessment was not properly considered. A holistic interpretation of the data for HFPO-DA support involvement of a PPARα mode of action in the liver that is not relevant to humans.

9 References

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EXHIBIT 3

Issues with the Uncertainty Factors in USEPA Toxicity Assessment (2021)

MARCH 16, 2022

ToxStrategies

Innovative solutions
Sound science

Issues with the Uncertainty Factors in USEPA Toxicity Assessment (2021)

MARCH 16, 2022

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Executive Summary

ToxStrategies, Inc. reviewed the rationale for the 3000-fold composite uncertainty factor used in the USEPA (2021a) risk assessment of HFPO-DA. Notably, this value *increased* 10-fold from 300 in a recent USEPA draft assessment from 2018. The increase was the result of a slight decrease in the duration of the study used as the basis of the RfD as well as an unusual increase in the database uncertainty factor (UF_D). Based on our review, the large UF is not consistent with the available studies on HFPO-DA and nor is it consistent with recent USEPA risk assessments on other PFAS. As will be discussed, the 10-fold database uncertainty factor (UF_D) should have been no more than 3 given the available data on HFPO-DA. Similarly, the availability of several studies of different durations does not support the need for a 10-fold uncertainty factor to account for the use of a subchronic study (UF_S) in the derivation of the RfD. Moreover, USEPA guidance indicates that the use of an endpoint in maternal rodents in a developmental toxicity study does not necessitate the application of a UF_S. The available data indicate that the mode of action for the liver effects serving as the basis of the RfD is not relevant to humans and therefore the application of a 3-fold interspecies uncertainty factor (UF_A) to account for potential sensitivity in humans is not necessary. As such, the composite uncertainty factor for the endpoint USEPA selected as the basis of the RfD should be 30.

1 Lack of Justification for the Increased Composite and Database Uncertainty Factors

The composite uncertainty factor used in the derivation of the reference dose (RfD) for HFPO-DA increased 10-fold between the USEPA (2018) draft toxicity assessment and the USEPA (2021a) final assessment. **Table 1** compares the individual and composite uncertainty factors in the two USEPA assessments.

Table 1. Uncertainty Factors in USEPA (2018 & 2021a)

Uncertainty Factor	**2018**	**2021a**
Interspecies extrapolation (UF_A)	3	3
Human variability (UF_H)	10	10
Subchronic-to-chronic extrapolation (UF_S)	3	10
Database uncertainty (UF_D)	3	10
Composite uncertainty	300	3000

The method USEPA uses to compute the composite uncertainty factor means that the next composite uncertainty factor above 3000 is 10000.[1] USEPA guidance on the derivation of RfD values recommends that 3000 should be the maximum composite uncertainty factor applied in a chronic RfD (USEPA, 2002). The rationale for this limit is that applying more uncertainty (e.g., 10000) implies that there is too little known about the chemical to derive

[1] Possible composite uncertainty factor values are 1, 3, 10, 30, 100, 300, 1000, 3000, or 10000.

a meaningful toxicity value. Notably, ~90% of the 557 RfD values listed in the IRIS database used a composite uncertainty factor less than 3000. In selecting a 3000-fold composite uncertainty factor for HFPO-DA, USEPA (2021a) is incorrectly signaling that so little is known about HFPO-DA that it was almost not possible to derive an RfD. **Table 2** lists some of the studies that have been conducted on HFPO-DA. As shown in the table, there are numerous toxicity studies of multiple durations in multiple species. Not only are there a number of guideline toxicity studies, but there is considerable evidence that HFPO-DA and other PFAS act as PPARα activators (Chappell et al., 2020; Klaunig et al., 2012). Many of the effects of PPARα activators, especially in the liver, do not occur in humans (Corton et al., 2018). As such, some of the studies that USEPA considers deficient in the database have limited utility for human health risk assessment. For example, the absence of a chronic bioassay in a second rodent species, specifically mice, is of limited value here because it is well-accepted that the PPARα-mediated liver effects observed in shorter-term mouse studies are likely to result in liver tumors in long-term studies. Again, these effects are not relevant to humans and, as such, their absence in the database should not be considered a deficiency.

Table 2. Select Toxicity Studies on HFPO-DA

Study Type	**Reference**
OECD Guideline Studies	
28-day OECD 407 Acute Oral Toxicity Study (Rats)	DuPont-24447 (2008)
28-day OECD 407 Acute Oral Toxicity Study (Mice)	DuPont-24459 (2008)
90-day OECD 408 Subchronic Oral Toxicity Study (Rats)	DuPont-17751-1026 (2009)
90-day OECD 408 Subchronic Oral Toxicity Study (Mice)	DuPont-18405-1307 (2010)
OECD 421 Reproduction/Developmental Toxicity Study (Mice)	DuPont-18405-1037 (2010)
OECD 414 Prenatal Developmental Toxicity Study (Rats)	DuPont-18405-841 (2010)
OECD 453 Combined Chronic Toxicity/Oncogenicity 2-year Study (Rats)	DuPont-18405-1238 (2013)
Published Toxicity Studies	
Combined Chronic Toxicity/Oncogenicity 2-year Study (Rats)	Caverly-Rae et al. (2015) Toxicol Reports 2:939
28-day Immunotoxicity Study (Mice)	Rushing et al. (2017) Tox Sci 156:179
Reproductive and Developmental Toxicity Study (Rats)	Conley et al. (2019) EHP 127: 037008
Reproductive and Developmental Toxicity Study (Mice)	Blake et al. (2020) EHP 128: 027006
Reproductive and Developmental Toxicity Study (Rats)	Conley et al. (2021) Env Int 146:106204

Despite the relatively large number of studies conducted on HFPO-DA that seemingly preclude a 10-fold UF_D (see above), including both an OECD 414 prenatal developmental toxicity study and OECD 421 reproductive/developmental toxicity study), USEPA (2021a) provides the following as their justification for the 10-fold UF_D:

> "Specifically, a value of 10 was selected for the UF_D to account for the uncertainty surrounding reproductive or developmental effects of concern occurring at similar dose levels to the liver effects (maternal GWG, placental lesions indicative of placental insufficiency, changes in thyroid hormones) or effects that observed to occur with exposure to other PFAS (e.g., PFOA) but have not been studied or do not have published studies currently for GenX chemicals (skeletal ossification, changes in thyroid hormones, mammary gland development, and altered metabolism in the mouse)."

These endpoints will be addressed below; however, it should be noted that USEPA (2002) specifically states, "If the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproduction study is missing, or a factor of 10 may be applied if both are missing (Dourson et al., 1996)." As such, the lack of a full two-generation study may warrant the 3-fold UF_D in USEPA (2018), but the available database, which includes a prenatal toxicity study, does not support a 10-fold UF_D. USEPA guidance (2002) states:

> "If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment and their potential to affect the POD for the particular duration RfD or RfC under development."

USEPA (2021a) uses the above quote to attempt to justify the increased database uncertainty factor; however, many of the items articulated in the preceding quote would be addressed in a 2-generation study and therefore it is inappropriate to compile a list of supposed unexamined endpoints to justify a 10-fold UF_D.

1.1 Maternal Gestational Weight

With respect to gestational weight, exposure to HFPO-DA caused decreases in gestational weight gain in rats and increases in mice. Rather than use these observations as justification to increase the UF_D, USEPA should have instead carefully evaluated the data and, if required, developed candidate RfD values based on these effects. Analysis of maternal bodyweight gain in rats from Conley et al. (2019) indicates a $BMDL_{1SD}$ of 28 mg/kg-day and an RfD of 0.23 mg/kg (**Table 3**). This RfD is much higher than USEPA's final RfD for HFPO-DA, and as such does not support increasing the UF_D.

Acknowledging that female rats appear to have much higher clearance of HFPO-DA than other species, there might be concern for similar effects to occur in mice at lower exposure levels. However, Blake et al. (2020) reported *increases* in maternal gestational weight gain. Exposure of mice to 2 and 10 mg/kg HFPO-DA caused non-significant increases in maternal bodyweight at embryonic day 0.5 (E0.5) and 11.5. Blake et al. (2020) reported that the % change in bodyweight between these two time points was significantly higher in the 10 mg/kg group. Stated differently, HFPO-DA did not significantly alter maternal bodyweight at two timepoints (E0.5 and E11.5), but USEPA considered the slight increase in bodyweight *gain* between the two time points in treated versus non-treated mice as a

potential concern. At E11.5, the absolute difference in maternal bodyweight from the control mice and mice exposed to 2 and 10 mg/kg was 0.4 and 2.1 grams, respectively. Notably, the maternal liver weight was also significantly increased at 2 and 10 mg/kg relative to control mice, and the absolute difference in liver weight from the control mice in these two groups was 0.9 and 2.0 grams, respectively. These data suggest that the differences in maternal bodyweight and bodyweight gain are likely driven by the increases in maternal liver weight as a result of PPARα activation. When similar data were collected at E17.5, there were no differences in maternal bodyweight or maternal gestational weight gain from E0.5 to E17.5. The absolute difference in maternal liver weight from the control mice at 2 and 10 mg/kg was 0.8 and 1.9 grams, which were nearly identical to the differences at E11.5, suggesting that the liver weight changes had plateaued. Taken together, the data suggest that the early increases in maternal bodyweight and bodyweight gain were driven by the liver and that the changes were non-significant by E17.5.

In contrast to the straightforward interpretations of the maternal weight data above, Blake et al. (2020) report results of a mixed effect model suggesting that after accounting for litter weight, and embryonic day, there was a significant effect of HFPO-DA on gestational weight gain at both E11.5 and E17.5. Given this, we attempted to model the maternal bodyweight data to determine if these effects would result in a lower RfD. Importantly, Blake et al. (2020) did not report the mean and standard deviation for the absolute bodyweight gain (in grams) during gestation, but rather reported the mean and standard deviation for the % change in bodyweight. This is unusual and it is not entirely clear what difference in % change in bodyweight should be considered relevant (i.e., the benchmark response). We therefore used the default benchmark response (BMR) of 1 standard deviation for continuous endpoints for determining a POD for RfD calculation. Although HFPO-DA only significantly increased the % weight gain at day 11.5, we modeled the data at both time points since Blake et al. (2020) reported significant effects at E11.5 and E17.5 with their mixed effects model. At E11.5, the BMD_{1SD} was above the range of observation at 12.3 mg/kg with a $BMDL_{1SD}$ of 6.8 mg/kg. At E17.5, the BMD_{1SD} was above the range of observation at 19.1 mg/kg with a $BMDL_{1SD}$ of 8.6 mg/kg. Candidate RfD values for these endpoints are shown in **Table 3**. For this exercise, the 10-fold UF_D was retained so that we could make relevant comparisons. However, the UF_S was reduced from 10 to 1 based on guidance that maternal effects are inherently short-term effects occurring in a specific window of sensitivity (USEPA, 2002; USEPA, 1991). This highlights the importance of not simply comparing PODs, but rather comparing candidate RfD values because the composite uncertainty factor is not the same for all endpoints. Both RfD values are higher than USEPA's RfD based on liver lesions in mice; as such, there is no need to increase the UF_D based on concerns for HFPO-DA affecting maternal gestational weight gain. Moreover, the increase in gestational weight is likely due to PPARα mediated responses in the liver that have little/no human relevance.

In conclusion, the effects of HFPO-DA on maternal gestational weight in both mice and rats result in substantially higher PODs and therefore higher RfD values than USEPA's RfD of 0.000003 mg/kg. It is therefore inappropriate to cite concerns for effects on maternal gestational weight gain to support increasing the UF_D to 10.

Table 3. Draft Candidate RfD Values

Endpoint	$BMDL_{1SD}$ (LOAEL) (mg/kg)	HED (mg/kg)	Uncertainty Factors	RfD (mg/kg)
Mouse, female, liver*	**0.09**	**0.01**	**3000** (UF_A=3, UF_H=10, UF_S=10, UF_D=10)	**0.000003**
Rat, decreased maternal bodyweight (Conley et al. 2019)	28	7	300 (UF_A=3, UF_H=10, UF_S=1, UF_D=10)	0.023
Mouse, increased maternal gestation weight gain E11.5 (Blake et al. 2020)	6.8	0.97	300 (UF_A=3, UF_H=10, UF_S=1, UF_D=10)	0.003
Mouse, increased maternal gestation weight gain E17.5 (Blake et al. 2020)	8.6	0.0041	300 (UF_A=3, UF_H=10, UF_S=1, UF_D=10)	0.004
Mouse, abnormal placentas at E17.5 (Blake et al. 2020)	(2)	0.29	3000 (UF_A=3, UF_H=10, UFL = 10, UF_S=1, UF_D=10)	0.0001

*RfD in USEPA (2021a); note: a 10-fold UF_D was retained for comparison purposes only

1.2 Placental Insufficiency

USEPA (2021a) also cites Blake et al. (2020) for concerns of placental insufficiency. Although USEPA acknowledges that Blake et al. (2020) reported no effects on the number of implantation sites, viable embryos, non-viable embryos, or resorptions, we nevertheless attempted to model the number of "abnormal placentas" in Supplemental Table S10 from Blake et al. (2020). No acceptable model fits were achieved, likely due to the sharp increase in incidence between control (1/41) and 2 mg/kg (18/31) groups. If we consider the 2 mg/kg a LOAEL, a candidate RfD of 0.0001 mg/kg is derived (**Table 3**). This candidate RfD is considerably higher than USEPA's RfD and therefore there is no scientific justification to increase the UF_D based on concerns for HFPO-DA affecting the placenta at lower doses. Any concerns for effects in a multigeneration study are already accounted for with a 3-fold UF_D.

It was also informative to investigate whether placental effects are a common basis for RfD values in the IRIS database. A search of the IRIS database selecting for chemicals with noncancer RfD values based on toxicities in the reproductive or developmental organ system resulted in 60 records. Broadening the search by unchecking boxes "noncancer", "oral", and "RfD" resulted in 88 records. Both datasets were exported as csv files and the "Critical Effect" column was searched for the term "placent" for placenta or placental. No records indicated critical effects based on placental toxicity. This result suggests that no oral RfD values have been developed based on placental lesions in the IRIS database. Considering (i) that placental lesions have not served as the basis of any RfD, (ii) that Blake et al. (2020) reported no effects on implantations and embryo viability following exposure to HFPO-DA, and (iii) the fact that an RfD based on placental lesions in Blake et al. (2020)

would result in a higher RfD than USEPA selected, USEPA's concern for placental effects does not warrant an increase in the UF_D from 3 to 10.

1.3 Thyroid Hormone Changes

It is well established that changes in thyroid hormones can result from changes in the expression of enzymes in the liver that regulate thyroid hormone homeostasis. Changes in serum thyroid levels generally occurred concurrently at doses that significantly increased liver weight (Conley et al., 2019). Given that USEPA (2021a) identified the liver as the most sensitive organ, it is reasonable to infer that hormone changes in rodents are a consequence of PPARα mediated liver changes that have no human relevance. As such, concern for such effects in mice does not justify an increase in the UF_D.

1.4 Effects Observed with Other PFAS

Although USEPA (2002) guidance allows for consideration of other chemicals within a class for informing the UF_D, USEPA (2021a) relies heavily on PFOA and PFOS, which are longer chain PFAS and thus their study databases and toxicity profiles may not be directly relevant for HFPO-DA. Concerns for immunotoxicity expressed in USEPA (2021a) are thus overstated. An immunotoxicity study by Rushing et al. (2017) states,

> "Our study is the first to report on the potential immunotoxicity of oral 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate [HFPO-DA] exposure in C57Bl/6 mice. Unlike PFOA, the test compound did not potently suppress the TDAR, even at doses that would induce high mortality in mice given PFOA."

The Rushing et al. (2017) study was conducted in mice and was assessed but not carried forward for RfD consideration by USEPA (2018, 2021a). As such, concern for immunotoxicity in mice does not justify an increase in the UF_D from 3 to 10. USEPA (2021a) also alludes to a lack of data for reduced antibodies. This may be in reference to endpoints being considered by USEPA for PFOA and PFOS; however, the veracity of these endpoints for PFOA and PFOS remain to be determined, and should not impact the UF_D for HFPO-DA.

In summary, rationale for the 10-fold UF_D provided in the USEPA (2021a) assessment is not compelling and the stated concerns do not justify the increase in the UF_D from 3-fold to 10-fold between the 2018 and 2021 assessments. There are no new studies justifying an increase in the UF_D. In contrast, there is stronger support for the effects in rodents being mediated by PPARα and therefore mitigating concerns for humans.

Finally, two recent PFAS toxicity assessments released by USEPA IRIS (for PFBA and PFHxA) had UF_D values of 3 (USEPA, 2021b; USEPA, 2022). Unlike the USEPA (2021a) assessment for HFPO-DA, these two recent IRIS assessments explicitly described the basis for the UF_D within a table format (excepted in **Table 4** below). The database deficiencies for PFHxA are similar to HFPO-DA in that PFHxA only has a single chronic bioassay in rats and lacks a multigenerational study, and the UF_D for PFHxA is 3. **Table 5** compares the reproductive/developmental toxicity studies available for HFPO-DA, PFHxA, and

PFBA. Overall, HFPO-DA has a more robust reproductive and developmental toxicity database and yet has a UF_D of 10, whereas PFHxA and PFBA each have a 3-fold UF_D.

Table 4. UF_D Justifications in IRIS Assessments of PFHxA & PFBA*

PFHxA (USEPA, 2022 p.5-21)
A UF_D of 3 is applied because the evidence base for hepatic, hematopoietic, and developmental endpoints included **two subchronic studies and one chronic study in Sprague-Dawley rats** and **developmental/reproductive studies in Sprague-Dawley rats and Crl:CD1 mice**. Limitations, as described in U.S. EPA (2002c) were used as the basis for a $UF_D = 3$. These *limitations* included a lack of informative human studies for most outcomes, subchronic or **chronic toxicity studies in more than one species**, or a **multigenerational study**. For developmental outcomes, pups were indirectly exposed via the dam (i.e., via placental or lactational transfer); thus, the dose received by the pups is unclear and might be significantly less than that administered to the dams.
PFBA (USEPA, 2021b p. 5-13)
A UF_D of 3 is applied because, although the PFBA database is relatively small, high confidence **subchronic and developmental toxicity studies are available in mice and rats**. Although these high confidence studies are available for PFBA, the database has some deficiencies, including the **lack of information on developmental neurotoxicity and other endpoints**; see the text below for further discussion.

* **emphasis added**

Table 5. Reproductive/Developmental Toxicity Databases for HFPO-DA, PFHxA, & PFBA

Study Type	**Reference**
HFPO-DA	
OECD 421 Reproduction/Developmental Toxicity Study (Mice)	DuPont-18405-1037 (2010)
OECD 414 Prenatal Developmental Toxicity Study (Rats)	DuPont-18405-841 (2010)
Reproductive and Developmental Toxicity Study (Rats)	Conley et al. (2019) EHP 127: 037008
Reproductive and Developmental Toxicity Study (Mice)	Blake et al. (2020) EHP 128: 027006
Reproductive and Developmental Toxicity Study (Rats)	Conley et al. (2021) Env Int 146:106204
PFHxA	
Reproductive and Developmental Toxicity Study (Mice)	Iwai & Hoberman (2014) Int. J. Toxicol 33:219
Reproductive and Developmental Toxicity Study (Rats)	Lovelace et al. (2009) Toxicol 265:32
*PFBA**	
Reproductive and Developmental Toxicity Study (Mice)	Das et al. (2008) Tox Sci 105:173

***** USEPA assessment indicates that two high quality studies evaluated reproductive organ weights in rats; however, these do not appear to be reproductive/developmental toxicity studies

1.5 Misinterpretation of HFPO-DA Pharmacokinetic Data

In Section 7.3 of the USEPA (2021) toxicity assessment, USEPA mentions potential bioaccumulation of HFPO-DA in the embryo stating the following:

> "Blake et al. (2020) demonstrated accumulation of HFPO dimer acid in whole mouse embryos from E1.5 to E11.5 to E1.5 to E17.5. The lack of studies evaluating these endpoints at or below doses included in the critical study identifies this as a significant gap in the understanding of the developmental toxicity of GenX chemicals." [end of paragraph]

The so-called bioaccumulation reported in Blake et al. (2020) and accepted by USEPA is a gross overinterpretation of the findings. **Table 6** below recapitulates HFPO-DA levels reported in Blake et al. (2020). Notably, PFAS including HFPO-DA are found primarily in the serum and liver. **Table 6** shows that dosing of pregnant mice with either 2 or 10 mg/kg/d HFPO-DA from E1.5 to E11.5 results in *higher* liver and serum HFPO-DA concentrations than does dosing from E1.5 to E17.5. Thus, there is no evidence of bioaccumulation in maternal liver or serum after 16 days of dosing.

Table 6. HFPO-DA levels from Blake et al. (2020)

Measurement	Embryonic day	2 mg/kg/day	10 mg/kg/day
Amniotic fluid (µg/mL)	11.5	3.6 ± 2.2	9.3 ± 2.0
	17.5	NQ	NQ
Maternal liver (µg/g)	11.5	5.45 ± 3.43	19.9 ± 4.2
	17.5	4.56 ± 2.80	14.2 ± 7.6
Maternal serum (µg/ml)	11.5	33.5 ± 15.7	118.1 ± 10.4
	17.5	22.9 ± 17.1	58.5 ± 34.5
Whole Embryo (µg/g)	11.5	0.91 ± 0.22	3.21 ± 0.51
	17.5	3.23 ± 1.28	7.69 ± 2.92
Maternal serum to embryo/fetus ratio (not calculated in Blake et al.)	11.5	36.8	36.8
	17.5	7.1	7.6

It is highly likely that the decreases in maternal liver and serum HFPO-DA and the increases in whole embryo/fetus HFPO-DA between E11.5 and E17.5 represent a relative change in partitioning of HFPO-DA to the maternal and embryo/fetal compartments, due to a change in body composition of the embryo/fetus over that time. The ratio of maternal serum to embryo HFPO-DA on E11.5 is 36.8 at both dose levels (2 and 10 mg/kg), while the ratios are lower but quite similar at E17.5 (7.1 and 7.6 for 2 and 10 mg/kg, respectively). This change from 36.8 to ~7 is indicative of redistribution of HPFO-DA from the mother to the litter during late gestation. The E11.5 mouse embryo is a much different organism than the E17.5 fetus (see **Figure 1**, E10.5 – 12.5 vs E16.5). For example, the liver of the E11.5 mouse embryo is just beginning to grow at E11.5, while it is much larger and mature by E17.5 (**Figure 1**, E14.5 – E16.5). Hepatocyte differentiation does not begin until around E15 in the mouse. Given that HFPO-DA partitions to liver (as observed in the maternal

liver, see **Table 6**), the increasing percentage of the liver to the body weight of the mouse embryo/fetus over E11.5-17.5 likely underlies the increasing concentration of HFPO-DA measured in the in the embryo and fetus over that period. This partitioning is based on the changes in the tissue composition of the developing fetus, not bioaccumulation.

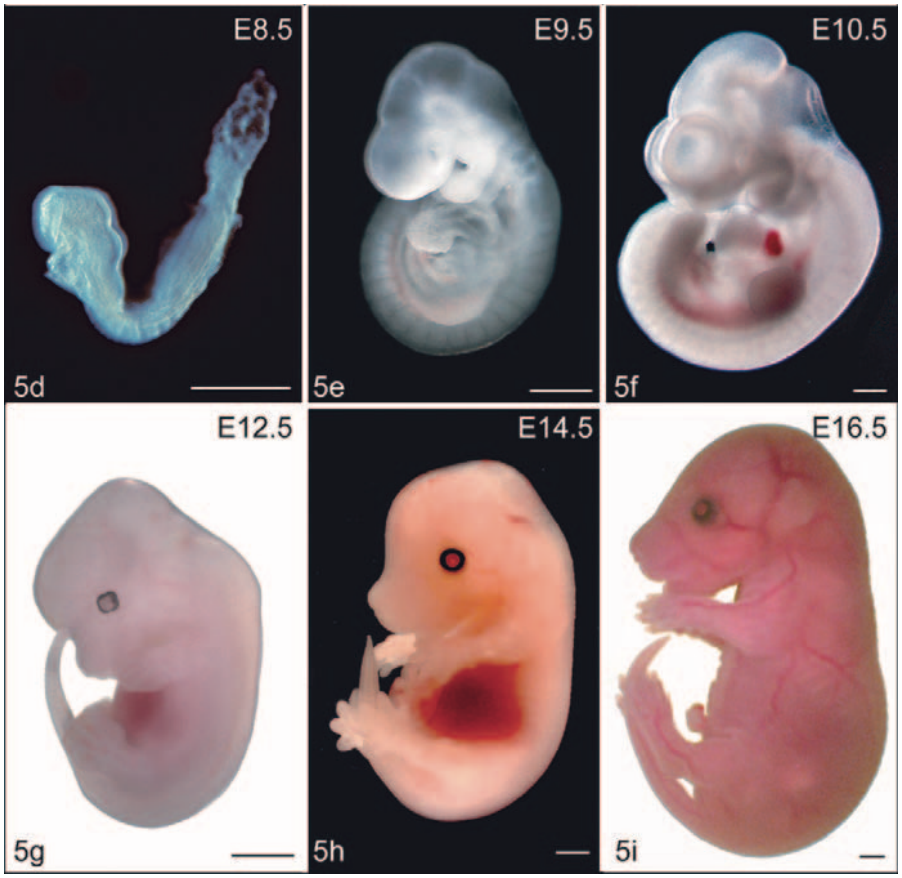

Figure 1. Development of the mouse embryo/fetus from E8.5 – E16.5.
Source: Papaioannou and Behringer (2012).

Another contributor to the higher HFPO-DA levels in the E17.5 fetus compared to the E11.5 embryo is the developmental stage of the placenta. Blake et al. (2020) state that they chose to examine E11.5 embryos "because it overlaps a critical period of placental development in the mouse where the placenta undergoes vascularization with the uterine wall and chorioallantoic branching of vessels begins". The immature vascularization of the placenta at E11.5 means that there is less maternal blood flow, the source of HFPO-DA, to the placenta and fetus on E11.5 versus E17.5 when the placenta is fully formed and vascularized.

Given the above, it is not appropriate to interpret an increase in embryo/fetal HFPO-DA over the developmental period of E11.5 – E17.5 as bioaccumulation. The difference in body composition between a E11.5 mouse embryo and a E17.5 mouse fetus are substantial and preclude evaluation of "bioaccumulation" over time of a chemical in a tissue. As such,

the so-called bioaccumulation in Blake et al. (2020) is likely a misinterpretation of normal changes that would be expected during xenobiotic exposure and should not be used to support USEPA's increase in the database uncertainty factor UF_D.

2 Lack of Justification for the Increased Subchronic-to-Chronic Uncertainty Factor

In the USEPA draft assessment (2018), single cell necrosis (SCN) was considered the most sensitive effect and it was observed in the mouse 28-day, 90-day, and reproductive/developmental toxicity studies. USEPA (2018) applied a 3-fold UF_S, arguing that the 0.1 mg/kg NOAEL for SCN in male mice of the reproductive/developmental toxicity study was within an order of magnitude of the 1 mg/kg NOAEL for liver effects in the chronic *rat* bioassay. USEPA (2018) further noted that mice were more sensitive to HFPO-DA and therefore the UF_S was warranted. Because SCN in mice was considered the critical effect, USEPA could have analyzed data on SCN in studies of different duration to inform the need or magnitude of a UF_S. **Table 7** contains the NOAEL and $BMDL_{10}$ values for SCN in several mouse studies. These values show no clear indication of a progression in sensitivity (e.g., reduction in NOAEL or $BMDL_{10}$ values) in male mice from the 28 to 90 days of exposure, where the NOAEL values ranged from 0.1 to 0.5 mg/kg and the $BMDL_{10}$ values ranged more narrowly from 0.2-0.3 mg/kg. In female mice, the NOAEL values for 28 and 90 days of exposure were 3 and 5 mg/kg, respectively. The female mice from the reproductive study (exposed for 60 days) were not included in this analysis as these mice were recently pregnant and nursing and therefore represent a different population from non-pregnant mice in the 28-day and 90-day studies. Overall, **Table 7** provides no clear evidence for an increase in sensitivity of SCN in either male or female mice with increased exposure duration.

Table 7. Comparison of NOAEL and $BMDL_{10}$ values for SCN Across Study Duration

Study	Sex	Doses (mg/kg)	NOAEL (mg/kg-day)	$BMDL_{10}$ (mg/kg-day)
28-day	Male	0.1, 3, 30	0.1	0.3
90-day		0.1, 0.5, 5	0.5	NA
Repro/dev (~90 days)		0.1, 0.5, 5	0.1	0.2
28-day	Female	0.1, 3, 30	3	NA
90-day		0.1, 0.5, 5	5	NA

* NA = no model fits

In the USEPA (2021a) final assessment, liver lesions were still used as the critical effect, albeit a "constellation of lesions" was used instead of SCN. Concerns related to the "constellation of lesions" are discussed in a separate expert report submitted as part of the Request for Correction. Importantly, the "constellation of lesions" is related to the SCN endpoint that showed no clear evidence of progression over time (see above). As such, there is no basis to increase the UF_S to 10-fold. USEPA (2021a) argues that the 54-64 day exposures of female mice in the reproductive/developmental toxicity study are "well below the 90-day exposure window typically employed in a subchronic study." It is difficult to

assess the progression of the "constellation of lesions" endpoint over time because the USEPA did not ask the NTP PWG to evaluate liver sections from the 28-day mouse study, despite the fact that USEPA (2018) developed a candidate RfD value based on SCN in the 28-day study. Therefore, to investigate whether the liver changes increased over time, we compared the $BMDL_{10}$ values for the incidence of "constellation of lesions" for various datasets in Appendix D of USEPA (2021a). Notably, USEPA (2021a) considered the ~60 day exposure to be "well below" the ~90 day exposure and considered this as justification, in part, for increasing the UF_S from 3-fold to 10-fold. **Table 8** lists the $BMDL_{10}$ values reported in USEPA (2021a) as well as our $BMDL_{10}$ for females in the 90-day study (not modeled in USEPA (2021a)). As stated above, it may be inappropriate to compare female mice in the reproductive study to non-pregnant mice; nevertheless, these data do not indicate a significant progression of adversity for this endpoint as the $BMDL_{10}$ values ranged narrowly between 0.09 to 0.2 mg/kg.

Table 8. Comparison of $BMDL_{10}$ Values for Constellation of Lesions Across Study Duration

Study	**Sex**	**Doses (mg/kg)**	**$BMDL_{10}$ (mg/kg-day)**	**Notes**
28-day	Male	0.1, 3, 30	--	Not assessed by NTP PWG
90-day		0.1, 0.5, 5	--	Same duration as repro/dev study
Repro/dev (~90 days)		0.1, 0.5, 5	0.14	Derived by USEPA
28-day	Female	0.1, 3, 30	--	Not assessed by NTP PWG
Repro/dev (~60 days)		0.1, 0.5, 5	0.09	Derived by USEPA
90-day		0.1, 0.5, 5	0.2*	Derived by ToxStrategies

* our own modeling; -- = not modeled

USEPA (2021a) also cites evidence that rats exposed to HFPO-DA for up to one year did not exhibit liver lesions, whereas lesions were observed at two years. These observations in rats were known at the time of the 2018 draft assessment, so these observations cannot justify an increase in the UF_S to 10-fold in the final assessment.

USEPA (2021a) further states, "Additionally, Blake et al. (2020) did not find clear evidence of changes in maternal liver serum enzymes (i.e., ALP, ALT or AST) or increases in liver necrosis as compared to control after 10-16 days of dosing at 2 mg/kg/day." Here, USEPA appears to be arguing that the absence of effects in a *subacute* study (i.e., 10-16 days) and their presence in subchronic studies is evidence of progression supporting the Agency's UF_S. However, USEPA (2002) guidance states that "No chronic reference value is derived if neither a subchronic nor chronic study is available. The application of a UF to less-than-subchronic studies is not part of the current practice…" This USEPA guidance indicates that the absence of lesions at 10-16 days should not play a role in the determination of the UF_S.

In summary, there is no strong indication of a progression of liver lesions with longer exposure duration, and the purported justifications for the 10-fold UF_S provided in the

USEPA final assessment (2021a) are not compelling and do not support the increase in the UF_S from 3-fold to 10-fold between the 2018 draft and 2021 final assessments.

3 The Liver Lesions in Maternal Mice from the Reproductive/Developmental Toxicity Study Do Not Require a UF_S

Between the USEPA draft (2018) and USEPA final (2021a) assessments, USEPA changed the basis of the RfD from liver lesions in male mice to liver lesions in female mice in the DuPont reproductive/developmental toxicity study. USEPA's 1991 Guidelines for developmental toxicity risk assessment states (**emphasis added**):

> "Uncertainty factors (UFs) for developmental and **maternal toxicity** applied to the NOAEL generally include a 10-fold factor for interspecies variation and a 10-fold factor for intraspecies variation. **In general, an uncertainty factor is not applied to account for duration of exposure.**"

This USEPA guideline indicates that the liver lesions in maternal mice used as the basis for the RfD do not require a UF_S. Therefore, the UF_S applied in the USEPA final assessment (2021a) should have been 1 instead of 10.

4 The Liver Lesions in Mice from the Reproductive/Developmental Toxicity Study Do Not Require a UF_A

Data strongly indicate that the liver lesions in male mice (USEPA, 2018) and female mice (USEPA, 2021a) are the result of a PPARα MOA (see accompanying expert report submitted as part of the Request for Correction). Because such lesions have no human relevance, they should not be the basis of the RfD. However, if under an abundance of extreme caution, the USEPA chose to use these lesions as the basis of the RfD, then after making interspecies pharmacokinetic adjustment to the dose (via allometric scaling), the remaining 3-fold interspecies uncertainty factor (UF_A) that accounts for additional uncertainty—primarily related to pharmacodynamics—should have been set to one because there is no reason to believe that humans are more susceptible to PPARα activators like HFPO-DA than rodents (see accompanying expert report).

5 The Appropriate Composite Uncertainty Factor for Liver Lesions Serving as the Basis of the USEPA (2021a) RfD is 30 Instead of 3000

Based on strong evidence for involvement of a PPARα MOA, we do not believe that the liver lesions in mice should serve as the basis of an RfD for HFPO-DA. However, if an RfD were to be based on liver lesions in female mice from the reproductive/developmental toxicity study, then, based on all of the reasons set forth in sections 1-4 (above), the

appropriate composite UF for the endpoint USEPA (2021a) selected should be 30 (**Table 9**).

Table 9. Appropriate Uncertainty Factors for USEPA (2021a)

Uncertainty Factor	2021	Rationale
Interspecies extrapolation (UF_A)	1	Allometric scaling accounts for interspecies differences in pharmacokinetics; Data support involvement of PPARα for liver lesions, for which rodents are more sensitive than humans
Human variability (UF_H)	10	
Subchronic-to-chronic extrapolation (UF_S)	1	Use of maternal effects in the reproductive and developmental toxicity study
Database uncertainty (UF_D)	3	Lack of 2-gen study, but availability of numerous other studies
Composite uncertainty	30	

6 Conclusion

Based on the consideration above, there is no justification for the 10-fold increase in composite uncertainty factor between the 2018 and 2021 USEPA risk assessments of HFPO-DA. Relatedly, there is no justification for a 3000-fold composite uncertainty factor. The database for HFPO-DA studies is as robust or more robust than other recent risk assessments with a UF_D of 3 and composite uncertainty factor of 300. The liver lesions in mice are the result of a mode of action that is not relevant to humans and therefore should not serve as the basis on the RfD. However, if the lesions observed in female mice exposed to HFPO-DA during pregnancy were to serve as the basis of the RfD, the appropriate composite uncertainty factor would be 30.

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EXHIBIT 4

Inappropriate Use of the Database Uncertainty Factor in the US EPA Human Health Toxicity Values for "GenX Chemicals"

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The EPA published the final human health toxicity assessment for hexafluoropropylene oxide (HFPO) dimer acid and its ammonium salt (also known as "GenX" or herein "HFPO-DA") that includes hazard and dose response assessments.[1] These assessments led EPA to develop chronic and subchronic oral reference doses (RfDs). I previously commented on the draft RfD values that EPA issued in 2018 expressing my concern about two critical errors EPA made in that draft assessment and I provided EPA with a report that discussed this, and other information related to deriving an RfD and a drinking water health advisory limit for HFPO-DA.[2,3] The EPA subsequently revised the draft toxicity assessment and released the final version in 2021. EPA's final toxicity assessment dramatically lowered the point of departure (POD) for a human equivalent dose (HED) and the RfD values. In this brief comment on the final toxicity assessment for HFPO-DA, I will confine my discussion to one of the changes that EPA made – increasing the database uncertainty factor (UF_D) that EPA applied to the POD (HED) to arrive at the final RfD values. As I demonstrate below, the EPA considered and judged new information on the toxicity of HFPO-DA to justify raising the uncertainty factor, when in fact, this new information greatly *reduced* uncertainty regarding HFPO-DA toxicity.

In the draft toxicity assessment, EPA selected a database uncertainty factor value of 3. In the response to public comments, the EPA increased the UF_D to 10 and based their decision on three newer studies that became available after the draft toxicity assessment was issued.[4] EPA stated

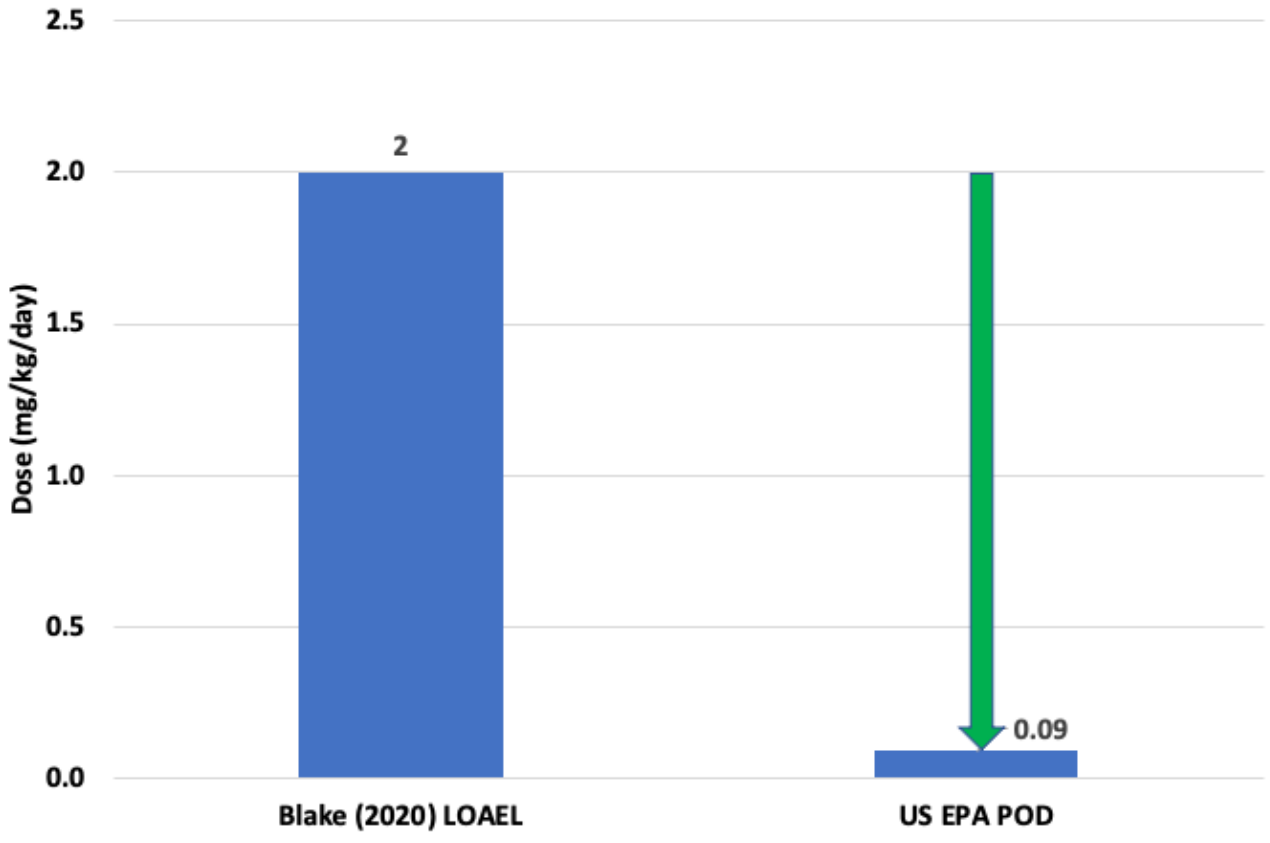
> *"As stated above, a number of commenters pointed out the deficiency of the GenX chemical database pertaining to human, immunotoxicity, and reproductive and developmental data. Recently published toxicokinetic and toxicological findings after Gen X chemicals exposure of Blake et al. (2020) and Conley et al. (2019, 2021) heighten concerns regarding the impact of GenX chemicals exposure on reproduction, development, and neurotoxicity. To address the information provided by the commenters and in recently published studies, EPA has increased the UFD from 3 to 10 in the final assessment. These points that justify the selection of a UFD of 10 are summarized in brief in this response (above) as well as in section 7.3 of the assessment (EPA, 2021a)."*

[1] EPA (U.S. Environmental Protection Agency). 2021a. *Final Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known As GenX Chemicals*. EPA 822R21010. EPA, Office of Water, Health and Ecological Criteria Division, Washington, DC.
[2] Comment on the US EPA GenX Toxicity Assessment by Damian Shea, PhD. Submitted to the US EPA 01/22/2019.
[3] Shea D. 2019. Proposed Drinking Water Health Advisory Value for GenX: 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid.
[4] EPA Response to Public Comments on Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals" (Docket ID No. EPA-HQ-OW-2018-0614) p24.

As discussed below, the studies EPA uses to support increasing the value of the database uncertainty factor do not justify such an increase. Although I strongly disagree with how EPA derived the POD, I will use the most conservative value (0.09 mg/kg/day) to compare to the no-observed-adverse-effect-level (NOAEL) and the lowest-observed-adverse-effect-level (LOAEL) of the newer studies that EPA uses to justify changing the uncertainty factor from 3 to 10. The comparison of the BMDL-derived POD to the LOAEL (or NOAEL) is to illustrate the margin between the dose where effects are actually observed (or not observed) and the EPA POD, to assess the impact of the new data on uncertainty.

Blake et al. (2020) evaluated maternal, embryo, and placental effects in mice following exposure to PFOA and HFPO-DA.[5] Two doses of HFPO-DA were used, 10 mg/kg/day and 2 mg/kg/day. Nearly every measurement made in this study found no statistical difference between the lowest dose of HFPO-DA (2mg/kg/day) and the control. The effects noted at 2 mg/kg/day were maternal liver weight gain, lipid composition and placental abnormalities and these were not consistently observed across time points. The liver and lipid effects were noted before and, in this Blake (2020) study the effect was observed at a dose 22 times above the POD the EPA is using in its final toxicity assessment (Figure 1).

Figure 1. Blake (2020) Found Effects at a Dose 22-fold Higher than the Point of Departure Used by US EPA

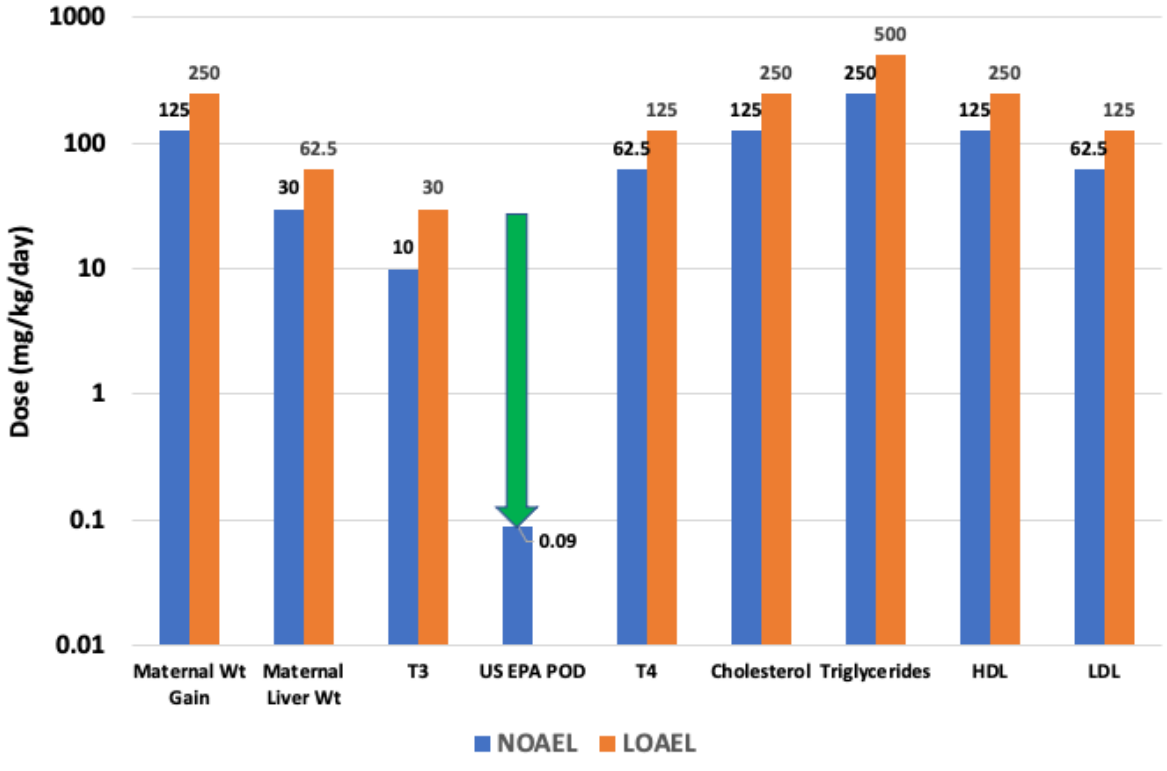


[5] Blake, B.E., H.A. Cope, S.M. Hall, R.D. Keys, B.W. Mahler, J. McCord, B. Scott, H.M. Stapleton, M.J. Strynar, S.A. Elmore, and S.E. Fenton. 2020. Evaluation of maternal, embryo, and placental effects in CD-1 Mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). Environmental Health Perspectives 128(2):027006. doi:10.1289/EHP6233.

Thus, the EPA is using a study that finds effects that mostly were previously identified – at a dose 22 times higher than the POD – to suggest there is now an increase in uncertainty due to this new information. To the contrary, the Blake (2020) study for the most part simply confirms effects we already knew could happen at a high dose. And it further confirms that the current EPA POD is protective of these observed effects with a value 22 times below the LOAEL in the Blake study. Thus, the Blake et al. (2020) study has *reduced uncertainty* in deriving a POD for HFPO-DA.

Conley et al. (2019) assessed maternal, fetal, and postnatal effects of HFPO-DA in rats.[6] Eight different effects were measured with the lowest LOAEL being 333-fold higher than the EPA POD (Figure 2). And the lowest measured NOAEL, where no effects are observed, is 111-fold higher than the POD. The observed effects have been noted before at similarly high doses. Thus, the EPA is using a study that finds effects previously identified – at a dose 333 times higher than the POD – to suggest there is now an increase in uncertainty due to this new information. As with the Blake et al. (2020) study, to the contrary, this Conley et al. (2019) study confirms effects we already knew could happen at a high dose. And it further confirms that the current EPA POD is fully protective of these observed effects with a value 333 times below the lowest LOAEL and 111 times below the lowest NOAEL. Thus, the Conley et al. (2019) study has further *reduced uncertainty* in deriving a POD for HFPO-DA.

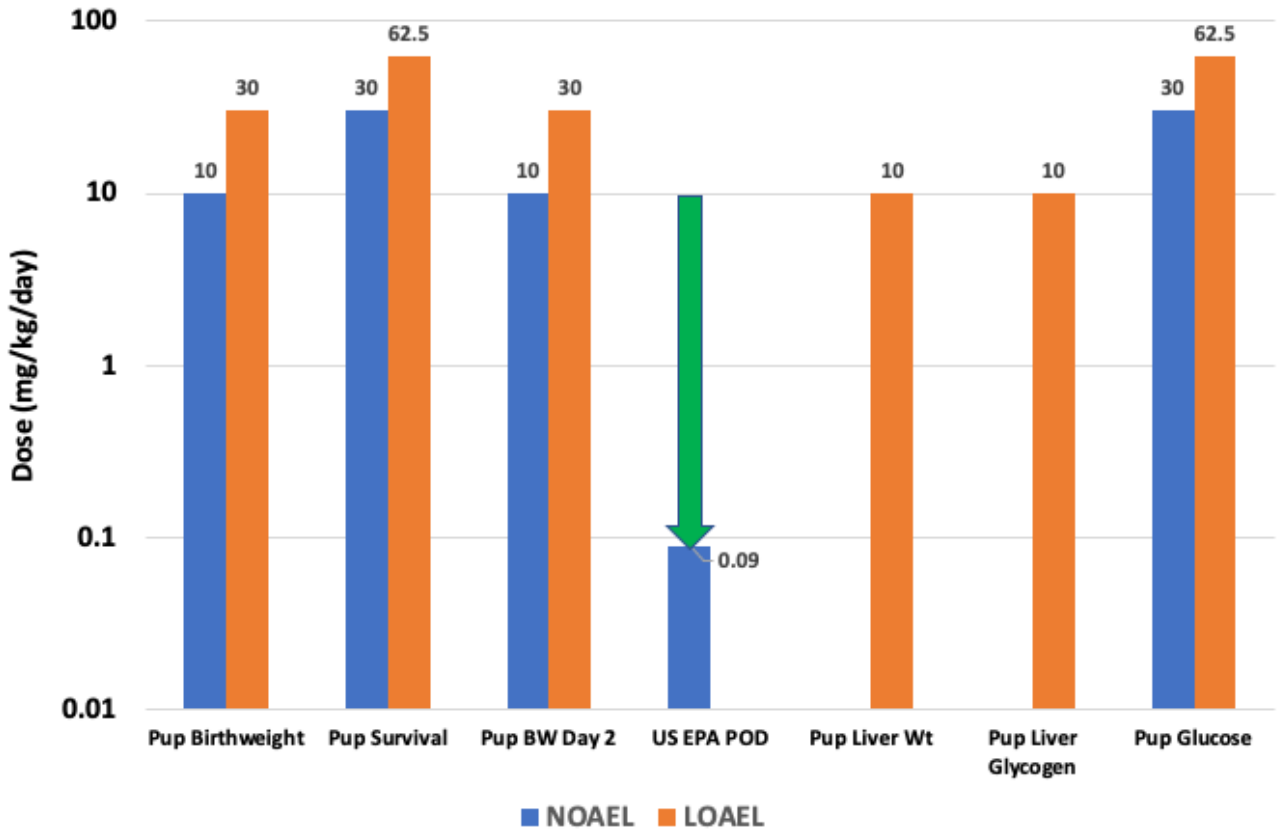
Figure 2. Conley (2019) Did Not Find Any Effects Until They Reached a Dose 333-fold Higher than the Point of Departure Used by US EPA



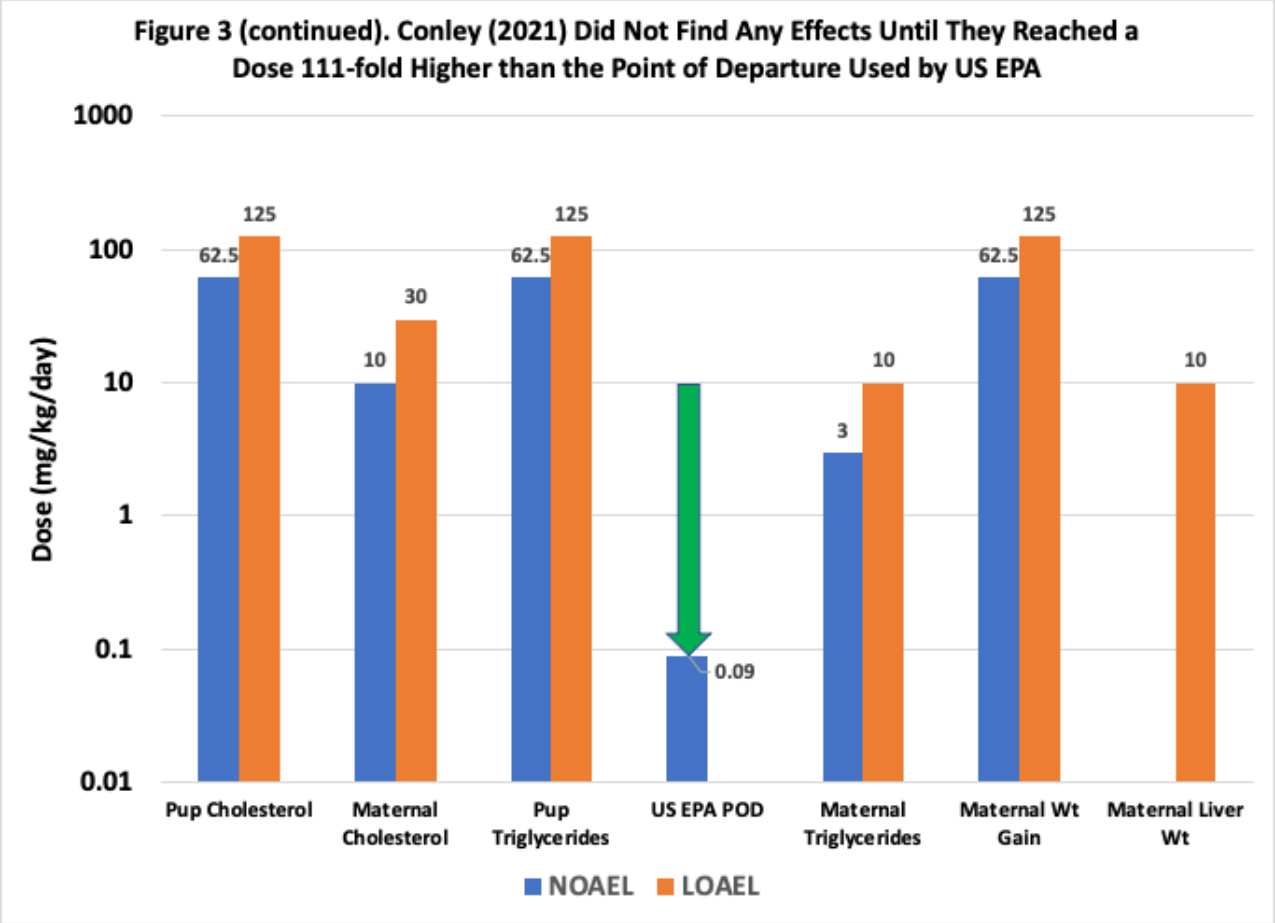
[6] Conley, J.M., C.S. Lambright, N. Evans, M.J. Strynar, J. McCord, B.S. McIntyre, G.S. Travlos, M.C. Cardon, E. Medlock-Kakaley, P.C. Hartig, V.S. Wilson, and L.E. Gray, Jr. 2019. Adverse maternal, fetal, and postnatal effects of hexafluoropropylene oxide dimer acid (GenX) from oral gestational exposure in Sprague-Dawley rats. Environmental Health Perspectives 127(3):037008. doi:10.1289/EHP4372.

Conley et al. (2021) exposed rats to HFPO-DA and assessed maternal and fetal glucose and lipids, among other measures.[7] Twelve different effects were measured with the lowest LOAEL being 111-fold higher than the EPA critical effect POD (Figure 3). And the lowest measured NOAEL, where no effects are observed, is 33-fold higher than the POD. Once again, similar observed effects have been noted before at similarly high doses. Thus, the EPA is using a study that finds effects at a dose 111 times higher than the critical effect POD to suggest there is now an increase in uncertainty due to this new information. As with the other studies noted above, this Conley et al. (2021) study confirms effects we already knew could happen at a high dose. And it further confirms that the current EPA POD is fully protective of these observed effects with a value 111 times below the lowest LOAEL and 33 times below the lowest NOAEL. Thus, the Conley et al. (2021) study has *reduced uncertainty* in deriving a POD for HFPO-DA.

Figure 3. Conley (2021) Did Not Find Any Effects Until They Reached a Dose 111-fold Higher than the Point of Departure Used by US EPA

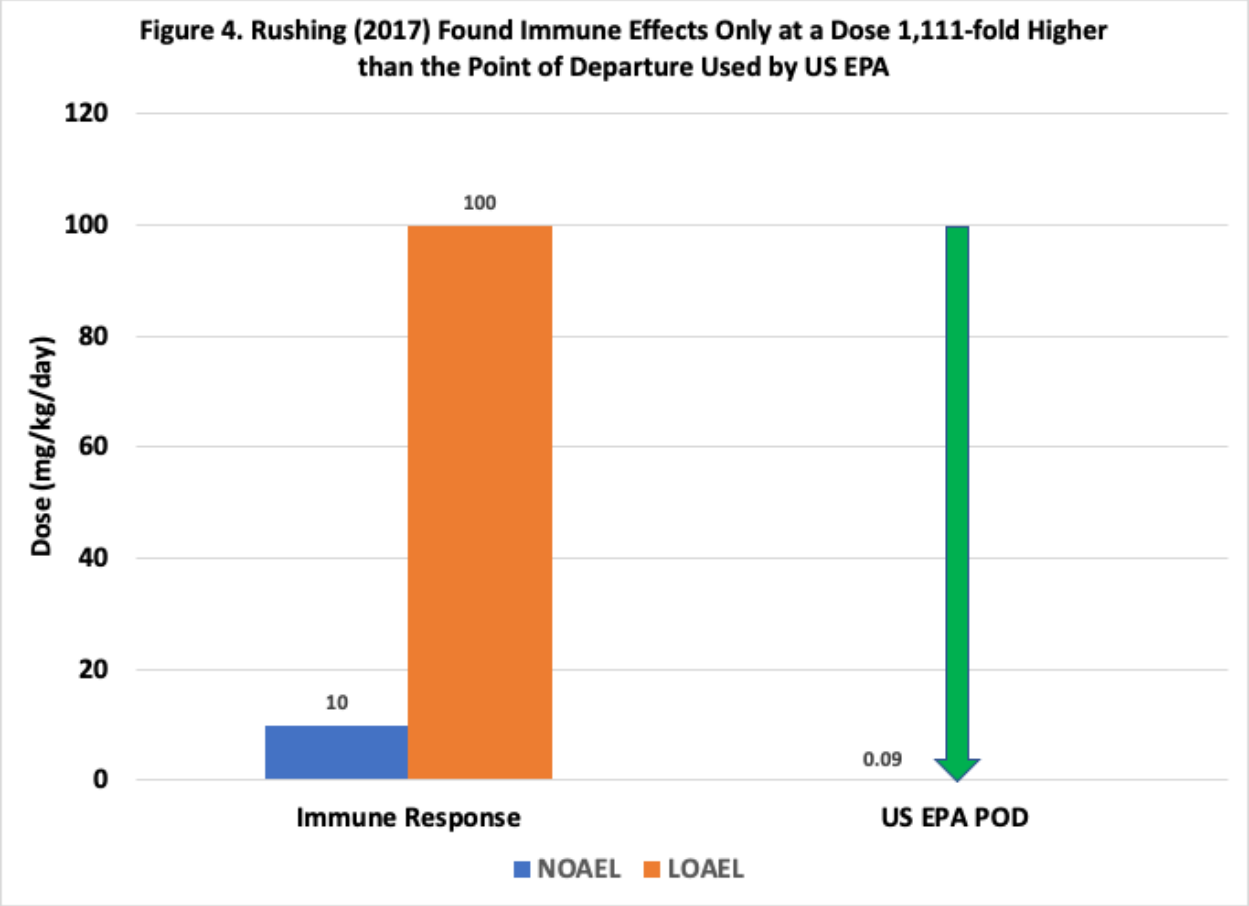


[7] Conley JM, Lambright CS, Evans N, McCord J, Strynar MJ, Hill D, Medlock-Kakaley E, Wilson VS, Gray LE Jr. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. Environ Int. 2021 Jan: 146:106204. doi: 10.1016/j.envint.2020.106204. PMID: 33126064.



In addition to the above three studies, EPA refers to a study by Rushing et al. (2017) as evidence for possible immunotoxicity of HFPO-DA and more uncertainty.[8] Once again, the results of the study clearly demonstrate that effects are only seen at a very high dose, in this case 100 mg/kg/day or 1,111-fold higher than the critical effect POD used by EPA (Figure 4). And the lowest measured NOAEL, where no effects are observed, is 111-fold higher than the POD. The Rushing et al. (2017) study confirms that the current EPA POD is fully protective of this observed effect with a value 1,111 times below the lowest LOAEL and 111 times below the lowest NOAEL. The Rushing et al. (2017) study further *reduces uncertainty* in deriving a POD for HFPO-DA by demonstrating that immunotoxicity effects are only observed at doses over 1000 times higher than the EPA POD for the critical effect.

[8] Rushing, B., Q. Hu, J. Franklin, R. McMahen, S. Dagnio, C. Higgins, M. Strynar, and J. DeWitt. 2017. Evaluation of the immunomodulatory effects of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in C57BL/6 mice. Toxicological Sciences 156(1):179–189. doi:10.1093/toxsci/kfw251.



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The fact that EPA chose these four studies to support increasing the value of the database uncertainty factor is not scientifically defensible. It is not surprising that previously identified effects would be found at doses far above the POD for the critical effect. And it is not surprising that a new effect might be found at high doses either – as in the case of immunotoxicity indicators at 100 mg/kg/day. These findings do not suggest increased uncertainty; they do the opposite. These findings tell us that all the new information since the draft EPA toxicity assessment for HFPO-DA confirm that the POD EPA derived for HFPO-DA in its draft toxicity assessment is protective of all new findings. There is no scientifically defensible way to justify increasing the database uncertainty factor based on these four studies.

EXHIBIT 5

Epidemiology of Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt

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Epidemiology of Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt

Summary

Per- and polyfluoroalkyl substances (PFAS) include thousands of chemicals that have differing physical, chemical, and toxicological characteristics, making them epidemiologically distinct. No published epidemiological studies have evaluated the potential human health effects of environmental or occupational exposure to hexafluoropropylene oxide dimer acid (HFPO-DA) and its ammonium salt, also known as "GenX chemicals." However, publicly available data from the North Carolina Department of Health and Human Services (NCDHHS), the U.S. National Cancer Institute (NCI), and the Centers for Disease Control and Prevention (CDC) do not indicate a pattern of increased cancer incidence or liver disease mortality in the populations surrounding the chemical facility in Fayetteville, North Carolina, that produces HFPO-DA, compared with geographically adjacent or socioeconomically matched populations elsewhere in the state. Specifically:

- Age-adjusted incidence rates of overall, liver, pancreatic, kidney, and male childhood (including testicular) cancers in 2014–2018 were similar in the counties surrounding the Fayetteville facility, compared with geographically adjacent counties, other North Carolina counties matched on socioeconomic status and total population size, the state of North Carolina, and the overall U.S.
- Age-adjusted incidence rates of overall cancer in 2005–2020 have not been increasing over time in the counties surrounding the Fayetteville facility.
- Age-adjusted mortality rates from liver disease in 2010–2020 were similar in the counties surrounding the Fayetteville facility, compared with geographically adjacent counties, other North Carolina counties matched on socioeconomic status and total population size, the state of North Carolina, and the overall U.S.

Therefore, these data sources do not support an adverse effect of HFPO-DA on cancer or liver disease in humans. This conclusion is consistent with findings from NCDHHS, which reported in 2017 that 20-year and recent cancer incidence rates in the Fayetteville region were similar to statewide rates.

Qualifications

I am an epidemiologist with particular research expertise in cancer epidemiology, surveillance, and prevention. I have conducted epidemiological studies of a wide range of exposures in association with cancer risk, including air pollutants, occupational exposures, infections, immunological biomarkers, medication use, reproductive factors, physical activity, body size, diet and nutrition, alcohol consumption, tobacco smoking, family structure, personal and family medical history, and genetic variation. I have published more than 200 peer-reviewed scientific articles and 12 book chapters, including systematic literature reviews on the epidemiology of perfluorooctanoic acid (PFOA) and

perfluorooctane sulfonic acid (PFOS) with respect to cancer and immune outcomes (Chang et al. 2014, 2016).

I earned my undergraduate degree at Harvard College in 1998 and my doctorate degree (Doctor of Science, Sc.D.) in epidemiology with a minor in biostatistics from the Harvard School of Public Health in 2003. I completed a postdoctoral fellowship in medical epidemiology and biostatistics at the Karolinska Institute in Stockholm, Sweden, in 2005. I am currently a Principal Scientist at Exponent, Inc., an international science and engineering consulting company. I am also an Adjunct Associate Professor in the Department of Epidemiology & Biostatistics at the University of California, San Francisco, and a Visiting Professor at the Sun Yat-sen University Cancer Center in Guangzhou, China. Before and during my time at Exponent, I was a Consulting Assistant Professor in the Division of Epidemiology, Department of Health Research and Policy at the Stanford University School of Medicine, and a member of the Stanford Cancer Institute.

Prior to joining Exponent in 2012, I was a research scientist at the non-profit Cancer Prevention Institute of California, where I conducted original research studies on cancer epidemiology and performed cancer surveillance research at a National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) population-based cancer registry. I was also the Chief Epidemiologist at the Asian Liver Center at Stanford University, where I conducted community-based research on hepatitis B and liver cancer awareness, detection, prevention, and management.

Epidemiology of HFPO-DA

PFAS comprise a class of thousands of different substances with distinct physical, chemical, environmental, ecological, toxicological, epidemiological, and other characteristics (U.S. Environmental Protection Agency (EPA) 2021b, National Institute of Environmental Health Sciences (NIEHS) 2022). Existing epidemiological and toxicological studies of certain PFAS, such as PFOA, PFOS, perfluorobutanoic acid (PFBA), perfluorohexane sulfonic acid (PFHxS), perfluorononanoic acid (PFNA), and perfluorodecanoic acid (PFDA), have yielded results that vary by PFAS type, indicating different potential human health effects of each substance (Agency for Toxic Substances and Disease Registry (ATSDR) 2020). Therefore, epidemiological findings for one type of PFAS cannot be generalized to another.

GenX is a trade name for HFPO-DA, a processing aid technology used to make high-performance fluoropolymers without PFOA. At present, as acknowledged by U.S. EPA (2021a), "[n]o human epidemiological studies for GenX chemicals are available," whether pertaining to occupational exposure among workers or environmental exposure among community members. Nevertheless, U.S. EPA has issued subchronic and chronic oral reference doses for HFPO-DA and its ammonium salt based on the results of an animal toxicity study of non-cancer liver effects in mice (U.S. EPA 2021a). In addition, U.S. EPA has concluded that there is "*Suggested Evidence of Carcinogenic Potential*" of oral exposure to these chemicals in humans, based on an animal toxicity study of liver and pancreatic tumors in rats (U.S. EPA 2021a).

In the absence of human epidemiological studies of HFPO-DA, information on the potential effects of HFPO-DA on cancer and non-malignant liver disease in humans can be gleaned from population-based health data on North Carolina residents living in the vicinity of the Chemours Fayetteville Works facility, which began manufacturing HFPO-DA in 2009. In particular, cancer incidence and liver disease mortality rates can be compared between residents of Bladen, Brunswick, Cumberland, New Hanover, and Pender Counties, North Carolina (henceforth classified as "exposed" counties), and residents of other North Carolina counties ("unexposed" counties), as well as the overall populations of North Carolina and the U.S. Accordingly, the remainder of this report describes analyses of data from the NCI and CDC to evaluate whether environmental exposure to HFPO-DA may have led to excesses of cancer incidence and liver disease mortality among residents of "exposed" counties in North Carolina.

Cancer incidence in North Carolina

Cancer incidence data collected by the North Carolina Central Cancer Registry can be accessed through the State Cancer Profiles, an interactive map engine produced in collaboration between the NCI and CDC (2021). We used these data to investigate potential differences in age-adjusted cancer incidence rates in 2014–2018 between "exposed" and "unexposed" counties in North Carolina. In particular, we considered cancers of the liver and pancreas to be of interest based on animal studies (U.S. EPA 2021a), and we considered cancers of the kidney and testes to be of interest based on prior studies of PFOA (Steenland et al. 2020). Because testicular cancer incidence rates are not reported in the State Cancer Profiles (NCI and CDC 2021), and because the age-specific incidence of testicular cancer rises steeply beginning at ages 10–14 years (NCI 2022), we instead evaluated childhood cancers in males under age 20 years.

Figure 1 shows the North Carolina counties identified for this analysis, including the five counties designated as "exposed" (Bladen, Brunswick, Cumberland, New Hanover, and Pender, indicated in blue); seven "unexposed" geographically adjacent counties (Columbus, Duplin, Harnett, Hoke, Onslow, Robeson, and Sampson, indicated in green); and five "unexposed" counties matched to the five exposed counties on percent of the population below federal poverty level (± 2%) and total population size (± 40%), based on 2019 data from the U.S. Census Bureau (2021, 2022). Comparator counties were chosen *a priori*, before accessing cancer incidence or liver disease mortality rates.

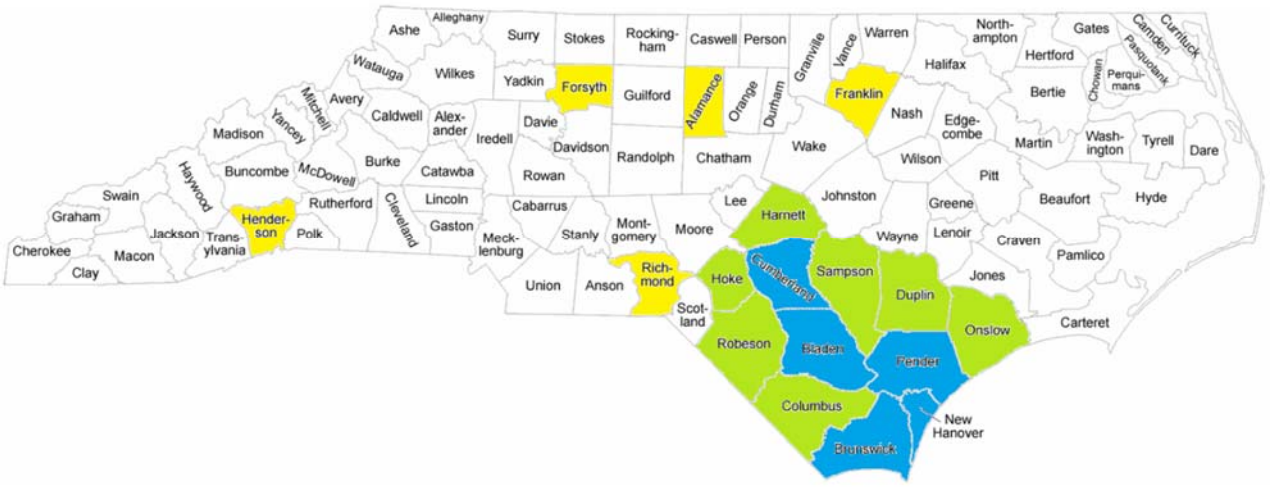

Figure 1. North Carolina counties included in analysis. The Fayetteville Works facility is located near the Bladen-Cumberland county line. Counties designated as "exposed" are indicated in blue. Geographically adjacent comparator counties designated as "unexposed" are indicated in green. Poverty- and population-matched comparator counties designated as "unexposed" are indicated in yellow.

As shown below in Figures 2 and 3, and summarized in Table 1, age-adjusted female and male cancer incidence rates in 2014–2018 were not systematically higher in the "exposed" counties than in geographically adjacent "unexposed" counties, other "unexposed" counties matched on percent of population below poverty level and total population size, the state of North Carolina, or the overall U.S. On the contrary, cancer incidence among females in the "exposed" counties (median: 421.2 per 100,000 person-years; range: 390.3–440.0) was comparable with or lower than that among females in adjacent "unexposed" counties (median: 426.1 per 100,000 person-years; range: 375.4–517.9), matched "unexposed" counties (median: 436.8 per 100,000 person-years; range: 405.7–470.8), North Carolina (433.3 per 100,000 person-years), and the overall U.S. (422.7 per 100,000 person-years) (Table 1). Based on comparisons using 95% confidence intervals, the female cancer incidence rate was not statistically significantly higher in any "exposed" county (and in Cumberland and New Hanover Counties was statistically significantly lower) than in its poverty- and population-matched "unexposed" counterpart. The annual average percent change in cancer incidence among females in all areas was generally stable between 2014 and 2018.

Likewise, among males, cancer incidence in the "exposed" counties (median: 508.8 per 100,000 person-years; range: 491.1–522.9) was comparable with or lower than in adjacent "unexposed" counties (median: 506.6 per 100,000 person-years; range: 451.0–590.7), matched "unexposed" counties (median: 521.6 per 100,000 person-years; range: 489.0–554.6), and North Carolina (521.1 per 100,000 person-years), while many of these rates were higher than that for men in the overall U.S. (487.4 per 100,000 person-years) (Table 1). Comparing poverty- and population-based "exposed" and "unexposed" counties, the male cancer incidence rate in Brunswick County was statistically significantly higher, but that in New Hanover County was statistically significantly lower, and otherwise no differences were observed. Time trends in all areas were generally stable or falling

between 2014 and 2018.

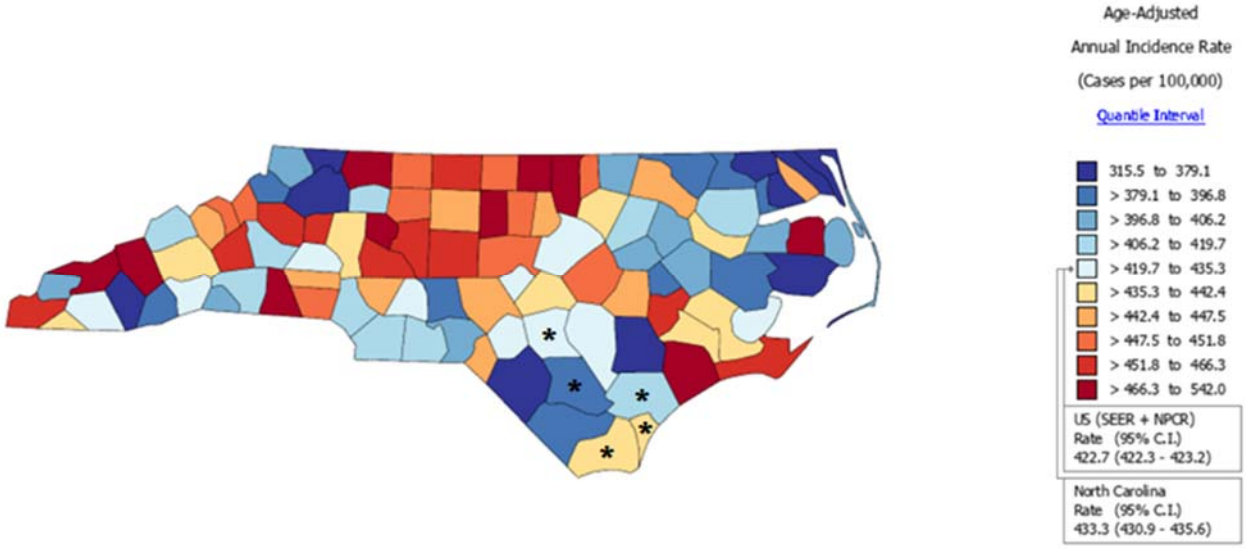

Figure 2. Age-adjusted incidence rates of all cancers in females by county, North Carolina, 2014–2018. "Exposed" counties are indicated with asterisks (*). Map generated at https://www.statecancerprofiles.cancer.gov/map/map.noimage.php (NCI and CDC 2021).

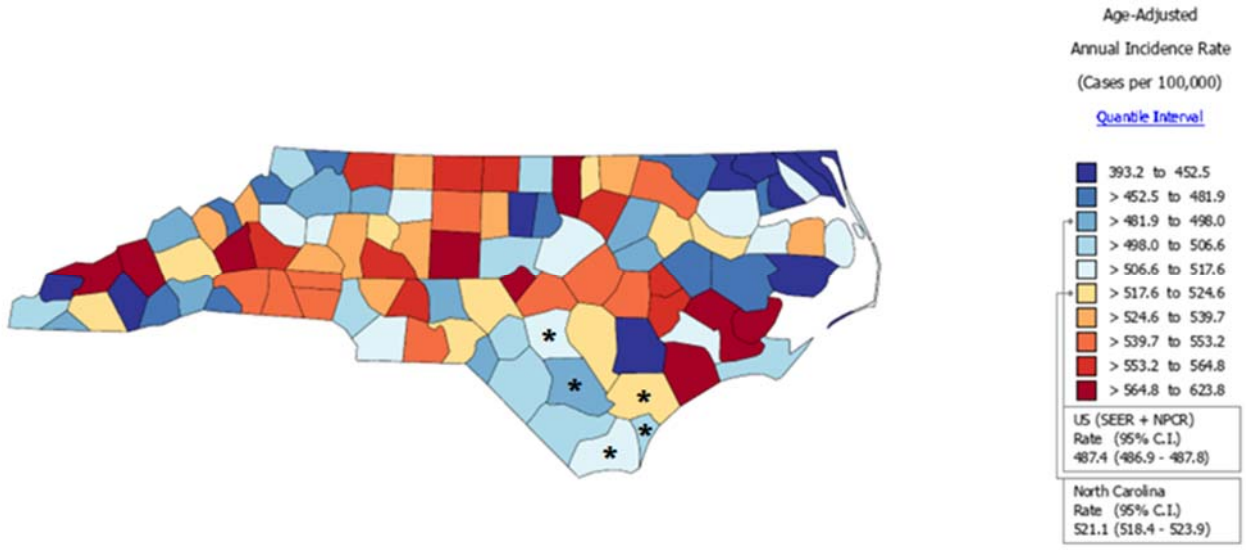

Figure 3. Age-adjusted incidence rates of all cancers in males by county, North Carolina, 2014–2018. "Exposed" counties are indicated with asterisks (*). Map generated at https://www.statecancerprofiles.cancer.gov/map/map.noimage.php (NCI and CDC 2021).

Table 1. Age-adjusted incidence rates of all cancers combined in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2014–2018

				All Cancers, Females			All Cancers, Males		
	Area	Population[a]	% Poverty[b]	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]
"Exposed"	Bladen County	32,722	24.3%	390.3 (354.2, 429.5)	Stable	1.1 (-0.1, 2.3)	491.1 (449.2, 536.2)	Falling	-1.3 (-2.1, -0.5)
	Brunswick County	142,820	11.8%	439.4 (419.3, 460.3)	Stable	0.6 (-0.1, 1.2)	514.3 (493.5, 535.9)	Stable	0.8 (-0.1, 1.6)
	Cumberland County	335,509	18.4%	421.2 (407.3, 435.4)	Stable	0.0 (-0.6, 0.6)	508.8 (491.5, 526.6)	Falling	-1.0 (-1.5, -0.4)
	New Hanover County	234,473	16.0%	440.0 (424.3, 456.1)	Stable	0.1 (-0.6, 0.7)	503.3 (485.5, 521.7)	Stable	-0.5 (-1.1, 0.2)
	Pender County	63,060	14.1%	407.8 (378.8, 438.5)	Stable	-0.4 (-2.0, 1.2)	522.9 (489.0, 558.6)	Stable	-0.4 (-1.3, 0.6)
"Unexposed" adjacent	Columbus County	55,508	22.8%	392.2 (364.0, 422.1)	Stable	0.4 (-0.6, 1.4)	504.9 (471.3, 540.4)	Falling	-1.2 (-1.8, -0.5)
	Duplin County	58,741	21.2%	376.4 (348.7, 405.9)	Stable	0.5 (-0.7, 1.7)	451.0 (419.3, 484.5)	Stable	-0.8 (-1.9, 0.3)
	Harnett County	135,976	15.8%	442.0 (419.7, 465.2)	Stable	0.5 (-0.1, 1.1)	543.5 (516.0, 572.0)	Stable	-0.6 (-1.3, 0.1)
	Hoke County	55,234	20.4%	426.1 (389.4, 465.2)	Stable	0.5 (-0.5, 1.6)	505.2 (459.3, 554.3)	Falling	-2.2 (-3.7, -0.6)
	Onslow County	197,938	13.2%	517.9 (494.8, 541.9)	Rising	1.7 (1.1, 2.2)	590.7 (563.4, 618.8)	Stable	-0.2 (-1.1, 0.6)
	Robeson County	130,625	27.7%	375.4 (356.1, 395.4)	Stable	-0.1 (-0.9, 0.7)	506.6 (481.9, 532.1)	Falling	-1.5 (-2.3, -0.8)
	Sampson County	63,531	20.9%	435.3 (406.2, 466.1)	Stable	0.9 (0.0, 1.9)	521.9 (488.7, 556.9)	Stable	-0.5 (-1.4, 0.5)
"Unexposed" matched	Richmond County	44,829	25.2%	405.7 (373.4, 440.3)	Stable	0.1 (-1.2, 1.4)	521.6 (482.1, 563.5)	Stable	-0.7 (-1.8, 0.4)
	Henderson County	117,417	10.9%	432.7 (412.7, 453.5)	Stable	0.0 (-0.6, 0.6)	489.0 (467.8, 511.1)	Falling	-2.6 (-3.5, -1.6)
	Forsyth County	382,295	16.8%	447.6 (435.4, 460.1)	Stable	0.1 (-0.4, 0.6)	515.3 (500.8, 530.1)	Falling	-2.6 (-3.4, -1.8)
	Alamance County	169,509	16.1%	470.8 (452.2, 490.1)	Rising	0.9 (0.4, 1.5)	535.5 (513.9, 557.9)	Falling	-1.0 (-1.6, -0.4)
	Franklin County	69,685	13.2%	436.8 (408.6, 466.5)	Stable	0.4 (-0.5, 1.3)	554.6 (520.0, 590.9)	Stable	2.4 (-2.4, 7.5)
Other	North Carolina	10,488,084	14.7%	433.3 (430.9, 435.6)	Stable	-0.7 (-2.1, 0.8)	521.1 (518.4, 523.9)	Falling	-0.8 (-1.6, -0.1)
	United States	328,239,523	13.4%	422.7 (422.3, 423.2)	Stable	-0.8 (-1.6, 0.1)	487.4 (486.9, 487.8)	Falling	-1.1 (-1.7, -0.5)

CI: confidence interval. NR: not reported to ensure confidentiality and stability of rate and trend estimates. Counties matched on poverty status and total population are color-coded.

[a]U.S. Census Bureau. County population totals: 2019 population estimate. Dataset: CO-EST2019-alldata

[b]U.S. Census Bureau. Poverty status in the past 12 months: percent below poverty level. American Community Survey 5-year estimates subject tables. Dataset: ACSST5Y2019

[c]Incidence rates (per 100,000 person-years) are age-adjusted to the 2000 U.S. standard population and include all ages, races, and invasive cancer stages: https://www.statecancerprofiles.cancer.gov/incidencerates/.

[d]Trend is "rising" when 95% CI of average annual percent change is above 0, "stable" when 95% CI includes 0, and "falling" when 95% CI is below 0.

[e]Annual average percent changes are calculated by the Joinpoint Regression Program (https://surveillance.cancer.gov/joinpoint/) and are based on annual percent changes.

Site-specific cancer incidence data are presented in Table 2 (liver and intrahepatic bile duct cancer), Table 3 (pancreatic cancer), Table 4 (kidney and renal pelvis cancer), and Table 5 (male childhood cancers). Sex-stratified incidence rates for each cancer site were also evaluated, but are not shown here.

For liver cancer, incidence rates were comparable across the "exposed," adjacent "unexposed," and matched "unexposed" counties (medians: 8.4, 8.8, and 8.0 per 100,000 person-years, respectively; ranges: 6.3–10.2, 5.2–10.1, and 6.8–9.3, respectively), as well as North Carolina (8.6 per 100,000 person-years) and the U.S. (8.6 per 100,000 person-years), with mostly stable and occasionally rising rates between 2014 and 2018 in all areas (Table 2). The incidence rate of 10.2 per 100,000 in Bladen County was based on small numbers (mean: 5 cases per year), making estimates statistically unstable and insufficiently robust to calculate time trends. Liver cancer incidence rates did not differ statistically significantly between any matched "exposed" and "unexposed" counties. No noteworthy patterns were evident for liver cancer incidence in females or males after stratification by sex (results not shown).

Table 2. Age-adjusted incidence rates of liver and intrahepatic bile duct cancer in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2014–2018. Footnotes are the same as in Table 1.

		Liver and Intrahepatic Bile Duct		
	Area	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]
"Exposed"	Bladen County	10.2 (6.6, 15.5)	NR	NR
	Brunswick County	6.3 (4.8, 8.2)	Stable	3.0 (-1.6, 7.8)
	Cumberland County	8.4 (7.1, 10.0)	Stable	2.6 (-0.2, 5.5)
	New Hanover County	8.6 (7.1, 10.3)	Rising	3.5 (0.1, 7.0)
	Pender County	7.4 (5.0, 10.7)	Stable	2.0 (-3.3, 7.7)
"Unexposed" adjacent	Columbus County	8.9 (6.1, 12.7)	NR	NR
	Duplin County	5.2 (3.3, 8.1)	Stable	1.9 (-2.4, 6.4)
	Harnett County	10.1 (7.8, 12.9)	Stable	3.1 (-0.6, 7.0)
	Hoke County	NR	NR	NR
	Onslow County	8.7 (6.6, 11.2)	Stable	2.4 (-0.3, 5.2)
	Robeson County	6.8 (5.1, 9.0)	Stable	3.3 (-1.0, 7.9)
	Sampson County	9.2 (6.5, 12.8)	Rising	6.9 (1.4, 12.7)
"Unexposed" matched	Richmond County	6.8 (4.0, 10.8)	Stable	1.5 (-3.8, 7.0)
	Henderson County	7.8 (5.9, 10.1)	Rising	4.3 (1.2, 7.6)
	Forsyth County	8.3 (7.2, 9.6)	Rising	3.6 (0.4, 6.8)
	Alamance County	9.3 (7.5, 11.3)	Rising	4.4 (1.6, 7.3)
	Franklin County	8.0 (5.5, 11.4)	Stable	2.2 (-3.0, 7.7)
Other	North Carolina	8.6 (8.3, 8.8)	Stable	0.1 (-2.2, 2.4)
	United States	8.6 (8.5, 8.6)	Stable	-0.2 (-0.8, 0.3)

As shown in Table 3, pancreatic cancer incidence rates in the "exposed" counties (median: 12.9 per 100,000 person-years; range: 11.0–14.1) were generally similar to or lower than those in geographically adjacent "unexposed" counties (median: 14.7 per 100,000 person-years; range:

11.9–19.4), matched "unexposed" counties (median: 14.4 per 100,000 person-years; range: 13.8–17.5), North Carolina (13.2 per 100,000 person-years), and the U.S. (13.1 per 100,000 person-years). Comparing counties matched on percent below poverty and total population size, no statistically significant differences in pancreatic cancer incidence were observed in three county pairs, whereas incidence was significantly lower in Bladen and Brunswick counties than their matched counterparts. Most areas reported stable incidence rates of pancreatic cancer between 2014 and 2018, except for rising rates in New Hanover County, one matched "unexposed" county, and North Carolina and the U.S. as a whole. Due to a small number of cases (mean: 6 cases per year) statistically reliable time trends could not be calculated in Bladen County. Results for pancreatic cancer incidence were also unremarkable after stratification by sex (results not shown).

Table 3. Age-adjusted incidence rates of pancreatic cancer in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2014–2018. Footnotes are the same as in Table 1.

		Pancreas		
	Area	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]
"Exposed"	Bladen County	11.0 (7.3, 16.3)	NR	NR
	Brunswick County	11.6 (9.6, 14.1)	Stable	1.2 (-2.0, 4.5)
	Cumberland County	14.1 (12.2, 16.1)	Stable	1.7 (0.0, 3.3)
	New Hanover County	13.0 (11.1, 15.1)	Rising	2.8 (0.6, 5.2)
	Pender County	12.9 (9.5, 17.2)	Stable	0.6 (-2.6, 4.0)
"Unexposed" adjacent	Columbus County	15.0 (11.4, 19.5)	Stable	0.8 (-2.8, 4.5)
	Duplin County	12.1 (8.8, 16.3)	Stable	-0.3 (-3.6, 3.2)
	Harnett County	19.4 (16.0, 23.2)	Stable	-7.3 (-19.0, 6.2)
	Hoke County	11.9 (7.4, 17.9)	Stable	5.9 (-4.8, 17.8)
	Onslow County	14.7 (11.9, 17.9)	Stable	-0.1 (-2.3, 2.1)
	Robeson County	14.1 (11.5, 17.1)	Stable	-1.1 (-9.1, 7.6)
	Sampson County	16.1 (12.3, 20.6)	Stable	1.6 (-1.8, 5.1)
"Unexposed" matched	Richmond County	17.5 (13.0, 23.2)	Stable	2.9 (-0.3, 6.1)
	Henderson County	15.4 (13.0, 18.2)	Rising	3.4 (0.9, 6.0)
	Forsyth County	14.4 (12.8, 16.1)	Stable	0.8 (-0.9, 2.5)
	Alamance County	13.8 (11.6, 16.4)	Stable	1.9 (-0.1, 4.0)
	Franklin County	14.4 (10.8, 18.8)	Stable	1.0 (-3.0, 5.3)
Other	North Carolina	13.2 (12.9, 13.5)	Rising	1.4 (1.0, 1.7)
	United States	13.1 (13.0, 13.1)	Rising	0.9 (0.7, 1.0)

The analysis of kidney cancer incidence patterns did not reveal systematically higher rates in "exposed" counties (median: 17.8 per 100,000 person-years; range: 15.8–18.5), compared with geographically adjacent "unexposed" counties (median: 19.7 per 100,000 person-years; range: 17.3–20.9), other "unexposed" counties matched on percent below poverty and total population size (median: 16.4 per 100,000 person-years; range: 15.0–20.6), North Carolina as a whole (17.4 per 100,000 person-years) or the U.S. (median: 17.1 per 100,000 person-years) (Table 4). Comparison of matched pairs of counties showed that Brunswick County had a statistically significantly higher rate of kidney cancer than its matched county in 2014–2018, but rates in the

other four "exposed" counties did not differ statistically significantly from those in their counterparts. Time trends in kidney cancer incidence were stable in most areas, except for rising rates in the "exposed" county of New Hanover and a minority of "unexposed" counties in the "unexposed" groups. After stratification by sex, no clear patterns by exposure status were evident (results not shown).

Table 4. Age-adjusted incidence rates of kidney and renal pelvis cancer in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2014–2018. Footnotes are the same as in Table 1.

		Kidney and Renal Pelvis		
	Area	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]
"Exposed"	Bladen County	17.8 (12.3, 24.9)	Stable	4.0 (-0.5, 8.7)
	Brunswick County	18.5 (15.7, 21.7)	Stable	1.1 (-0.7, 3.0)
	Cumberland County	15.8 (13.9, 17.9)	Stable	1.3 (-0.3, 2.9)
	New Hanover County	18.4 (16.2, 21.0)	Rising	3.0 (1.8, 4.3)
	Pender County	17.3 (13.3, 22.2)	Stable	0.8 (-1.5, 3.1)
"Unexposed" adjacent	Columbus County	20.8 (16.2, 26.4)	Stable	3.2 (-0.9, 7.4)
	Duplin County	18.3 (14.1, 23.4)	Stable	-0.5 (-4.3, 3.4)
	Harnett County	19.5 (16.2, 23.2)	Rising	3.0 (0.8, 5.2)
	Hoke County	20.9 (15.3, 27.9)	Stable	0.2 (-3.4, 3.9)
	Onslow County	19.7 (16.5, 23.3)	Stable	0.7 (-1.6, 3.0)
	Robeson County	20.9 (17.7, 24.7)	Rising	2.8 (0.8, 4.8)
	Sampson County	17.3 (13.4, 22.0)	Stable	0.4 (-2.7, 3.6)
"Unexposed" matched	Richmond County	19.8 (14.9, 25.9)	Stable	2.7 (-2.0, 7.6)
	Henderson County	15.0 (12.3, 18.0)	Stable	-0.9 (-2.9, 1.1)
	Forsyth County	16.2 (14.5, 18.0)	Stable	-0.9 (-3.5, 1.7)
	Alamance County	20.6 (17.9, 23.8)	Rising	2.8 (0.7, 5.0)
	Franklin County	16.4 (12.7, 20.8)	Stable	2.6 (-2.1, 7.6)
Other	North Carolina	17.4 (17.0, 17.7)	Stable	0.4 (0.0, 0.7)
	United States	17.1 (17.0, 17.1)	Stable	0.2 (-0.6, 1.0)

Incidence rates of childhood cancer in males younger than 20 years were not reported in most counties due to small numbers (mean: ≤ 3 cases per year), which created statistically unstable results and concerns about data confidentiality (Table 5). Nevertheless, male childhood cancer incidence rates did not differ appreciably among the two "exposed" counties with reported data (median/mean: 18.6 per 100,000 person-years), the two geographically adjacent "unexposed" counties with reported data (median/mean: 19.4 per 100,000 person-years), the two matched "unexposed" counties with reported data (median/mean: 20.3 per 100,000 person-years), North Carolina (20.2 per 100,000 person-years), and the U.S. (19.9 per 100,000 person-years). Incidence rates were stable in most areas, except for rising trends in New Hanover County and North Carolina as a whole.

Table 5. Age-adjusted incidence rates of childhood cancer in males under age 20 years in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2014–2018. Footnotes are the same as in Table 1.

		Childhood, Males < 20 y		
	Area	Incidence Rate (95% CI)[c]	Trend[d]	5-y Trend (95% CI)[e]
"Exposed"	Bladen County	NR	NR	NR
	Brunswick County	NR	NR	NR
	Cumberland County	17.2 (12.2, 23.4)	Stable	-8.9 (-17.7, 0.8)
	New Hanover County	20.0 (12.9, 29.5)	Rising	6.2 (0.2, 12.5)
	Pender County	NR	NR	NR
"Unexposed" adjacent	Columbus County	NR	NR	NR
	Duplin County	NR	NR	NR
	Harnett County	NR	NR	NR
	Hoke County	NR	NR	NR
	Onslow County	15.3 (9.5, 23.2)	Stable	-1.1 (-5.7, 3.6)
	Robeson County	23.5 (14.9, 35.3)	Stable	3.5 (-3.1, 10.5)
	Sampson County	NR	NR	NR
"Unexposed" matched	Richmond County	NR	NR	NR
	Henderson County	NR	NR	NR
	Forsyth County	19.7 (14.6, 26.0)	Stable	0.4 (-3.7, 4.6)
	Alamance County	20.8 (13.2, 31.4)	NR	NR
	Franklin County	NR	NR	NR
Other	North Carolina	20.2 (19.2, 21.3)	Rising	1.5 (0.4, 2.6)
	United States	19.9 (19.7, 20.1)	Stable	-0.8 (-3.2, 1.5)

Finally, time trends in five-year average incidence rates of overall cancer in the five "exposed" counties from 2005–2009 to 2016–2020 do not show a pattern of rising cancer incidence following the production of HFPO-DA at the Fayetteville Works plant in 2009 (Figure 4) (NCDHHS 2022).

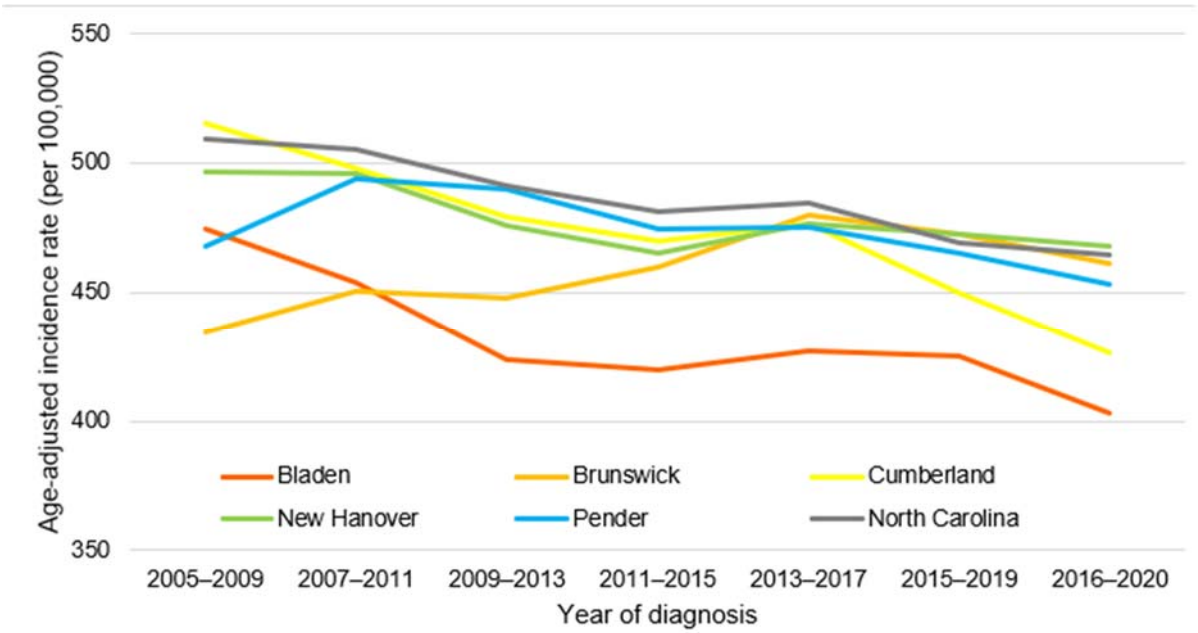

Figure 4. Time trends in age-adjusted (to 2000 U.S. standard) incidence rates of all cancers by county, North Carolina, 2005–2020. Data from https://schs.dph.ncdhhs.gov/data/cancer/incidence_rates.htm (NCDHHS 2022).

In summary, although limited by their ecological nature and the lack of individual-level information on exposure to HPFO-DA, risk factors for the cancers of interest, and residential history, and allowing for only up to a decade of putative latency since the introduction of HPFO-DA, available population-based data on cancer incidence in North Carolina do not support an effect of HPFO-DA on risk of overall cancer, liver cancer, pancreatic cancer, kidney cancer, or male childhood cancers (as a proxy for testicular cancer).

These results are consistent with findings from NCDHHS, which conducted its own investigation of pancreatic, liver, uterine, testicular, and kidney cancer incidence rates in Bladen, Brunswick, New Hanover, and Pender Counties in 1996–2015, and separately for each five-year interval therein (NCDDHS 2017). Based on its results, NCDHHS (2017) concluded that "[o]verall, cancer rates in the four counties were similar to state rates."

Liver disease mortality in North Carolina

Cause-specific mortality data from 1999–2020 are accessible through CDC's Wide-ranging OnLine Data for Epidemiologic Research (WONDER) database of public health information (CDC 2021). Prompted by EPA's identification of liver toxicity as the critical endpoint for development of oral reference doses for HPFO-DA (U.S. EPA 2021a), we used CDC WONDER to evaluate potential differences in age-adjusted liver disease mortality rates between "exposed" and "unexposed" counties in North Carolina in 2010–2020, using the same groupings as described in the cancer incidence analysis. We included deaths from all diseases of the liver (International Classification of Diseases, 10th Revision (ICD-10) codes K72–K76), except for alcoholic liver disease (ICD-10 code K70) and toxic liver disease (ICD-10 code K71).

As shown in Table 6, mortality rates from liver disease in 2010–2020 were similar across "exposed," geographically adjacent "unexposed," and poverty- and population-matched "unexposed" counties (medians: 10.6, 10.1, and 9.2 per 100,000 person-years, respectively; ranges: 9.6–13.0, 8.5–12.1, and 7.4–13.1, respectively), as well as North Carolina (9.5 per 100,000 person-years) and the U.S. (8.3 per 100,000 person-years). Based on comparisons using 95% confidence intervals, liver disease mortality in both sexes combined and among males was statistically significantly higher in Brunswick, Cumberland, and New Hanover Counties than their matched counties, but no such differences were observed in Bladen and Pender Counties. Moreover, liver disease mortality among females in Bladen County was statistically significantly lower than in its matched county, and no significant differences in female liver disease mortality were otherwise seen between matched county pairs.

Table 6. Age-adjusted liver disease mortality rates in "exposed" and "unexposed" geographically adjacent or poverty- and population-matched counties, North Carolina, and U.S., 2010–2020.

		Mortality Rate (95% Confidence Interval)[a]		
	Area	All	Females	Males
"Exposed"	Bladen County	11.1 (8.3, 14.5)	7.6 (4.7, 11.6)	15.1 (10.4, 21.4)
	Brunswick County	10.6 (9.0, 12.1)	8.7 (6.8, 10.9)	12.8 (10.4, 15.2)
	Cumberland County	9.6 (8.6, 10.7)	6.9 (5.7, 8.1)	13.1 (11.2, 15.0)
	New Hanover County	13.0 (11.6, 14.4)	9.4 (7.8, 10.9)	17.3 (15.0, 19.5)
	Pender County	9.7 (7.7, 12.1)	8.7 (6.1, 12.1)	10.9 (7.8, 14.8)
"Unexposed" adjacent	Columbus County	12.0 (9.6, 14.3)	10.0 (7.3, 13.5)	14.4 (10.8, 18.8)
	Duplin County	8.5 (6.6, 10.9)	7.8 (5.3, 10.9)	9.3 (6.4, 13.2)
	Harnett County	10.1 (8.4, 11.8)	9.3 (7.2, 11.7)	10.7 (8.2, 13.6)
	Hoke County	9.5 (6.8, 13.0)	"Unreliable" [n ≤ 20] (4.3, 11.8)	12.4 (7.7, 19.0)
	Onslow County	12.1 (10.4, 13.9)	9.9 (7.9, 12.4)	14.7 (11.8, 17.6)
	Robeson County	11.3 (9.6, 13.0)	10.8 (8.7, 13.2)	12.0 (9.5, 14.9)
	Sampson County	9.9 (7.9, 12.4)	8.3 (5.9, 11.5)	11.6 (8.4, 15.6)
"Unexposed" matched	Richmond County	13.1 (10.4, 16.3)	11.6 (8.2, 16.0)	14.8 (10.7, 20.0)
	Henderson County	7.4 (6.1, 8.8)	7.0 (5.2, 9.1)	8.1 (6.3, 10.3)
	Forsyth County	8.4 (7.5, 9.2)	7.9 (6.8, 9.0)	9.1 (7.8, 10.4)
	Alamance County	10.3 (8.9, 11.7)	9.0 (7.3, 10.8)	12.1 (9.8, 14.3)
	Franklin County	9.2 (7.2, 11.5)	6.3 (4.2, 9.1)	12.5 (9.2, 16.7)
Other	North Carolina	9.5 (9.4, 9.7)	7.9 (7.7, 8.2)	11.4 (11.2, 11.7)
	United States	8.3 (8.3, 8.4)	6.9 (6.8, 6.9)	10.0 (9.9, 10.0)

[a]Mortality rates (per 100,000 person-years) from liver disease, excluding alcoholic and toxic liver disease, are age-adjusted to the 2000 U.S. standard population and include all ages and races: https://wonder.cdc.gov/.

In summary, although these findings are limited by the ecological nature of the data, the lack of individual-level information on HPFO-DA exposure, liver disease risk factors and prognostic factors, or residential history, and the restriction to liver disease mortality rather than incidence, available population-based cause-specific mortality data in North Carolina do not support an effect of HPFO-DA on liver disease in humans.

Conclusions

In the absence of any published epidemiological studies of HPFO-DA, virtually the only available data with which to evaluate the potential human health effects of HPFO-DA are public-use population-based datasets on cancer incidence and cause-specific mortality from NCDHHS, NCI, and CDC. Comparing these ecological data between designated "exposed" counties surrounding the Fayetteville Works plant and "unexposed" reference counties, the entire state of North Carolina, or the U.S. as a whole, no apparent pattern of excess cancer risk or mortality from liver disease was detected among residents in the vicinity of Fayetteville, North Carolina. Thus, available epidemiological data from these sources are not consistent with a carcinogenic or hepatotoxic effect of HPFO-DA in humans.

The findings in this report are stated with a reasonable degree of scientific certainty, and are based on information that is publicly available from the cited sources at the present time.

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EXHIBIT 6

ToxStrategies

Innovative solutions
Sound science

In Vitro Human and Rodent Hepatocyte Study Protocol

Chemours is conducting an *in vitro* study to provide additional information concerning the mode of action underlying the non-neoplastic liver changes associated with exposure to HFPO-DA. Broadly, the study follows design elements described in McMullen et al. (2020). In the Chemours *in vitro* study, human and rodent hepatocytes will be exposed to various concentrations of known agonists of PPARα and PPARγ, as well as to known cytotoxic agents for three timepoints (to be determined) and their transcriptomic responses measured by templated oligomer sequencing technology. These agents will be considered the control or "benchmark" agents to which parallel studies with HFPO-DA will be compared. Experiments will be conducted on pooled human hepatocytes (from 10 donors), Crl:CD1(ICR) mice, PPARα null mice on 129/Sv genetic background, 129/Sv mice, and Sprague Dawley SD:CD rats (from CRL). The use of rat hepatocytes is to compare the results to those in McMullen et al. (2020)[1] for a PPARα agonist. The Crl:CD1 mice are the same strain as used in various OECD guideline studies with HFPO-DA. Wild type and PPARα null mice will be used to further inform the mode of action. The overall objective is to compare the molecular signature of HFPO-DA to agents with known modes of action, as well as to compare responses in rodent and human hepatocytes to inform the mode of action of HFPO-DA in the liver. The hepatocyte generation and *in vitro* exposures will be conducted by Xenotech, Inc., while the transcriptomic processing will be conducted at BioSpyder Technologies, Inc. The bioinformatics will be conducted by ToxStrategies, Inc. In addition to these studies, an *in vivo* element, like that described in McMullen et al. (2020), will also be conducted to compare responses observed *in vitro* with rodent hepatocytes to those in the rodent liver.

[1] McMullen PD, Bhattacharya S, Woods CG, Pendse SN, McBride MT, Soldatow VY, Deisenroth C, LeCluyse EL, Clewell RA, Andersen ME. 2020. Identifying qualitative differences in PPARα signaling networks in human and rat hepatocytes and their significance for next generation chemical risk assessment methods. Toxicol In Vitro. 64:104463.

Preliminary In Vitro Study Design

	McMullen et al. (2020)	Chemours *In Vitro* Study
PPARα agonist (positive control)	GW7647	(e.g., GW7647)
PPARγ agonist (positive control)	NA	(e.g., glitazones)
Cytotoxic agent (positive control)	NA	(e.g., acetaminophen)
PFAS	NA	HFPO-DA
Human cells	Human primary hepatocytes	Human primary hepatocytes
Rodent cells	Rat primary hepatocytes	Rat primary hepatocytes Mouse primary hepatocytes (WT, 2 strains) Mouse primary hepatocytes (PPARα-null)
Concentrations	0.001 – 10 μM	TBD
Exposure Duration	2, 6, 12, 24, and 72 h	TBD
Transcriptomic Platform	Affymetrix Rat Genome 230 Affymetrix Human Genome U133	TempO-Seq whole genome (human, rat, mouse)