BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
PROPOSED AMENDMENTS TO	)	R2022-018
GROUNDWATER QUALITY	)	(Rulemaking – Public Water Supply)
(35 Ill Adm. Code 620)	)	

NOTICE

TO: SEE ATTACHED CERTIFATE OF SERVICE LIST

PLEASE TAKE NOTICE that I have today electronically filed with the Office of the Clerk of the Illinois Pollution Control Board the AMERICAN CHEMISTRY COUNCIL'S RESPONSE to the PRE-FILED QUESTIONS submitted by the Board, the Illinois Environmental Protection Agency, and 3M in the matter of the Illinois Environmental Protection Agency's proposed amendments to groundwater quality, a copy of which is herewith served upon you.

Dated: November 23, 2022

Respectfully Submitted,

AMERICAN CHEMISTRY COUNCIL

By: /s/ Stephen P. Risotto

Stephen P. Risotto
Senior Director
Chemical Products and Technology Division

700 2nd Street, Northeast
Washington, DC 20002
(202) 249-6727
srisotto@americanchemistry.com

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RESPONSE TO PRE-FILED QUESTIONS
TO THE AMERICAN CHEMISTRY COUNCIL'S PRE-FILED TESTIMONY

Response to Pre-Filed Questions from the Illinois Pollution Control Board

37. *On pages 5 through 8, you raise several concerns regarding USEPA's 2021 Assessment of HFPO-DA and PFBS. Please clarify whether you are referring to the updated toxicity assessments published in April 2021.*

The referenced portions of my pre-filed are based on the final USEPA assessment for PFBS released on April 2021 (EPA/600/R-20/345F) and the USEPA final assessment for HFPO-DA issued in October 2021 (EPA Document 822R-21-010).

38. *Please comment on whether USEPA's toxicity assessment process allows for public comment and expert peer review prior to final publication.*

USEPA conducted letter peer reviews of the draft PFBS and HFPO-DA Human Health Toxicity Value (HHTV) assessments in the summer of 2018 and released the draft assessments for public comment in November 2018. USEPA conducted a subsequent letter peer review of a revised HHTV for PFBS in 2020; a peer review of the revised assessment for HFPO-DA was conducted in 2021. USEPA subsequently released a final assessment for PFBS in January 2021 and a revised final assessment for the chemical in April 2021. USEPA released the final assessment for HFPO-DA in October 2021.

Despite making some changes to the 2018 public drafts of the documents before issuing the final assessments, USEPA did not make the revised documents available for review by the public. In the case of the April 2021 PFBS assessment, USEPA revised its approach to calculating the human equivalent dose (HED) and removed the lower end of the range of toxicity values. For its final assessment of HFPO-DA, USEPA used a health effects metric that it has never used before and increased the total uncertainty factor to 3000 - despite having received additional data from public commenters.

a. If so, did ACC or any other researchers/groups raise the "underlying" concerns noted in your testimony (pages 5-8) during the public comment/peer review process?

ACC was among several groups who submitted comments on the public drafts of the PFBS and HFPO-DA assessments in January 2019. The issues raised in my pre-filed testimony on the IEPA proposal address the changes made to the assessments subsequent to the closing of the public comment period. ACC and other stakeholders did not have an opportunity to comment on the changes prior to the release of the final assessments.

b. If concerns noted in your testimony were raised, how did USEPA respond to them. Please submit any relevant documents from the USEPA toxicity assessment process into the record.

. Although USEPA has released its response to comments from the peer reviewers, its response to comments on the HHTV for PFBS submitted by ACC and other stakeholders have not been made publicly available. ACC did not have an opportunity to review the changes made to the HHTV for HFPO-DA prior to its finalization.

I have attached ACC's comments on the public drafts of USEPA's PFBS and HFPO-DA assessments. I also have attached a request for correction of the final HFPO-DA assessment filed pursuant to the Information Quality Act.

39. *On pages 9 through 13, you raise several concerns regarding ATSDR minimum risk levels (MRLs) for PFHxS, PFNA and PFOS that were used by IEPA to propose Class I/II standards.*

a. *Please comment on whether the process for developing MRLs at ATSDR allows for peer review and public comment prior publication of the MRL.*

The MRLs for PFHxS, PFNA, and PFOS are contained in ATSDR's Toxicological Profile for Perfluoroalkyls which was finalized in May 2021. The Toxicological Profile did undergo peer review and was made available for public comment.

b. *If so, did ACC or any other researchers/groups raise the concerns noted in your testimony (pages 8-13) during the public comment/peer review process of MRL development?*

ACC submitted written comments on the draft Toxicological Profile in August 2018. The concerns expressed in my pre-filed testimony on the IEPA proposal are consistent with the ACC's August 2018 comments to ATSDR.

c. *If concerns noted in your testimony were raised during MRL development, how did ATSDR respond to them. Please submit any relevant documents from the ATSDR MRL development process into the record.*

ATDSR has not made its response to stakeholder comments publicly available. In addition, the comments of the peer reviewers and ATSDR's response to those comments, are not publicly available.

40. *On page 4, you state, "the calculation of an acceptable daily exposure (ADE) for a child between the ages of 0 and 6 years of age is similarly not appropriate for PFNA and PFOS for which the ATSDR MRL is based on developmental effects among laboratory animals in utero."*

a. *Please elaborate on why the use of ATSDR MRLs are inappropriate.*

As noted in my pre-filed testimony, ATSDR based its derivation of MRLs for PFNA and PFBS on developmental effects in laboratory animal studies resulting from *in utero* exposures. In the case of PFNA, the key effect was decreased body weight and developmental delays in the offspring; for PFOS, the key effects were decreased body weight and delayed eye opening in the pups. Since these effects result from exposure during gestation, the MRL should be based on daily exposures to the pregnant female, not on exposures to the child after birth. This approach is consistent with the approach taken by USEPA in deriving its 2016 lifetime Health Advisory (LHA) for PFOS.

b. *What would you recommend that the Board consider as the bases for establishing groundwater standards for PFNA and PFOS that would be protective of children between ages of 0 to 6 years instead of ATSDR MRLs?*

I do not believe that sufficient data are available to derive a groundwater standard for PFNA. In the animal study selected by ATSDR for deriving the MRL, the researchers reported toxic effects in the pregnant females that make it difficult to interpret the effects in the offspring.[1] In addition, there is evidence from another study that the developmental effects used by ATSDR result from a mechanism that is unique to the laboratory animals that may be of limited relevance to humans.

For PFOS, ATSDR's analysis ignored the conclusions of the authors of the study selected for deriving the MRL when identifying the dose at which adverse effects were seen in the animal offspring.

As noted above, the calculation of the groundwater standard also should be based on exposure to the pregnant female not on the fetal animal's exposure.

Response to Pre-Filed Questions from the Illinois Environmental Protection Agency

1. *Do you disagree with U.S. EPA's RSC assessment using its Decision Tree that data is insufficient to allow for a quantitative characterization of different exposure sources? Please explain.*

I believe that sufficient data are available to more definitively characterize exposure to the PFAS included in the proposal, as described in USEPA's Decision Tree. Data from the Centers for Disease Control and Prevention (CDC) demonstrate that blood levels of

[1] USEPA. Guidelines for Developmental Toxicity Risk Assessment. Risk Assessment Forum. EPA/600/FR-91/001 (December 1991). (USEPA Developmental Toxicity Guidelines)

PFOA and PFOS have declined precipitously as a result of the decision by US manufacturers to phase out production of these two substances in the early 2000s. Levels of PFNA and PFHxS also have declined as these substances are no longer produced in the US. This decline in serum levels signals a significant drop in exposure to these substances – as manufacturers have switched to the use of other substances. As other sources of exposure have declined, the contribution of drinking water to total exposure has increased. As a consequence, the default assumption of an RSC of 0.2 – is no longer applicable for these legacy PFAS. Several state agencies including those in MI, NH, NY, and PA have reached this same conclusion.

2. *Are products containing PFOA, PFOS or other PFAS present in homes and businesses in Illinois that allow for exposure to PFAS?*

PFAS are a broad class of substances with vastly different physical and chemical properties. Although there are many uses of PFAS in products manufactured for homes and businesses, it is wholly inappropriate to suggest that all PFAS present an equivalent level of concern. For the six PFAS for which IEPA has proposed groundwater standards, exposure in product present in homes and businesses is likely to minimal.

Before PFOA manufacture ceased, it was used as a processing aid in the production of various fluoropolymers. It was not used in the production of products for homes and businesses. The same is true for HFPO-DA which replaced PFOA as a processing aid in fluoropolymer production.

US production of PFOS ceased nearly two decades ago. While it was widely used before being phased out, exposures have declined dramatically as evidenced by the CDC serum data. The same is true for PFNA and PFHxS, which while not as widely used as PFOS, have been phased out as well.

3. *Can these products provide humans, especially young children, a route for exposure to PFAS?*

Given the context of this rulemaking, the ACC assumes this question asks about potential groundwater exposure from PFAS-containing products. The ACC is not aware of such an exposure route. Moreover, as noted above, it is inappropriate to suggest that exposure to all PFAS presents a health concern.

4. *What do you consider the "applicable adult population" for calculating the HTTAC?*

The selection of an applicable adult population is dependent on the health endpoint on which the assessment of hazard is based. For example, as noted above, for effects resulting from *in utero* exposure, females of child-bearing age are the appropriate population.

5. *What would the appropriate daily water intake of liters per kilogram body weight per day be for an applicable adult population?*

As indicated above, the applicable population is dependent on the health endpoint of concern. According to USEPA's Exposure Factors Handbook,[2] the 50th percentile of water intake for adults is between 0.012 and 0.015 L/kg per day. The 95th percentile ranges from 0.037 to 0.047 L/kg per day.

6. *Is the applicable adult population daily water intake protective of sensitive populations, such as pregnant or lactating females, and young children?*

The selection of applicable adult population is dependent on the health endpoint on which the assessment of hazard is based. For example, as noted above, for effects resulting from *in utero* exposure, females of child-bearing age are the appropriate population for which USEPA assumes a daily water intake of 0.043 l/kg per day.

7. *Section 620.410 – Groundwater Quality Standards, discusses concerns with the PFAS toxicity assessments. Did you file your concerns regarding the PFOA toxicity assessment with California EPA during its peer-review and Public Comment sessions?*

IEPA's proposed groundwater standard for PFOA is based on an analysis conducted by California's Office of Environmental Health Hazard Assessment's (OEHHA) as part of its recommendation to the State Water Resources Control Board (SWRCB) for a Notification Level for PFOA in drinking water.[3] The OEHHA recommendation document was not made available for public comment and, to the ACC's knowledge, was not subject to peer review.

ACC submitted comments on the study that was basis for its recommendation to the SWRCB in response to OEHHA's call for information for the development of a Public Health Goal (PHG) for PFOA in January 2020. OEHHA released a draft PHG in July 2021 that used a different key study as a basis for its analysis. ACC submitted comments on the draft PHG in October 2021. OEHHA has not yet finalized the PHG or released a second draft for public comment.

a. *If yes, please provide a copy of your comments submitted to California EPA and California EPA's response to your comments.*

A copy of ACC's response to the call for information for the development of a PHG for PFOA is attached. (See Attachment 1.)

b. *If no, please explain why you are bringing up these concerns during Part 620 rulemaking and did not during the toxicity assessment.*

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2 https://www.epa.gov/expobox/about-exposure-factors-handbook

3 OEHHA. Notification Level recommendations: perfluorooctanoic acid and perfluorooctane sulfonate in drinking water. California Environmental Protection Agency (August 2019).

8. *Did you file your concerns regarding the PFBS toxicity assessment with U.S. EPA during its peer-review and Public Comment sessions during development of its Provisional Peer-Reviewed Toxicity Value (PPRTV)?*

ACC submitted comments on the public draft of the toxicity assessment for PFBS in January 2019. USEPA released a final assessment in January 2021 and a revised assessment in April 2021. It did not seek comment on the changes that were made as part of the April 2021 revision.

a. *If yes, please provide a copy of your comments submitted to U.S.EPA and U.S. EPA's response to your comments.*

A copy of ACC comments on the public draft of the toxicity assessment for PFBS are attached. (See Attachment 2.)

b. *If no, please explain why you are bringing up these concerns during Part 620 rulemaking and did not during the toxicity assessment.*

9. *Did you file your concerns regarding the PFHxS, PFNA and PFOS toxicity assessments with CDC's Agency for Toxic Substances and Disease Registry (ATSDR) during its peer-review and Public Comment sessions during development of its Minimal Risk Levels (MRLs) for these chemicals?*

ACC submitted comments on the public draft of the Toxicity Profile for Perfluoroalkyls in August 2018.

a. *If yes, please provide a copy of your comments submitted to ATSDR and ATSDR's response to your comments.*

A copy of the ACC's August 2018 comment to ATSDR is attached. (See Attachment 3.)

b. *If no, please explain why you are bringing up these concerns during Part 620 rulemaking and did not during the toxicity assessment.*

10. *Did you file your concerns regarding the HFPO-DA toxicity assessment with U.S. EPA Office of Water during its peer-review and Public Comment sessions during development of its toxicity value?*

ACC submitted comments on the public draft of the toxicity assessment for HFPO-DA in January 2019. USEPA released a final assessment in October 2021 that contained significant changes to its 2018 draft, including the use of a controversial health endpoint that USEPA had never used before. USEPA did not release a revised draft for public comment to seek input on the changes.

a. *If yes, please provide a copy of your comments submitted to U.S.EPA Office of Water and U.S. EPA Office of Water's response to your comments.*

A copy of the ACC's comments on the public draft of the toxicity assessment for HFPO-DA is attached. (See Attachment 4.)

b. *If no, please explain why you are bringing up these concerns during Part 620 rulemaking and did not during the toxicity assessment.*

11. *On what Method is the U.S. EPA's MRLs based?*

In presentations to drinking water utilities in June 2022,[4] USEPA's Office of Water indicated that the minimum reporting level (MRL) for PFOA and PFOS in drinking water is 4 parts per trillion (ppt). These MRLs are based on the requirement for the Fifth Unregulated Contaminant Monitoring Rule (UCMR 5) to use EPA Analytical Method 533 to measure the six PFAS included in IEPA's proposal. Under UCMR 5, public water systems in Illinois and throughout the country will be required to sample for 29 PFAS between 2023 and 2025 using Method 533.[5]

According to USEPA, the MRL is the minimum quantitation level that, with 95 percent confidence, can be achieved by a capable analyst at 75 percent or more of the laboratories using the specific analytical method.

12. *Does Method 537.1 have MRL of 0.000002 mg/L for each of the proposed PFAS?*

No. Method 537.1 provides single "laboratory lowest concentration minimum reporting levels" (LCMRLs) for the six PFAS in drinking water between 0.82 and 6.3 nanograms per liter (ng/L).[6] These LCMRLs values are not equivalent to the MRLs which EPA has determined can be reliably measured for the purposes of the UCMR 5 national survey. A copy of Method 537.1 is attached. LCMRLs are typically used to help develop MRLs but are not, and cannot be used as, MRLs.

13. *Does Method 537.1 provide the lowest concentration minimum reporting levels in potable water for PFAS?*

The reported LCMRLs for Method 537.1 are lower than those reported for Method 533, the other USEPA-validated method for analyzing potable water. The LCMRLs for Method 533 are reported to range from 3.4 to 4.8 ng/L. As noted above, USEPA will require public water systems in Illinois to use Method 533 for the six PFAS as part of the UCMR 5 survey.

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4 https://www.epa.gov/sdwa/drinking-water-health-advisories-pfoa-and-pfos

5 https://www.epa.gov/dwanalyticalmethods/method-533-determination-and-polyfluoroalkyl-substances-drinking-water-isotope

6 https://www.epa.gov/pfas/epa-pfas-drinking-water-laboratory-methods

Response to Pre-Filed Questions from 3M

1. *IEPA chose toxicity values for each PFAS from toxicity assessments conducted by other agencies according to a specific hierarchy. Did IEPA adequately consider the strengths and limitations of the underlying data for these toxicity assessments?*

   No. IEPA's selection of toxicity values appears to be based solely on the hierarchy of human sources of toxicity information health toxicity values data sources outlined by USEPA's Office of Solid Waste and Emergency Response (OSWER). IEPA has not provided information suggesting that it conducted an independent review of the underlying data.

2. *IEPA emphasizes relying on the most recent data when selecting toxicity values. Do the most recent studies always represent the most reliable and relevant data?*

   No. Although it is important to include the most recent data in an assessment of toxicology, the data must be viewed in the context of all of the available data to evaluate the weight of the scientific evidence. A study, whether new or old, may have methodological limitations that diminish its value to the assessment.

   IEPA's dependence on the OSWER hierarchy has resulted in its failure to consider more recent data and more recent assessments that incorporate these newer data. This is a major shortcoming of IEPA's use of the hierarchy that should be corrected. A rigid dependence on the hierarchy for selection of toxicity values may lead to use of older, less comprehensive assessments. This is a particular concern given the slow pace at which USEPA updates IRIS assessments.

3. *IEPA uses the default relative source contribution of 0.2 for calculating the proposed standards for five of the PFAS. Is the use of this default value appropriate, and if not, why not?*

   Sufficient data are available to more definitively characterize exposure to the PFAS included in the IEPA's proposal. Data from the CDC demonstrate that blood levels of PFOA and PFOS have declined precipitously as a result of the decision by US manufacturers to phase out production of these two substances in the early 2000s. Levels of PFNA and PFHxS also have declined as these substances are no longer produced in the US. This decline in serum levels signals a significant drop in exposure to these substances – as manufacturers have switched to the use of other substances. As other sources of exposure have declined, the contribution of drinking water to total exposure has increased. As a consequence, the default assumption of an RSC of 0.2 is no longer applicable for these legacy PFAS. This is the conclusion of several state agencies, including those in MI, NH, NY, and PA.

4. *IEPA uses the U.S. EPA's HFPO-DA toxicity value. Are there issues with the endpoint selected as the basis for the HFPO-DA toxicity value?*

Yes. These concerns are summarized in the attached request for correction filed by Arnold & Porter of USEPA's final assessment for HFPO-DA issued in October 2021. The concerns include:

- the use of health effects in animals that are of limited relevance,
- use of a new and unprecedented toxicological endpoint,
- misapplication of scientific criteria in identifying adverse effects,
- use of evaluation criteria that have not been peer reviewed, and
- use of improper and significantly inflated uncertainty factors.[7]

These changes to USEPA's October 2021 final assessment resulted in a significant lowering of the toxicity value. They were not included in the public draft of the assessment and were not made available for public comment prior to finalization of the assessment.

5. *The toxicity values that IEPA chose to use for calculating the proposed standards for HFPO-DA, PFBS, PFNA, and PFOS all used database uncertainty factors or modifying factors due to concerns that there is a lack of information regarding whether other effects, such as reproductive and developmental toxicity or immunotoxicity, are observed at lower exposure levels than the critical effects upon which the toxicity values were based. Are these database uncertainty factors appropriate?*

The uncertainty factors applied to derive the toxicity values selected by IEPA include the following:

	Uncertainty Factor				
	Animal to Human (UFA)	Human Variability (UFH)	Subchronic to Chronic (UFS)	Database Uncertainty/ (UFD)	Total
HFPO-DA	3	10	10	10	3000
PFBS	3	10	1	10	300
PFHxS	3	10	1	10	300
PFOS	3	10	1	10	300
PFNA	3	10	1	10	300

Although the application of a UFA of 3 and a UFH of 10 is consistent with standard practice, use of a UFD (or modifying factor) of 10 is not. Guidance developed by USEPA explains that a UFD is to be applied when reproductive and developmental toxicity studies are missing since they have been found to provide useful information for

[7] See Attachment 5.

establishing the lowest no adverse effect level.[8] USEPA guidance also notes that, for a reference dose (RfD) 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.[9] In deciding whether to apply an UFD, the guidance advises that the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages.

Because robust data is available on the reproductive and developmental effects of PFOS, PFBS, and HFPO-DA, IEPA's application of a UFD of 10 is wholly inconsistent with USEPA guidance (which, again, posits that a UFD should be used in the absence of reproductive and developmental toxicity studies). Although studies of the reproductive/developmental effects of PFHxS and PFNA are lacking, their absence is reflective of a larger general dirth of information on these substances. USEPA is currently developing IRIS assessments for these two substances. IEPA should defer the development of groundwater standards for at least these two substances until the assessments are available and peer reviewed.

For all five substances, the reviewing agency (USEPA or ATSDR) also suggests concerns about immunotoxicity as a basis for applying a UFD or modifying factor reflects. These concerns appear based on equivocal data available for PFOA and PFOS. Certainly, in the case of PFOS, there are data available to evaluate immunotoxicity. For the other four substances, in the absence of chemical-specific data to suggest immune effects, applying an uncertainty factor on the basis of this health effect is inappropriate.

It is worth noting that the total uncertainty factor of 3000 used to derive the toxicity value for HFPO-DA is the maximum value that USEPA could have conceivably used. USEPA has previously stated that any greater factor is considered too uncertain for toxicity assessment and for calculation of a reference dose.[10]

6. *The IEPA proposal mentioned both critical effects and adverse effects.*

   a. *Are all of the critical effects that form the bases for the toxicity values chosen by IEPA considered adverse effects?*

According to USEPA, adverse effects are those that cause harm to the normal functioning of a plant or animals due to exposure to a substance. For the reviews chosen by IEPA for PFOS, HFPO-DA, and PFNA, the effects considered critical by the reviewing agency (USEPA or ATSDR) should not be considered adverse.

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8 EPA Risk Assessment Forum. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F (December 2002). https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

9 Ibid, at 4-45.

10 Ibid, at 4-41.

For PFOS, ATSDR ignored the conclusion of the authors of the key study that the effects seen at the lowest dose were transient and not considered adverse. In ignoring this conclusion, ATSDR selected a significantly higher dose as the no observed adverse effect level (NOAEl), which significantly impacted its calculation of the toxicity value.

For PFNA, the adverse effects seen in the offspring of female mice occurred at a dose that also caused maternal toxicity. As noted by USEPA guidance, "at doses that cause excessive maternal toxicity (that is significantly greater than the minimal toxic level), information on developmental effects may be difficult to interpret and of limited value."[11]

As explained in greater detail in the attached correspondence from Arnold & Porter, USEPA's use of a "constellation of liver effects" is unprecedented and misapplies scientific criteria in determining whether the observed effects should be considered adverse.

b. Are the stated critical effects considered relevant to humans?

In addition to the questions about whether the observed effects in laboratory animal studies should be considered adverse, there is also a concern about whether the animal effects are relevant to humans. Many of the effects observed in the rodent studies, particularly liver and developmental effects, involve the activation of the peroxisome proliferator activated receptor (PPARα) or other nuclear receptors. Activation of the PPARα receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver.[12] The proliferation of peroxisomes has been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, and decreased pup survival and immune effects. Since humans and non-human primates have been found to be less responsive to PPARα agonists than rodents,[13] the relevance of the rodent findings to humans is highly questionable.

c. *Do you have any concerns with using non-adverse, non-human-relevant effects as the bases for toxicity values used in calculating groundwater standards? If so, what are they?*

Yes. The use of observed effects in laboratory animal studies that are non-adverse and/or of limited, or no, relevance to humans can lead to incorrect toxicity assessments. Selection of non-adverse effects of little relevance to humans can lead to overly conservative toxicity values that can result in public confusion, greater effort and additional, unnecessary costs.

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[11] USEPA Developmental Toxicity Guidelines, at 6.

[12] Kennedy GL *et al.* The toxicology of perfluorooctanoate. *Crit Rev Toxicol* 34(4):351-384 (2004).

[13] Corton JC *et al.* Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPARα) as a case study. *Crit Rev Toxicol* 44(1):1–49 (2014).

7. *What did the 2019 National Toxicology Program (NTP) 28-day toxicity studies of various PFAS show with respect to the human relevance of the reported effects of these substances?*

NTP's 28-day study exposed Sprague-Dawley (SD) rats to various concentrations of seven PFAS by gavage – PFBS, PFHxA, PFHxS, PFOA, PFOS, PFNA, and PFDA. An additional group of animals was exposed to Wyeth-14,643 (a PPARα agonist) for qualitative comparison to the PFAS-exposed groups. The researchers evaluated clinical pathology, thyroid hormones, expression of PPARα and another nuclear receptor CAR, liver enzymes, blood concentrations, and histopathology. They reported that many of the effects observed in the liver of the PFAS-exposed animals were also observed in the rats administered Wyeth-14,643 – indicating that these effects are likely mediated by PPARα and thus may not be of relevance to humans.

8. *IEPA chose a cancer toxicity value for PFOA from an assessment of PFOA carcinogenicity by OEHHA, which was based on a carcinogenicity study conducted by NTP.*

The NTP bioassay study reported liver adenomas in male SD rats and pancreatic adenomas in male and female rats exposed to PFOA in food.[14] In the study, male rats were exposed post-weaning to up to 80 parts per million (ppm) while females were exposed to up to 1000 ppm.[15] The study also reported significant increases in hepatocyte cytoplasmic alteration and hypertrophy in the males in all the exposure groups. The study also noted a significant increase in pancreatic hyperplasia - a potentially preneoplastic lesion - in all the male groups, including the control group in which hyperplasia was reported in 36 percent of the animals.

The high background rate observed in this study is consistent with the historical sensitivity of the Sprague-Dawley rats compared to other rat stains – and more significantly when compared to humans.

a. Are the results of this study reliable?

According to the report, the male portion of the study was repeated using significantly lower exposures after "unanticipated toxicity" was observed in male rats exposed to 150 and 300 ppm after 16 weeks. In light of the fact that male SD rats tolerated doses as high as 300 ppm in a previous chronic study,[16] the reports of unanticipated toxicity at

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[14] NTP. Technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid administered in feed to Sprague-Dawley rats. Technical Report 598. Department of Health and Human Services. Research Triangle Park, North Carolina (2019).

[15] The study included groups of animals exposed to PFOA perinatally and post-weaning to assess the potential impact of gestational and lactational exposure but reported very few significant differences between the response in animals exposed post-weaning only to those with both perinatal and post-weaning exposure.

[16] Butenhoff JL *et al.* Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicol* 298(1–3): 1–13 (2012).

comparable levels in the male rats in the NTP study raise concerns about the overall confidence in the study.

b. Are the tumors observed in this study relevant to humans?

Likely not. The tumor types observed in the NTP study – liver, pancreas – have been observed with other substances that are PPARα agonists. Because of key toxicodynamic and biological differences in responses between rodents and humans, PPARα activators are considered unlikely to induce liver and pancreatic tumors in humans.

For liver tumors, this conclusion is based on minimal or no effects observed on growth pathways, hepatocellular proliferation and liver tumors in humans and/or species (*e.g.*, hamsters, guinea pigs and *Cynomolgous* monkeys) that are more appropriate animal model surrogates than mice and rats. The relevance of the liver tumor data from laboratory studies is further called into question as a result of a clinical study of a subpopulation of cancer patients with normal liver function exposed to weekly PFOA doses as high as 1,200 milligrams which reported no differences in clinical hepatic measures. [17]

For the induction of rat pancreatic tumors by PFOA, the available mechanistic data are less robust, but also point to the importance of PPARα activation in the liver. The high background rate observed of pancreatic hyperplasia in the NTP study is consistent with the historical sensitivity of the Sprague-Dawley rats compared to humans.

c. Is there any evidence to indicate that PFOA is carcinogenic to humans?

PFOA has been reported to cause liver tumors in laboratory animal studies, but the available epidemiology evidence does not support an association with liver cancer in humans. Reports of kidney cancer in epidemiology studies are conflicting, and not supported by the results of the animal bioassays.

9. *IEPA's proposal uses the terms "minimum reporting level," "quantification limit," and "method detection limit."*

a. What is the method detection limit (or MDL)?

USEPA defines the MDL as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured analyte concentration is distinguishable from method blank results. This is a statistical determination of precision, and accurate quantitation is not expected at this level.

b. What is a minimum reporting level?

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[17] Convertino M *et al.* Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systematic health risk of perfluorooctanoate (PFOA). *Toxicol Sci* 163(1) 293-306 (2018).

According to USEPA, the minimum reporting level, or MRL, is the minimum quantitation level that, with 95 percent confidence, can be achieved by a capable analyst at 75 percent or more of the laboratories using the specific analytical method.

c. What is a quantification limit?

USEPA defines the quantification limit, or minimum level of quantitation, as the lowest concentration at which an analyte can be measured with a known level of confidence.

10. *What is the difference between a method detection limit and a minimum reporting level?*

While the MDL indicates the level at which the method can determine whether the substance is present in a sample, the MRL indicates the level above which the substance can be reliably measured. In other words, an MRL can be used for reliable quantitative purposes while and MDL cannot.

11. *What are the method detection limits and minimum reporting levels for the PFAS at issue in the IEPA proposal?*

EPA Method 533, the method USEPA will require for reporting under the UCMR 5 national survey for the six PFAS included in the IEPA proposal, provides the following single laboratory LCMRLs:

	Calculated LCMRL
HFPO-DA	3.7 nanograms per Liter (ng/L)
PFBS	3.5
PFHxS	3.7
PFOA	3.4
PFOS	4.4
PFNA	4.8

MRLs and detection limits are not provided for the method.

Method 537.1 has the following limits for the six PFAS of the IEPA proposal:

	Detection Limit	LCMRL
HFPO-DA	1.9 ng/L	4.3 ng/L
PFBS	1.8	6.3
PFHxS	1.4	2.4
PFOA	0.53	0.82
PFOS	1.1	2.7
PFNA	0.70	0.83

MRLs are not provided.

12. *Does the accuracy or reliability of a testing method change depending on how close the result is to the method detection limit?*

Although the qualitative accuracy of the method is not affected by the proximity to the method detection limit, results below the MRL are quantitatively unreliable. As noted above, measurements below the MRL should not be considered to reflect the actual concentration.

13. *Does the accuracy or reliability of a measurement of a substance change depending on how close the result is to the minimum reporting level?*

Results below the MRL cannot be viewed to reliably represent a sample's actual concentration. For results above the MRL, the accuracy of results can be expected to be less reliable at lower concentrations. This is due to the fact that, according to USEPA, the MRL is determined by the sensitivity of the method, as well as the capabilities of the laboratory and the analyst. The lower the concentration in a sample, the greater the possibility that measurement will exceed the analyst's or laboratory's capabilities.

14. *Is there a point at which the accuracy or reliability of a measurement of a substance falls below 50%?*

The closer the concentration is to the MDL, the lower the reliability of the measurement.

15. *What testing methods have been approved and validated for use to measure PFAS in drinking water? How about groundwater?*

USEPA has validated two testing methods for measure PFAS in drinking water. Method 537.1 can measure 18 individual substances; Method 533 can measure 25 substances, including 14 substances measured by Method 537.1.

USEPA has validated one method for measuring PFAS in non-potable water. Method 8327 can measure 24 PFAS. A second method for measuring PFAS in aqueous samples (Method 1633) has not yet been finalized. It can reportedly measure 40 PFAS.

16. *Does U.S. EPA's method 537.1 for PFAS in drinking water provide information regarding the accuracy and precision of PFAS measurements for any PFAS at various concentrations?*

According to USEPA, the detection limit of the method provides a statistical determination of the method's precision. Those values are provided above.

CERTIFICATE OF SERVICE

I, the undersigned, certify that I have today filed the attached NOTICE OF FILING and PRE-FILED QUESTIONS to the PRE-FILED TESTIMONY OF DR. ROBYN PRUEITT in PCB R2022-18 upon the below service list by electronic mail.

Dated: November 23, 2022

Respectfully Submitted,

AMERICAN CHEMISTRY COUNCIL

By: /s/ Stephen P. Risotto

Stephen P Risotto
Senior Director
Chemical Products and Technology Division

700 2nd Street, Northeast
Washington, DC 20002
(202) 249-6727
srisotto@americanchemistry,com

Service List

Melissa S. Brown
Melissa.brown@heplerbroom.com
HeplerBroom LLC
4340 Acer Grove Drive
Springfield, IL 62711

Sara Terranova, Assistant Counsel
sara.terranova@illinois.gov
Nicholas E. Kondelis, Assistant Counsel
Nicholas.E.Kondelis@illinois.gov
Illinois Environmental Protection Agency
1021 North Grand Avenue East
PO Box 19276
Springfield, Illinois 62794

Don Brown, Clerk of the Board
don.brown@illinois.gov
Vanessa Horton, Hearing Officer
Vanessa.Horton@Illinois.gov
Chloe Salk, Hearing Officer
Chloe.Salk@illinois.gov
Illinois Pollution Control Board
100 West Randolph Street
Suite 11-500
Chicago, Illinois 60601

Jorge T. Mihalopoulos, Head Assistant Attorney
jorge.mihalopoulos@mwrd.org
Susan T. Morakalis, General Counsel
morakaliss@mwrd.org
J. Mark Powell, Senior Attorney
PowellJ@mwrd.org
Metropolitan Water Reclamation District of Greater Chicago
100 E. Erie Street
Chicago, IL 60611

Frederic P. Andes
fandes@btlaw.com
Barnes & Thornburg
One North Wacker Drive
Suite 4400
Chicago, Illinois 60606

Claire A. Manning
cmanning@bhslaw.com
Anthony D. Schuering
aschuering@bhslaw.com
Brown, Hay & Stephens, LLP
205 South Fifth Street
Suite 700
Springfield, IL 62705

Nessa Coppinger
ncoppinger@bdlaw.com
Daniel Schulson
dschulson@bdlaw.com
Matthew Schneider
mschneider@bdlaw.com
Beveridge & Diamond, PC
1900 N Street, NW
Washington, DC 20036

Renee Snow, General Counsel
renee.snow@illinois.gov
Illinois Department of Natural Resources
One Natural Resources Way
Springfield, Illinois 62702-1271

Ellen F. O'Laughlin, Senior Assistant Attorney General
Ellen.Olaughlin@ilag.gov
Jason James, Assistant Attorney General
Jason.James@ilag.gov
Office of the Attorney General
69 West Washington Street
Suite 1800
Chicago, IL 60602

Sandra Carey
sandracarey@imoa.info
International Molybdenum Association
454-458 Chiswick High Road
London W4 5TT
United Kingdom

Joshua R. More
Joshua.More@afslaw.com
Bina Joshi
Bina.Joshi@afslaw.com
Sarah L. Lode
Sarah.Lode@afslaw.com
ArentFox Schiff LLP
233 South Wacker Drive
Suite 6600
Chicago, IL 60606

James M. Morphew
jmmorphew@sorlinglaw.com
Sorling Northrup
1 North Old State Capitol Plaza
Suite 200
Springfield, IL 6270

BY ELECTRONIC MAIL

January 22, 2019

Docket EPA-HQ-OW-2018-0614
The Honorable David P. Ross
Assistant Administrator, Office of Water
US Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Re: Request for Public Review and Comment: Draft Human Health Toxicity Assessments for Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt (GenX Chemicals) and for Perfluorobutane Sulfonic Acid (PFBS) and Related Compound Potassium Perfluorobutane Sulfonate (83 *Fed. Reg.* 58768, November 21, 2018)

Dear Assistant Administrator Ross:

The Chemical Products and Technology Division of the American Chemistry Council (ACC- CPTD) submits the following comments on the draft human health toxicity assessments for hexafluoropropylene oxide dimer acid (HFPO) and its ammonium salt (GenX chemicals) and perfluorobutane sulfonic acid (PFBS) and potassium perfluorobutane sulfonate. ACC-CPTD represents companies interested in ensuring that the evaluation of perfluoroalkane substances (PFAS) such as GenX and PFBS incorporate the best available science.

ACC-CPTD appreciates EPA's efforts to engage stakeholders in the development of toxicity values for these two substances. We also support the transparent presentation of the Agency's systematic reviews of the available data for these products in the drafts. We note, however, that the Agency uses two separate approaches to systematic review in the documents, and urge EPA to ensure that the approaches are not in conflict and maximize the best of both approaches. Given the precedent that these documents likely will set, moreover, it is critical that the drafts be reviewed by the Agency's Science Advisory Board prior to their finalization.

In addition to the need for a broader review of these draft documents, we submit the following comments on the documents.

General Comments

ACC/CPTD supports the use of the default approach of body-weight scaling to estimate the human equivalent dose (HED) for the selected animal studies. Although the data are not sufficient to model external dose and clearance in humans, the information available for GenX and PFBS suggest that the substances are eliminated from the body relatively rapidly and will not accumulate as with PFOA, PFOS, and other legacy PFAS. As a result, body-weight scaling is the appropriate approach to estimating the HED.

In addition to the need to eliminate potential contradictions and increase consistency in the systematic review approaches taken in the two assessments, it is important that the results of the reviews be wholly transparent to promote confidence in the results – particularly since many of the studies considered are not publicly available. In this regard, ACC-CPTD encourages EPA to revise the PFBS assessment to include a more robust presentation of the systematic review results similar to that provided in the GenX assessment. Although the PFBS assessment provides significant details of the Agency's review, the information is neither as readily accessible nor as clearly presented as that for the GenX chemicals. To some degree, the difference in presentation reflect differences in the EPA offices conducting the reviews, but some specific consideration of relative strengths of one approach over the other would aid in transparency across Agency offices.

For example, the incorporation of more thoughtfully developed exclusion criteria gives the GenX assessment greater transparency compared to the PFBS assessment. Another strength is the predesignated mathematical evaluation of included studies, which helps smooth the implications of skewed evaluation due to potential bias in selection. Consistent, thoughtful designation of weighting factors to account for the fact that some domains are more important to the quality and utility of study findings than others also gives the GenX method an advantage over the PFBS method.

One general concern with both approaches is that neither provides a method for truly integrating the various data streams in determining the most appropriate basis for defining a toxicity value. Both approaches allow for a single study to define the health outcome for regulatory purposes, even when other relevant data are available. In particular, eliminating data because it does not produce the most conservative value is, by definition, not taking into account **all available** information in the final assessment. This remains a major flaw in most systematic review processes, regardless of which Agency office is the author.

Comments on the Assessment of GenX Chemicals

EPA bases its toxicity value for the GenX chemicals on liver effects reported in a mouse reproductive/developmental toxicity screening study,[1] despite the fact that a 90-day subchronic study is available which provides additional, relevant hepatic measurements.[2] Although evidence for liver hypertrophy is often considered to be rodent-specific, indications of histological and clinical pathological changes warrant additional consideration as to the relevance to humans.[3] Both the reproductive/development and 90-day studies provide information on liver cell necrosis, but the 90-day study also includes information on key clinical chemistry – including alanine aminotransferase (ALT), alkaline phosphatase (ALP) and aspartate aminotransferase (AST). The elevation of these enzyme levels provides important clinical correlations to the observed changes in pathology. In its assessment, EPA dismisses the results from the 90-day study because of the smaller sample size[4] without addressing the other significant aspects of the two studies.

The longer exposure time in the 90-day study should improve the chances to observe necrosis, despite the lower statistical power. The consistency of the necrosis data with the liver enzyme results, moreover, provides a more complete picture of what is happening in the liver than the more limited data available from the reproductive/developmental study used in the assessment. EPA's concern about the statistical power of the 90-day study is further eroded by the fact that the authors did not observe necrosis in any of the animals exposed to levels of 0.5 mg/kg-day or less. The minimal necrosis reported at these levels in the reproductive/ developmental study may suggest an adaptive, non-adverse reaction in the mice or a response to other stressors for which no acknowledgement has been made.

The decision to reject the liver results from the 90-day study also raises concerns about the approach the Agency has taken in integrating data from the various studies as part of its systematic review. Both of the studies in question were assigned an overall quality level of "High" in the Agency's data evaluation tables.[5] In particular, both studies received the best possible weighted score of "1" in relation to the number of animals per group. Any concern about the number of animals in the 90-day study should have been reflected in the data

[1] E.I. du Pont de Nemours and Company. An oral (gavage) reproduction/developmental toxicity screening study of H-28548 in mice. U.S. EPA OPPTS 870.3550; OECD Test Guideline 421. Conducted by WIL Research Laboratories, LLC, Ashland, OH (2010). **DuPont-18405-1037**.

[2] E.I. du Pont de Nemours and Company. H-28548: subchronic toxicity 90-day gavage study in mice. OECD Test Guideline 408. E.I. du Pont de Nemours and Company, Newark, DE. (2010). **DuPont-18405-1307**.

[3] Hall AP *et al.* Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes – conclusions from the 3rd international ESTP expert workshop. *Toxicologic Pathol* 40:971-994 (2012).

[4] 10 animals/exposure group versus 24/group in the reproductive/developmental study.

[5] GenX assessment, Appendix B, at B91-21, B95-96.

evaluation and scoring, not as part of an arbitrary decision to choose one study over another based solely on generating a lower value.

Based on the liver effects reported in the 90-day study, the lowest observed adverse effect level (LOAEL) is 5.0 mg/kg-day and the no observed adverse effect level (NOAEL) is 0.5 mg/kg-day. A benchmark dose analysis of the data conducted by North Carolina's Department of Health and Human Services suggests the lower limit of the benchmark dose (BMD) for 10 percent extra risk ($BMDL_{10}$) of between 0.449 and 0.466 mg/kg-day.[6] Using these values as the point of departure would generate a HED of 0.067 to 0.070 mg/kg-day[7] - compared to the value of 0.023 mg/kg-day presented by EPA, reflecting more than a 300% difference based on this alternate interpretation of the available data.

The draft toxicity assessment also includes a data base uncertainty factor (UF_D) of 3 based on limited testing of developmental toxicity and immunological responses for HFPO. Although data from a 2-generation reproductive toxicity and additional immunotoxicity studies would be valuable, the available evidence suggests that any developmental and immune effects are likely to occur at exposure levels that are comparable to the liver effects that are the basis of the draft toxicity value. Two studies investigating developmental and reproductive effects are available – the mouse study previously discussed (Dupont-18405-1037) and a prenatal developmental toxicity study in rats.[8] While these studies have reported developmental effects, the LOAELs and NOAELs for the most sensitive effect (pup body weight in mice) are consistent with the liver results. Similarly, a study of immunological effects which suggests T cell-dependent antibody response (TDAR) suppression in mice treated with 100 mg/kg-day[9] – well above the NOAEL/LOAEL reported in the liver studies. Other studies reported decreases in spleen weight after 28 days, but again only when treated with 100 mg/kg-day. Based on these data, it is reasonable to conclude that toxicity values generated from the liver effects observed in the 90-day study will provide sufficient protection against potential developmental and immunotoxic effects, and obviates the need to assign an additional uncertainty factor for the GenX chemicals.

[6] NC Department pf Health and Human Services. Benchmark dose modeling report for GenX. Report to the NC Secretaries Science Advisory Board (June 8, 2018), at 500. Available at https://files.nc.gov/ncdeq/GenX/SAB/NC-DHHS-BMD-Report-Supplemental-Documentation-8Jun2018.pdf.

[7] Assuming a dosimetric adjustment factor of 0.15.

[8] E.I. du Pont de Nemours and Company. An Oral (Gavage) Prenatal Developmental Toxicity Study of H-28548 in Rats. U.S. EPA OPPTS 850.3700; OECD Test Guideline 414. Conducted by WIL Research Laboratories, LLC Ashland, OH (2010). **DuPont-18405-841**

[9] Rushing B *et al.* Evaluation of the immunomodulatory effects of 2,3,3,3-tetrafluoro-2(heptafluoropropoxy)-propanoate in C57BL/6 mice. *Toxicol Sci* 156(1):179– 189 (2017)

Comments on the Assessment of PFBS

EPA's draft report for PFBS presents the results of its BMD analysis of key studies using multiple benchmark response rates (BMRs), including 10 percent, 20 percent, 1 standard deviation, and 0.5 standard deviation. Although the draft suggests that the multiple BMRs are "for comparison purposes," it does not attempt to critically compare the significant biological or mathematical difference in the results. For example, the PFBS draft presents the BMD and BMDL for thyroid effects for response deviations of 20 percent and one standard deviation.[10] Although the lower limit for both deviation scenarios is about the same (1.15 vs 1.11 micrograms/milliliter), the BMDLs differ considerably. The document explains why it chose the two different response rates, but does not attempt to evaluate the reasons for the different results or their potential significance.

In calculating the toxicity value for PFBS, EPA includes a UF_D of 3 for the subchronic toxicity values and a UF_D of 3 or 10 for the chronic values.[11] According to the draft, the decision to include a data base uncertainty factor is based on a lack of information on neurodevelopmental and immunotoxicity effects. For PFBS, however, robust data are available on reproductive and developmental effects, including both a prenatal toxicity study and a two-generation reproduction study. EPA notes, moreover, that developmental effects appear to be "less sensitive than thyroid hormone perturbations in developing mice."[12]

Consequently, a toxicity value that protects against effects on thyroid hormones also will protect against developmental effects, particularly effects on neurodevelopment since EPA's stated concern is that perturbations in thyroid hormones may trigger neurodevelopmental effects. After pointing out the connection between thyroid hormones and neurodevelopment, EPA provides no rationale for why neurodevelopmental effects should then be considered separately. This should be revised and refined as the most sensitive endpoint was already selected.

The Agency's concern for the potential immunotoxicity of PFBS is based entirely on suggestions of immunotoxicity for other PFAS. In explaining the addition of the UF_D, the Agency suggests that "immunotoxicity is an effect of increasing concern across several members of the larger PFAS family." In fact, to date, EPA has critically evaluated the immunotoxicity data for only two PFAS. In each case, the Agency has concluded that the available data did not suggest

[10] PFBS assessment, at 56.

[11] The EPA assessment does not explain why it applies different data base uncertainty factors to calculate the two chronic toxicity values, despite providing the same rationale for applying a UF_D for both endpoints.

[12] PFBS Assessment, at 60.

that immune effects are a particularly sensitive health endpoint.[13] ACC-CPTD is not aware of other data that would suggest that immunotoxicity is a concern for PFBS, which -- as clearly demonstrated by EPA's analysis -- exhibits dramatically different properties than the two PFAS previously evaluated.

Given the above concerns about the draft assessments, ACC-CPTD urges the Agency to seek their review by the Science Advisory Board to ensure that they present a consistent approach to evaluating animal data and can provide a template for evaluating other PFAS in the future. Please feel free to contact me at 202-249-6727 or srisotto@americanchemistry.com if you have questions about the above information.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

cc: Dr. Jaime Strong, OW
Dr. Samantha Jones, ORD

[13] EPA. Drinking water health advisories for perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA).

Arnold & Porter

Brian D. Israel
+1 202.942.6546 Direct
Brian.Israel@arnoldporter.com

March 18, 2022

Via Electronic Mail (quality@epa.gov)
Information Quality Guidelines Staff
U.S. EPA Headquarters
1200 Pennsylvania Ave., NW
Mail Code 2821T
Washington, DC 20460

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/.

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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:

[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.

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- 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").

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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
>
> 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
>
> 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).

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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

PREPARED FOR:
Arnold & Porter
Washington, DC

PREPARED BY:
ToxStrategies, Inc.
9390 Research Blvd.
Suite 100
Austin, TX 78759

Report Authors

Haws, Laurie C., Ph.D., DABT, ATS; Managing Principal Scientist; ToxStrategies, Inc.

Klaunig, James E., Ph.D., ATS, FIATP; Professor Emeritus; Indiana University

Thompson, Chad M., Ph.D., M.B.A.; Senior Consultant; ToxStrategies, Inc.

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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.

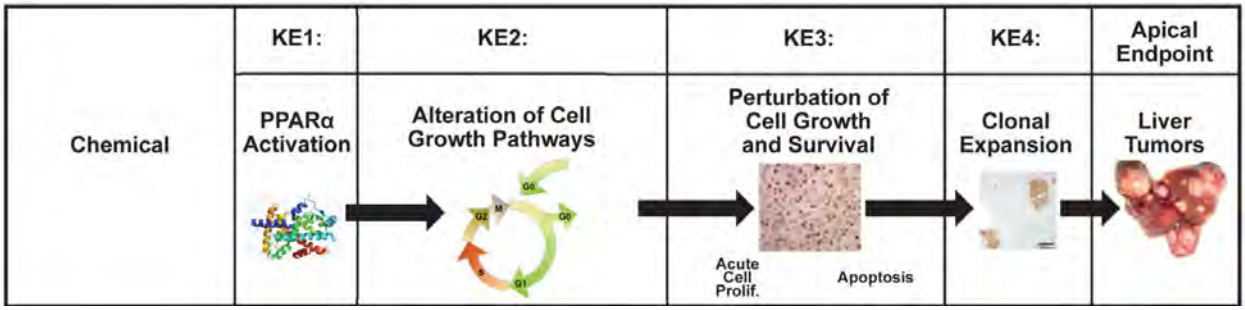

Figure 1. MOA for PPARα-induced liver tumors in mice and rats (Corton et al., 2018).

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.

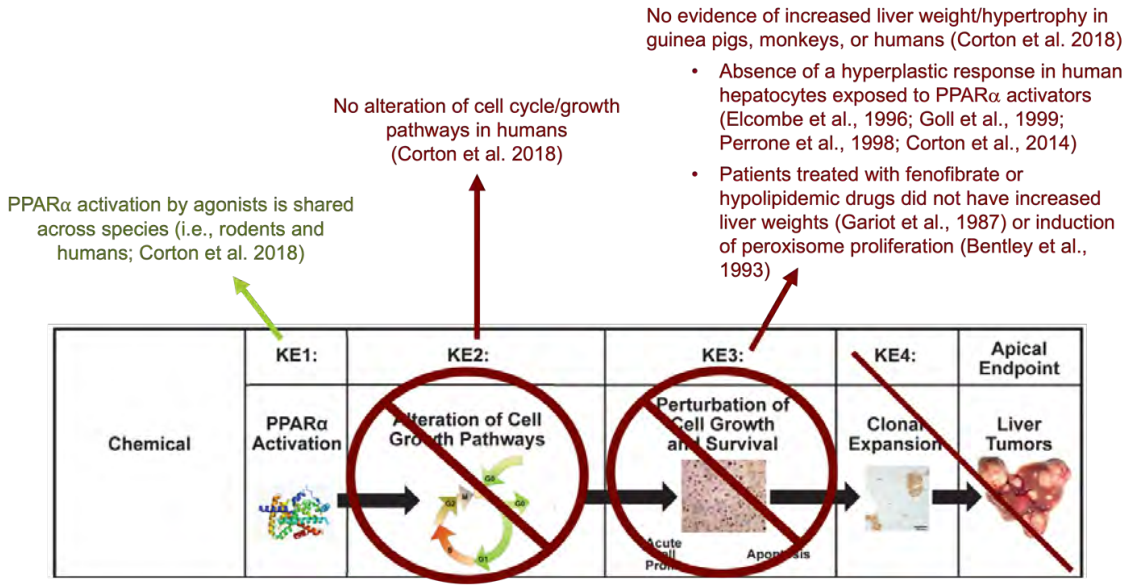

Figure 2. Human relevance of PPARα MOA in liver (modified from Corton et al. 2018).

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**).

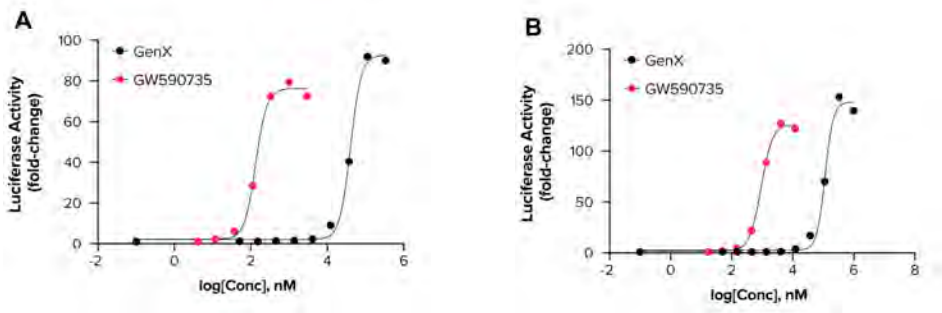

Figure 3. Activation of mouse (A) and rat (B) PPARα receptors by HFPO-DA in cell reporter assays (Chappell et al., 2020).

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.

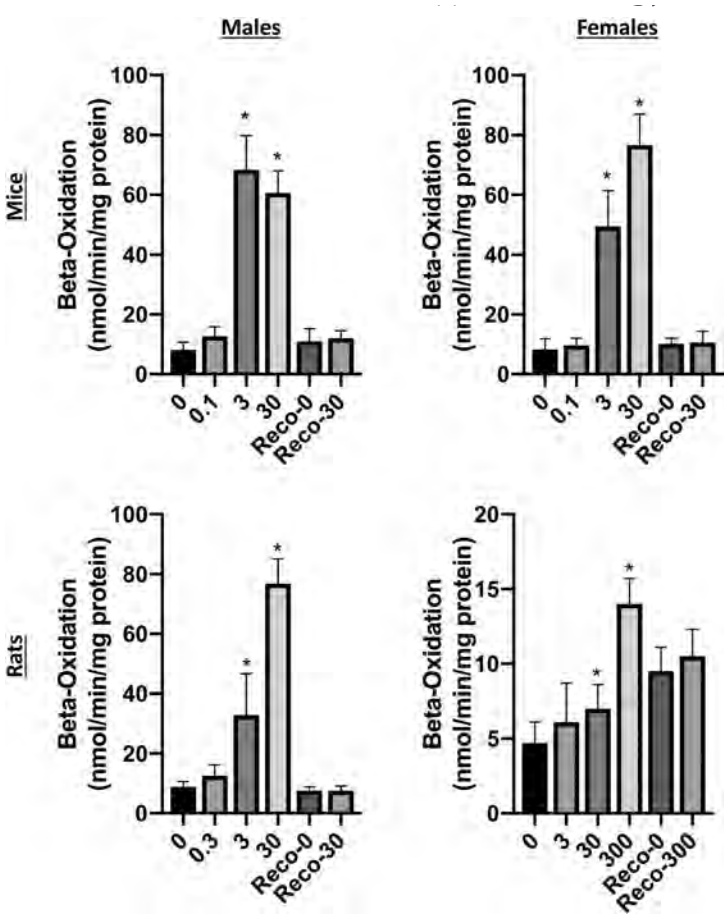

Figure 4. β-oxidation activity in mice and rats after 28-day exposure to HFPO-DA (Thompson et al., 2019).

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).

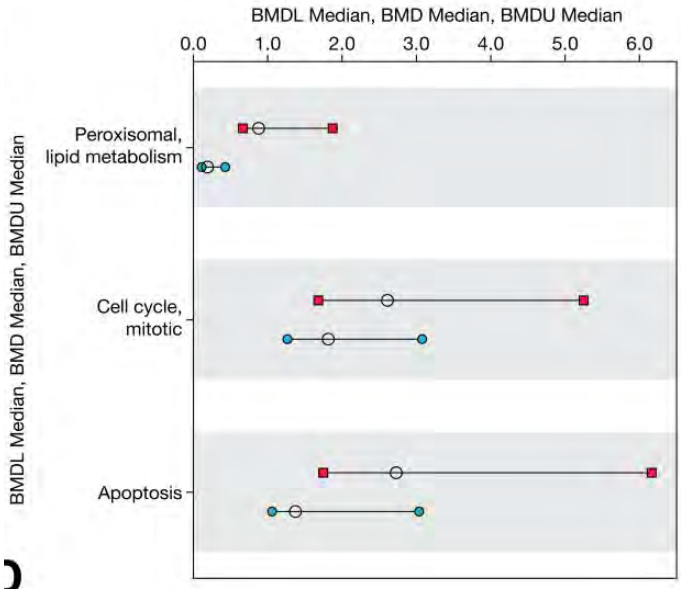

Figure 5. BMDL, BMD, and BMDU values for pathways related to peroxisomal lipid metabolism, cell proliferation, and apoptosis in mouse liver following 90 days of exposure to HFPO-DA (Chappell et al., 2020).

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.

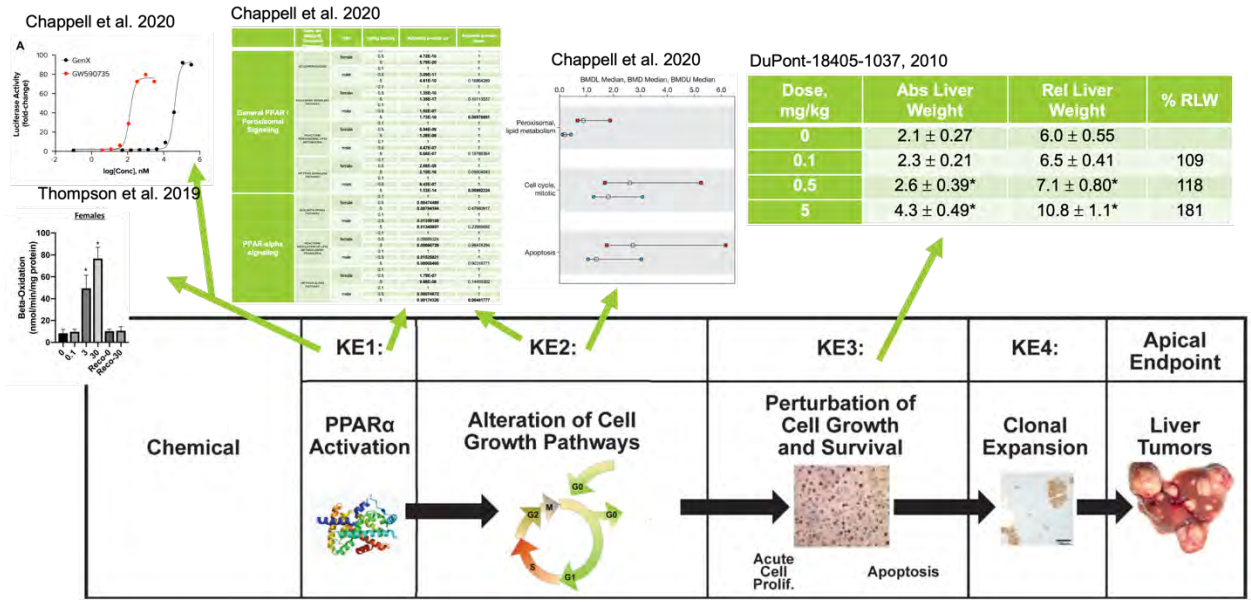

Figure 6. Empirical data supporting the PPARα MOA (from Corton et al. 2018) for HFPO-DA-induced liver effects in rodents.

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

ToxStrategies

Innovative solutions
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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

PREPARED FOR:
Arnold & Porter
Washington, DC

PREPARED BY:
ToxStrategies, Inc.
9390 Research Blvd.
Suite 100
Austin, TX 78759

Report Authors

Cullen, John M., Ph.D., V.M.D., FIATP; Professor of Pathology-Emeritus; North Carolina State University

Haws, Laurie C., Ph.D., DABT, ATS; Managing Principal Scientist; ToxStrategies, Inc.

Thompson, Chad M., Ph.D., M.B.A.; Senior Consultant; ToxStrategies, Inc.

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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 harm to the test animal.

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

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

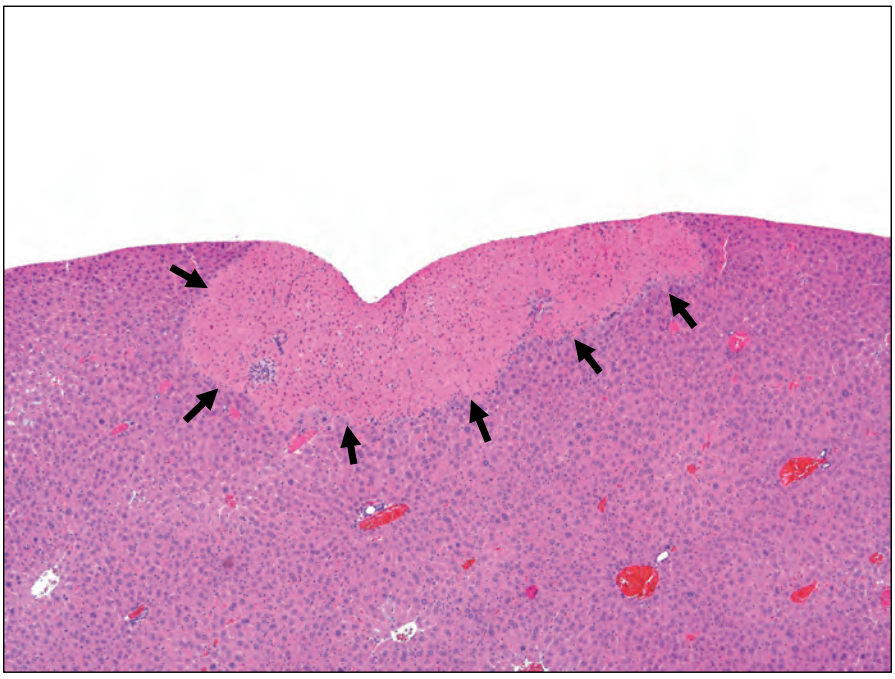


Figure 1. Focal necrosis (area delineated by arrows) in the liver of a Group 4 male mouse (animal 456) from the 18405-1307 subchronic study (as presented in USEPA (2021) Appendix D Figure 6).

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

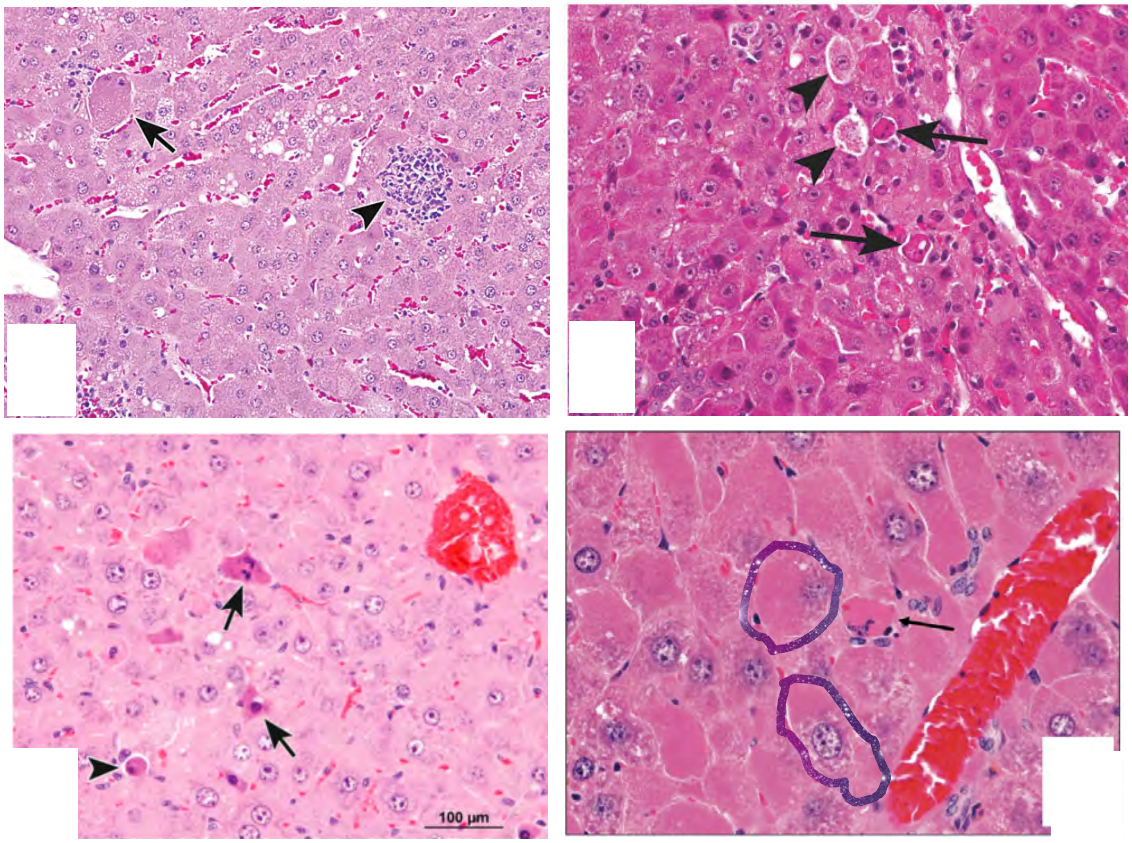
6.1 H&E staining intensity

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.

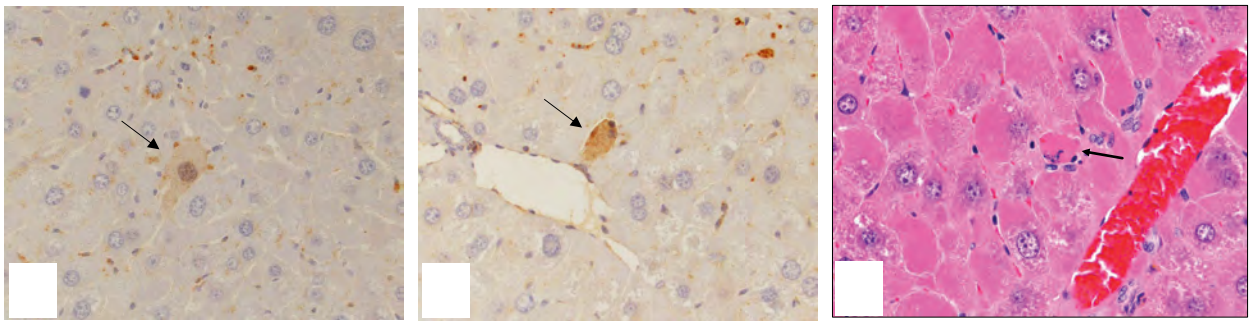


Figure 3. Examples of non-shrunken apoptotic hepatocytes. (A) Example of a swollen hepatocyte (arrow) with an irregular outline and with pale Caspase-3 staining. (B) Example of a caspase-3 stained hepatocyte (arrow) that has an oblong outline. (C) Example of a hepatocyte categorized as an individual 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.

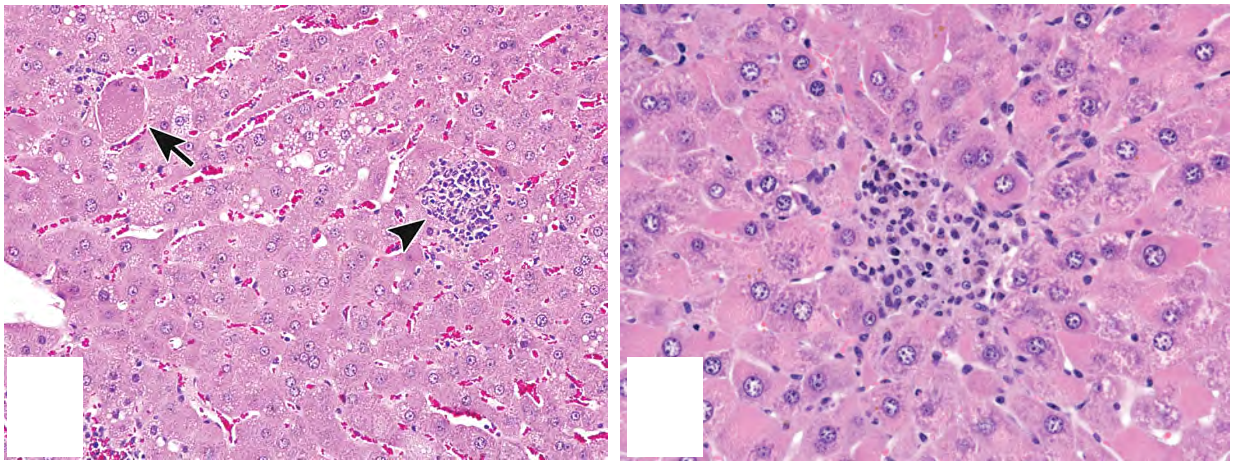


Figure 4. Examples of necrotic hepatocytes and mixed cell infiltrates. (A) "Examples of single cell necrosis in the liver. There is marked cell swelling and karyorrhexis in a necrotic hepatocyte (arrow) and a nearby small focus of inflammation (arrowhead), most likely secondary to cell rupture", *although 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.

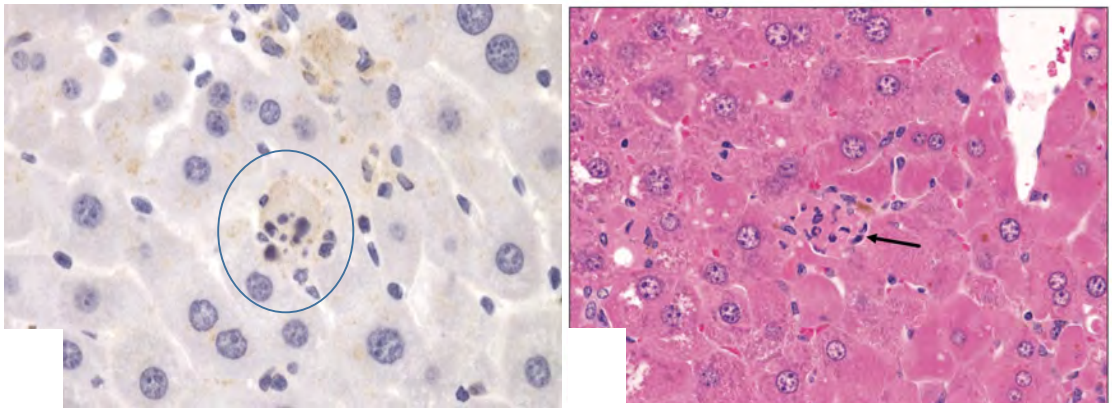


Figure 5. Examples of inflammation associated with an individual hepatocyte. (A) An example of a caspase-3 stained hepatocyte, indicative of apoptosis, with inflammatory cells adjacent (circled). Example of inflammatory cells in contact a with caspase-3 positive hepatocytes from a liver section of a 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

PREPARED FOR:
Arnold & Porter
Washington, DC

PREPARED BY:
ToxStrategies, Inc.
9390 Research Blvd.
Suite 100
Austin, TX 78759

Report Authors

Haws, Laurie C., Ph.D., DABT, ATS; Managing Principal Scientist; ToxStrategies, Inc.

Thompson, Chad M., Ph.D., M.B.A.; Senior Consultant; ToxStrategies, Inc.

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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.

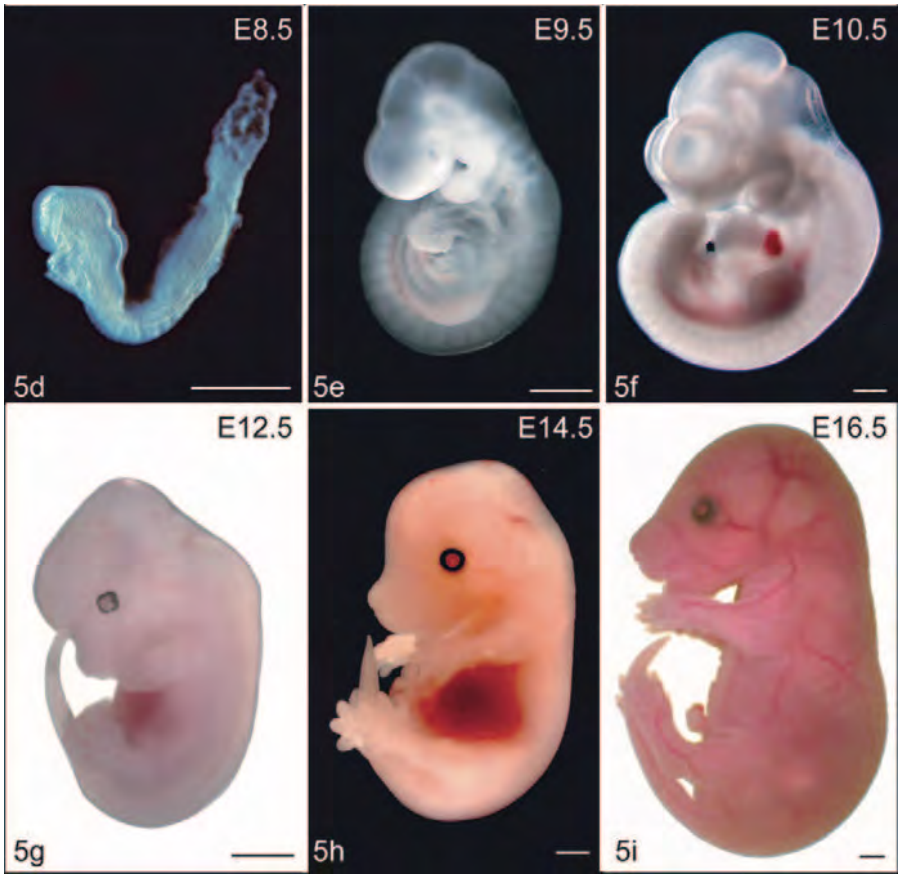

Figure 1. Development of the mouse embryo/fetus from E8.5 – E16.5.
Source: Papaioannou and Behringer (2012).

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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USEPA. 2021b. Toxicological Review of Perfluorobutanoic Acid (PFBA) and Related Compound Ammonium Perfluorobutanoic Acid [CASRN 375-22-4 CASRN 10495-86-0]. Environmental Protection Integrated Risk Information System, EPA/635/R-20/424a.

USEPA. 2022. Toxicological Review of Perfluorohexanoic Acid [CASRN 307244] and Related Salts. U.S. Environmental Protection Integrated Risk Information System, EPA/635/R-21/312a.

EXHIBIT 4

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

Damian Shea, Ph.D.
Statera Environmental
Raleigh, NC 27606

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

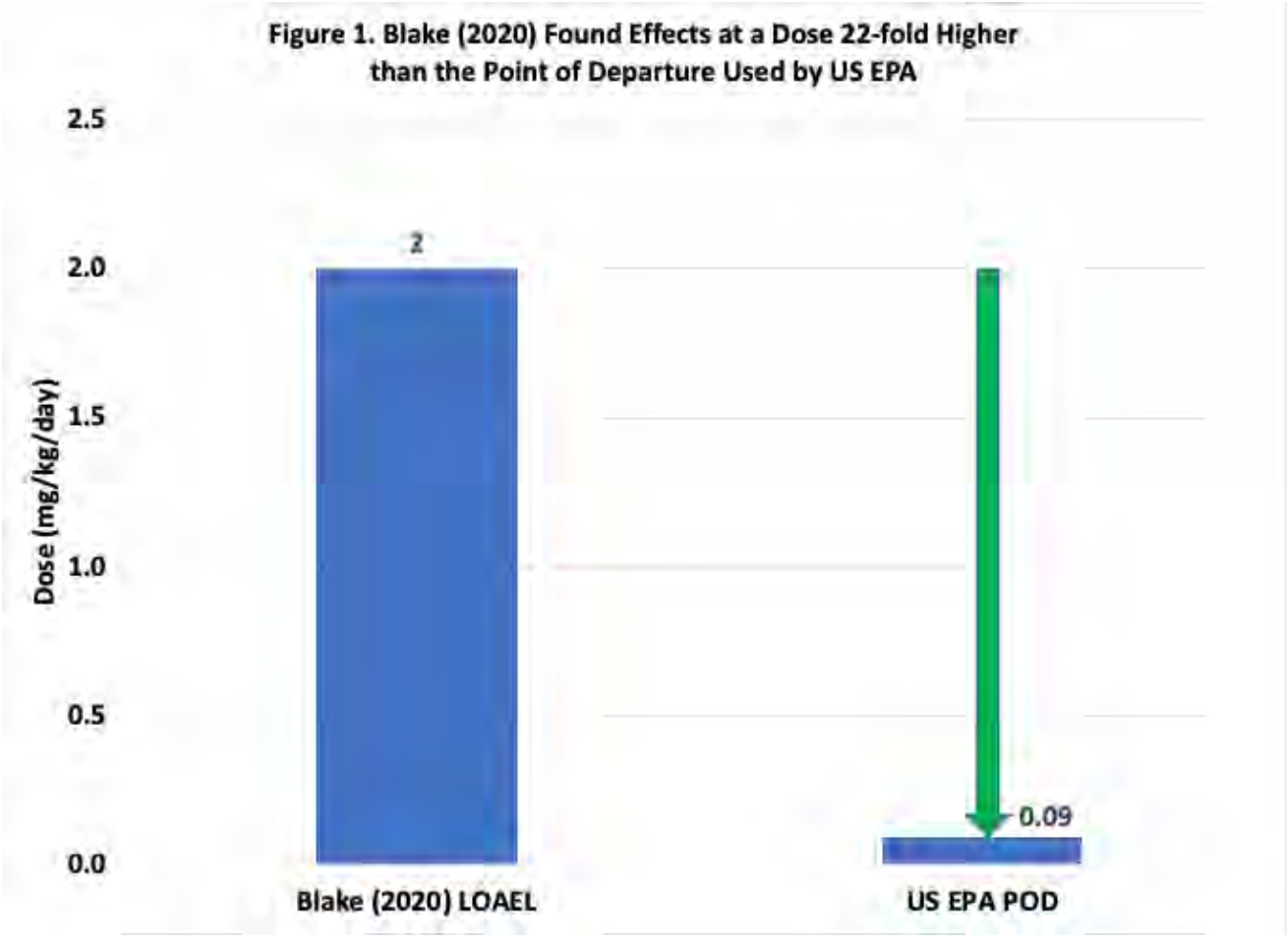
> *"As stated above, a number of commenters pointed out the deficiency of the GenX chemical database pertaining to human, immunotoxicity, and reproductive and developmental data. Recently published toxicokinetic and toxicological findings after Gen X chemicals exposure of Blake et al. (2020) and Conley 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

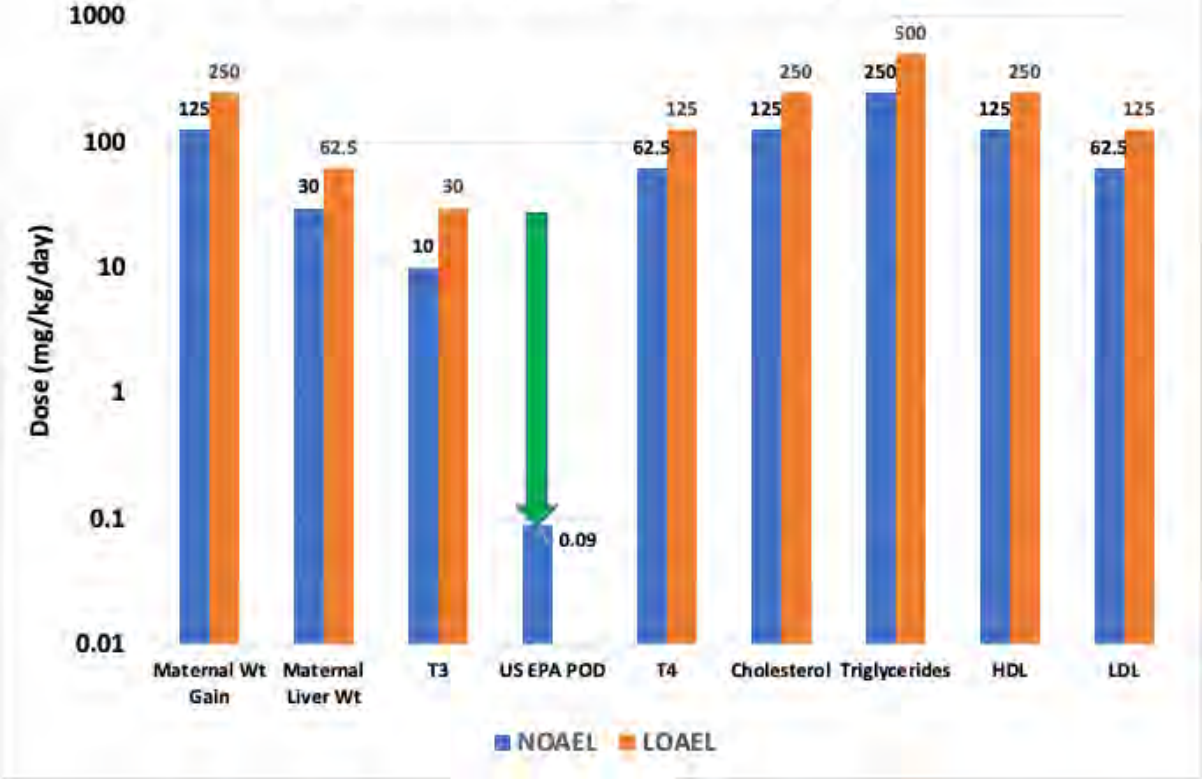


[5] Blake, B.E., H.A. Cope, S.M. Hall, R.D. Keys, B.W. Mahler, J. McCord, B. Scott, H.M. Stapleton, M.J. Strynar, S.A. Elmore, and S.E. Fenton. 2020. Evaluation of maternal, embryo, and placental effects in CD-1 Mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene 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.

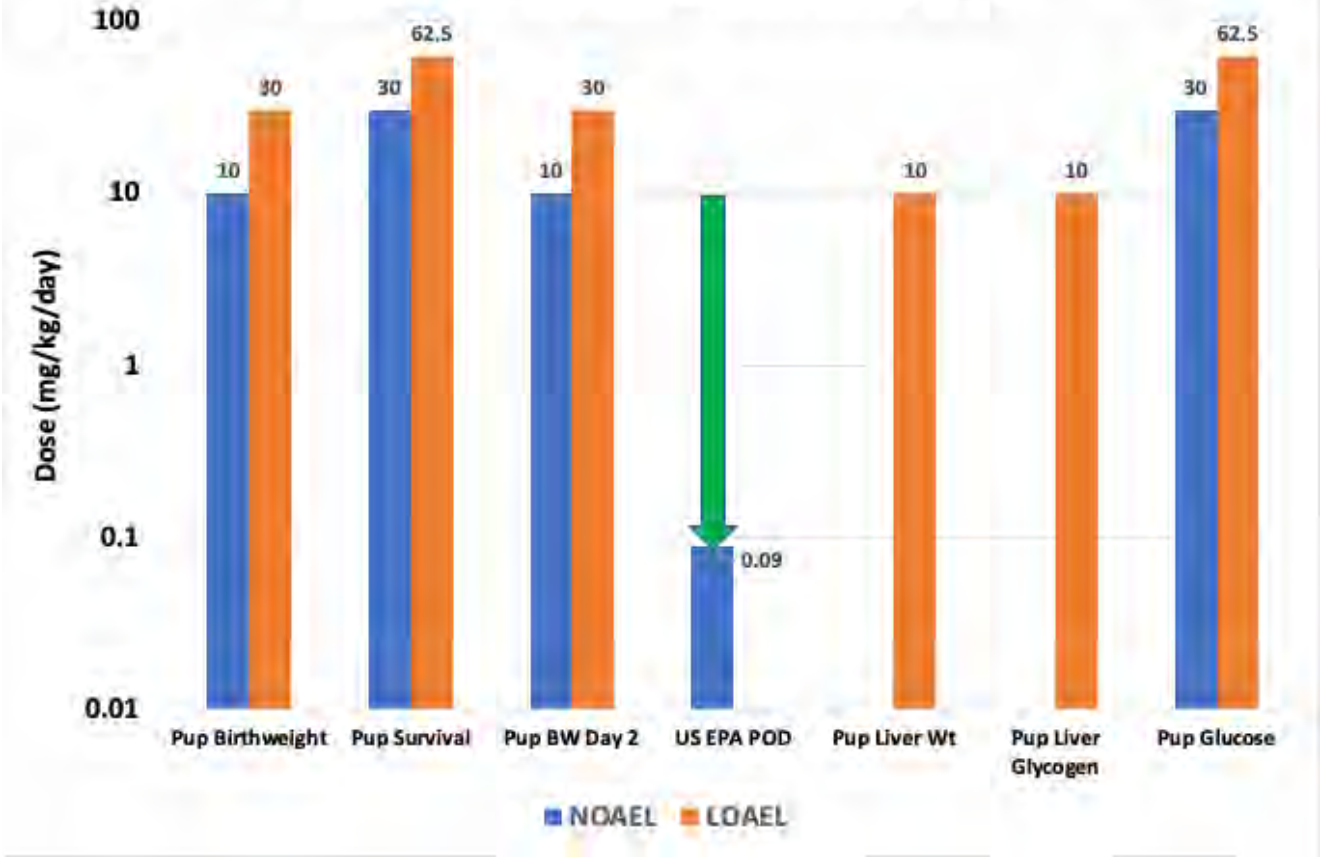
Figure 2. Conley (2019) Did Not Find Any Effects Until They Reached a Dose 333-fold Higher than the Point of Departure Used by US EPA



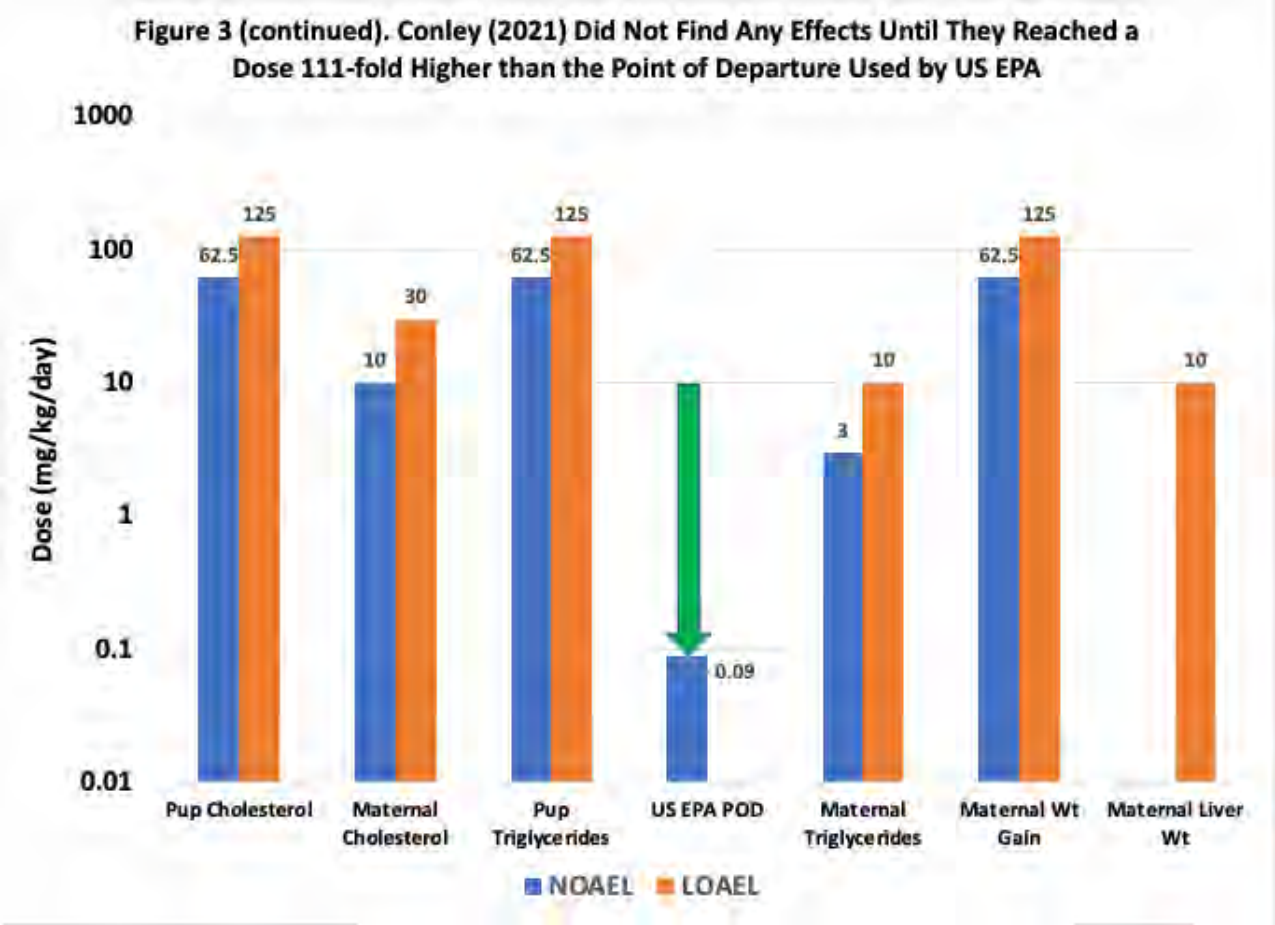
[6] Conley, J.M., C.S. Lambright, N. Evans, M.J. Strynar, J. McCord, B.S. McIntyre, G.S. Travlos, M.C. Cardon, E. Medlock-Kakaley, P.C. Hartig, V.S. Wilson, and L.E. Gray, Jr. 2019. Adverse maternal, fetal, and postnatal effects of hexafluoropropylene oxide dimer acid (GenX) from oral gestational exposure 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

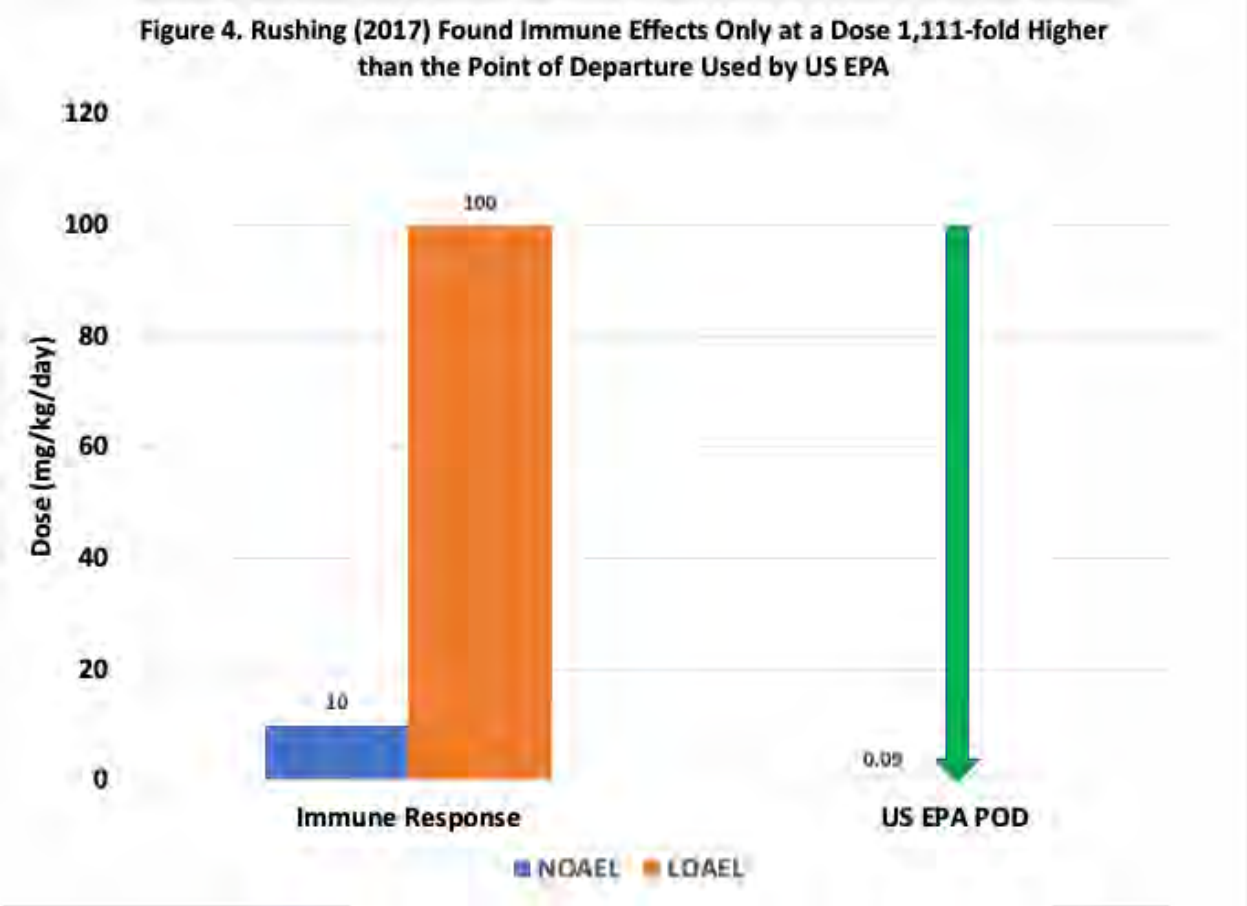


[7] Conley JM, Lambright CS, Evans N, McCord J, Strynar MJ, Hill D, Medlock-Kakaley E, Wilson VS, Gray LE Jr. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley 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

Exponent®

Epidemiology of Hexafluoropropylene Oxide Dimer Acid and Its Ammonium Salt

Prepared by

Ellen T. Chang, Sc.D.
Exponent, Inc.
149 Commonwealth Drive
Menlo Park, CA 94025

March 15, 2022

Ex™

Exponent
149 Commonwealth Drive
Menlo Park, CA 94025

telephone 650-326-9400
facsimile 650-326-8072
www.exponent.com

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.

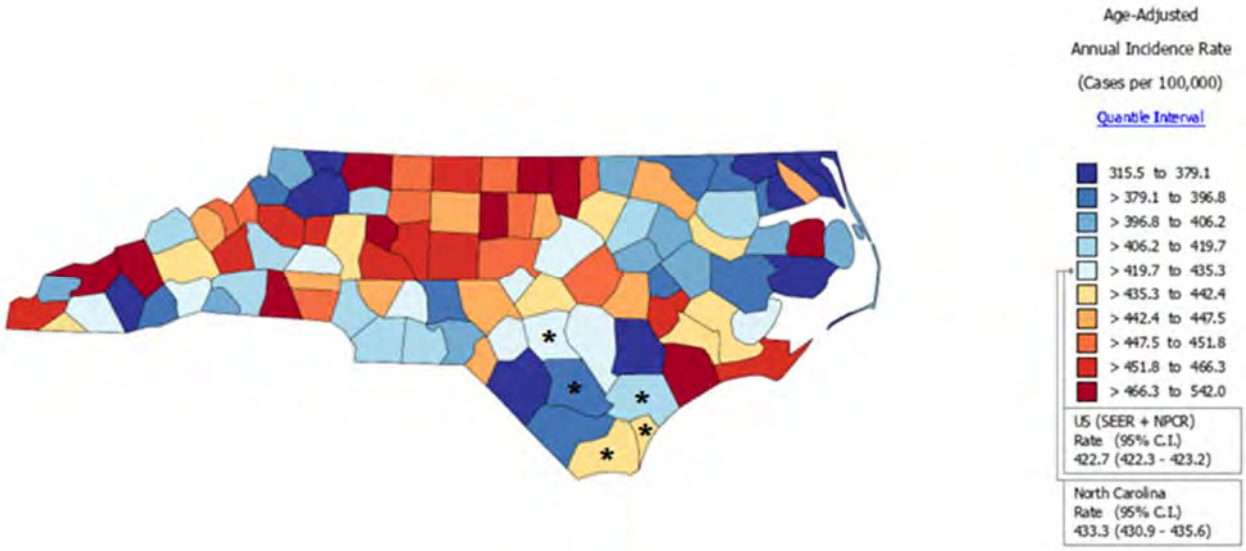

Figure 2. Age-adjusted incidence rates of all cancers in females by county, North Carolina, 2014–2018. "Exposed" counties are indicated with asterisks (*). Map generated at https://www.statecancerprofiles.cancer.gov/map/map.noimage.php (NCI and CDC 2021).

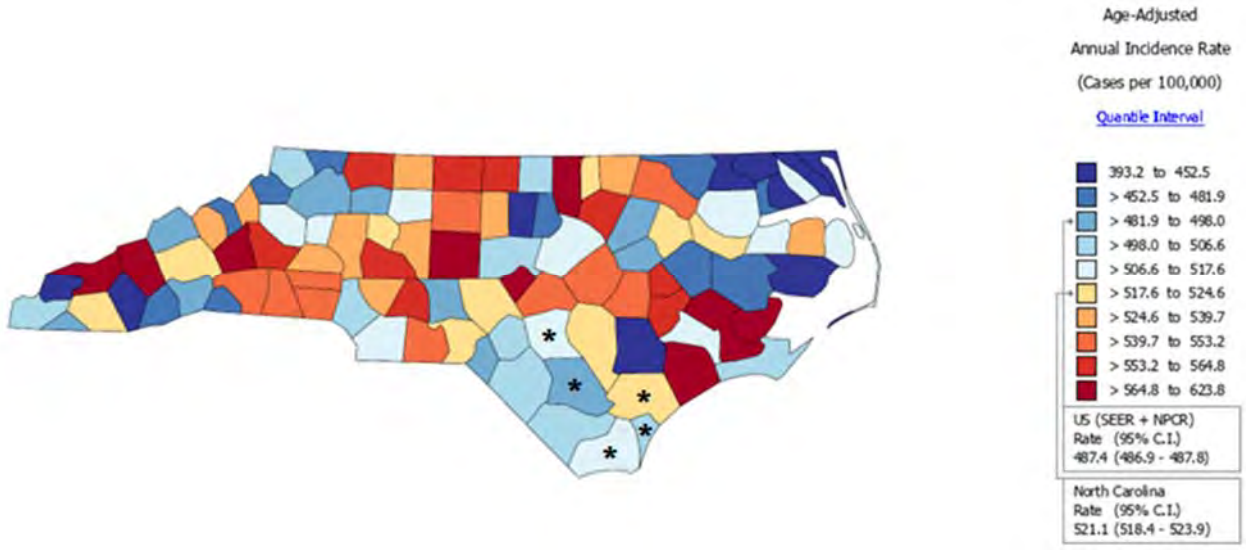

Figure 3. Age-adjusted incidence rates of all cancers in males by county, North Carolina, 2014–2018. "Exposed" counties are indicated with asterisks (*). Map generated at https://www.statecancerprofiles.cancer.gov/map/map.noimage.php (NCI and CDC 2021).

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).

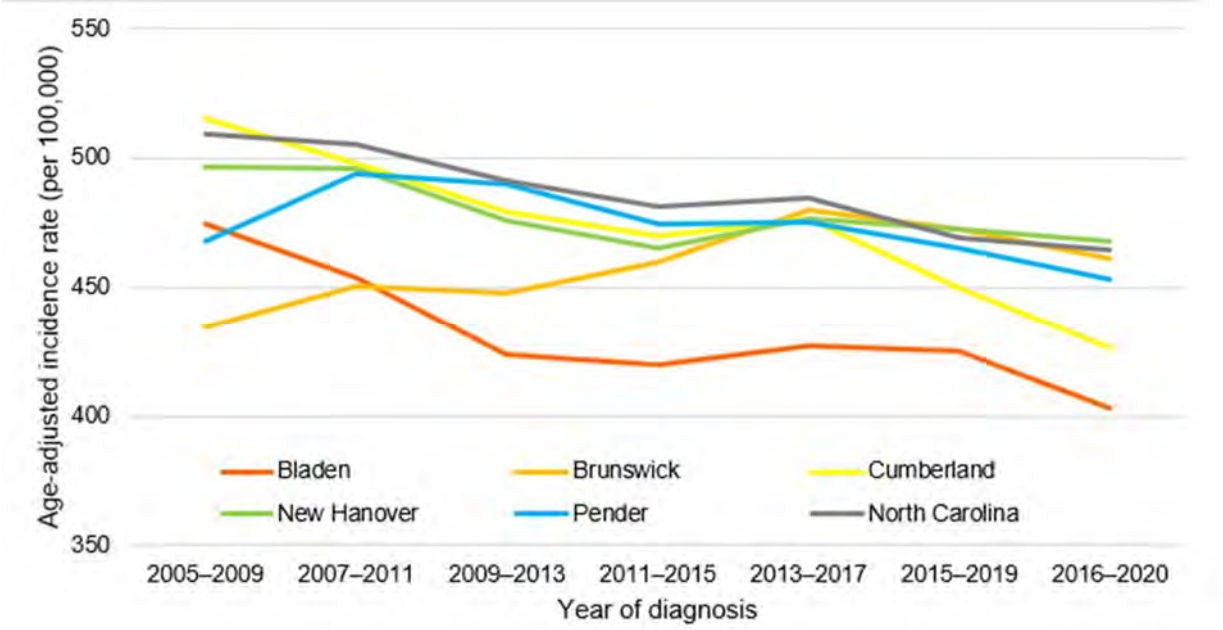

Figure 4. Time trends in age-adjusted (to 2000 U.S. standard) incidence rates of all cancers by county, North Carolina, 2005–2020. Data from https://schs.dph.ncdhhs.gov/data/cancer/incidence_rates.htm (NCDHHS 2022).

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)

January 15, 2020

Lauren Zeise, Ph.D.
Director
Office of Environmental Health Hazard Assessment
California Environmental Protection Agency
1001 I Street
Sacramento, California 95814

Re: Request for Relevant Information for the Development of Public Health Goals for Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonate (PFOS)

Dear Dr. Zeise:

The Chemical Products and Technology Division of the American Chemistry Council (ACC/CPTD)[1] submits the following information in response to the request for information on perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) to assist in the conducting risk assessments and in calculating public health goals (PHGs). ACC represents a number of companies with a strong interest in the science used to develop regulatory guidance for per- and polyfluorinated alkyl substances (PFAS) such as the PHGs to be developed by the Office of Environmental Health Hazard Assessment (OEHHA).

In addition to a list of relevant articles on PFOA and PFOS, we have enclosed comments on OEHHA's recent recommendations to the State Water Resources Control Board for Notification Levels (NLs) for these two substances.[2] The NL recommendations provide the latest insight into OEHHA's views of the risks associated with exposures to PFOA and PFOS. As outlined in the enclosed comments, OEHHA did not conduct a thorough review of the information available for PFOA and PFOS in developing the NLs and the analysis should not be used as a basis for developing PHGs for these substances.

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1 ACC represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing. ACC's Chemical Products and Technology Division is composed of a wide range of more than 60 self-funded product and sector groups that are focused on specific chemistries and related technologies. Members participating in these groups include large and small manufacturers, formulators, downstream users, distributors, suppliers and other trade associations.

2 OEHHA. Perfluorooctanoic Acid and Perfluorooctane Sulfonate in Drinking Water. Pesticide and Environmental Toxicology Branch (August 2019).

ACC/CPTD looks forward to engaging with OEHHA as it develops PHG's for these two substances in the coming months. Please do not hesitate to contact me if you have questions regarding the enclosed information.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director

Comments of the Chemical Products and Technology Division
of the American Chemistry Council
on the Notification Level Recommendations
for Perfluorooctanoic Acid and Perfluorooctane Sulfonate
in Drinking Water (August 2019)

Summary

The Office of Environmental Health Hazard Assessment (OEHHA) recommends that the Notification Levels (NLs) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS) be set at the lowest levels at which the two substances can be reliably detected, as a result of OEHHA's assessment of the carcinogenic risks associated with exposure to these substances.

For PFOA, the cancer assessment is based on the results of a chronic bioassay conducted by the National Toxicology Program which reported increased incidences of hepatocellular and pancreatic tumors in male rats in a dietary study. These tumors have not been observed in the epidemiological studies that have been conducted with PFOA, and the animal findings are consistent with a conclusion that the tumors result from a mechanism that is not relevant to humans. For PFOS, OEHHA's assessment is based on a bioassay reported in 2012 suggesting hepatocellular tumors in male and female rats and its "similarity in molecular structure" to PFOA. As with PFOA, the available human data do not support an association between PFOS exposure and the development of liver tumors. Moreover, the available data suggest the tumors result from a rodent-specific phenomena.

In developing the NLs, OEHHA also established reference levels (RLs) for noncancer effects. The study selected by OEHHA for PFOA reports altered protein expression in the livers of exposed mice, but does not provide histologic evidence of liver toxicity at the low level suggested by OEHHA. The results differ from earlier studies conducted with mice and rats. OEHHA's focus on immune effects in mice exposed to PFOS contradicts the conclusions reached by Health Canada and the US Environmental Protection Agency (USEPA) and suggests a correlation with effects in humans that are tenuous at best. OEHHA's analysis also underestimates human equivalent doses in extrapolating from the animal studies for both PFOA and PFOS, and understates the contribution of drinking water to overall exposure to these two substances.

PFOA – Cancer Studies

Several epidemiology studies and animal bioassays have been conducted to evaluate the carcinogenic potential of PFOA. The epidemiology studies have involved occupational, exposed community, and general populations and arguably provide some evidence for elevated

incidences of kidney and testicular cancer.[1] Animal bioassays have included dietary studies in rats and have reported that exposure to PFOA induces a "tumor triad" – liver, testis (Leydig cell), and pancreatic (acinar cell) tumors – consistent with that reported for several other substances known to activate the perioxisome proliferator-activated receptor α (PPAR α). The PPARα mechanism is well documented to be of little relevance for human health risk assessment.[2]

Epidemiology

Occupational studies examining cancer mortality have been conducted among workers in Minnesota and West Virginia focusing on kidney, bladder, liver, pancreatic, testicular, prostate, thyroid, and breast cancers. The results from the two study groups are conflicting and interpretation is limited by the small number of observed deaths and incident cases.

Raleigh et al. (2014) updated a study of cancer mortality among 4,668 PFOA workers in Minnesota followed through 2008.[3] Exposure estimates for inhalation exposures were calculated from work history records and industrial hygiene monitoring data; notably serum levels were not reported. The analysis reported no association between PFOA exposure and mortality from any cancer type. A slight elevation of bladder and pancreatic cancer incidence was reported although the confidence intervals were quite large; no association with kidney cancer incidence and PFOA exposure was reported.[4] The mean age of the workers was 29 years at the start of employment and 63 years at the end of follow-up.

Steenland and Woskie (2012) updated a cohort mortality study of 5,791 workers in West Virginia who had worked for at least 1 year between 1948 and 2002.[5] Mean duration of employment was 19 years. Exposure quartiles were assessed by estimated cumulative annual serum levels based on blood samples taken from 1,308 workers and time spent in various job categories. Referent groups included both nonexposed workers in the same region and the U.S. population. Overall, the mean cumulative exposure among the workers was 7.8 ppm-years and

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1 The available general population studies did not examine kidney or testicular cancer, but no associations were found in the general population between mean serum PFOA levels and several other cancer types.

2 Felter SP *et al.* Human relevance of rodent liver tumors: Key insights from a Toxicology Forum workshop on nongenotoxic modes of action. *Regul Toxicol Pharma 92:1-7* (2018).

3 Raleigh KK *et al.* Mortality and cancer incidence in ammonium perfluorooctanoate production workers. *Occup Environ Med* 71(7):500-506 (2014).

4 The authors report that the study had limited power to evaluate exposure response for testicular, bladder, liver, and pancreatic cancers.

5 Steenland K Woskie S. Cohort mortality study of workers exposed to perfluorooctanoic acid. *Am J Epidemiol* 176(10):909–917 (2012).

the estimated average annual serum level was 0.35 milligram per liter (mg/L).[6] The authors reported a significant positive trend for kidney cancer incidence among workers in the highest exposure quartile, while no association was reported between PFOA exposure and liver, pancreatic, testicular, or bladder cancer incidence.

Two studies involving communities in West Virginia and Ohio affected by contaminated drinking water (the C8 Health Project) reported a positive association between blood levels of PFOA and kidney and testicular cancers. Vieira *et al.* (2013) investigated incidences of 18 cancer types among residents supplied by six public water districts in Ohio and West Virginia contaminated with PFOA.[7] The analysis included over 25,000 cancer cases. Exposure levels and serum PFOA concentrations were estimated based on residence at time of diagnosis; exposures were categorized as very high, high, medium, low, or unexposed based on PFOA serum concentrations.

Among all cancer endpoints, the odds ratio for testicular cancer was elevated in one of the two areas with the highest concentration of PFOA in drinking water. There was no statistically significant increase in the odds ratio for testicular cancer in the total exposed population, however, or in the other districts, or in the other estimated dose-level categories. Kidney cancer incidence was increased significantly in one district in the two highest levels of individual exposure. Despite the large overall sample size, the authors noted that their analysis was limited by small numbers of individual cancers in the high-exposure groups. Moreover, there was little consistency across exposure categories, with no evidence of a dose response.

Barry et al (2012) conducted an analysis of cancer incidence among 32,254 individuals in the same geographic area as Vieira *et al.*, including 3,713 workers with occupational exposure to PFOA.[8] Cumulative PFOA serum concentrations were estimated based on historical regional monitoring data and individual residential histories. Based on measurements taken in 2005-2006, mean serum concentrations were 0.024 mg/L for community residents and 0.113 mg/L for workers. A total of 2,500 cancers were validated through a cancer registry or medical records. The authors reported that PFOA exposure was positively associated with kidney and testicular cancer across the exposure quartiles within the population, although the incidence of either tumor type was not elevated when compared to the US population.

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6 For comparison, the mean serum level of PFOA in the California Regional Exposure Study, Los Angeles County (CARE-LA) study was 0.001 mg/L.

7 Vieira VM *et al.* Perfluorooctanoic acid exposure and cancer outcomes in a contaminated community: a geographic analysis. *Environ Health Persp* 121(3):318-323 (2013).

8 Barry V *et al.* Perfluorooctanoic acid (PFOA) exposures and incident cancers among adults living near a chemical plant. *Environ Health Persp* 121(11-12): 1313-1318 (2013).

A review of the epidemiological evidence for cancer from 18 studies of occupational and general population exposure to PFOA reported a lack of concordance between community exposures and occupational exposures one or two magnitudes higher than those for the general population.[9] The authors evaluated the studies based on the study design, subjects, exposure assessment, outcome assessment, control for confounding, and sources of bias using Bradford Hill guidelines and concluded that the discrepant findings across the study populations were likely due to chance, confounding, and/or bias.

Animal Bioassays

Three chronic bioassays have been conducted in rats exposed to PFOA through diet. Although the results are not consistent, one or more of the studies have suggested liver tumors, testicular Leydig cell (LC) tumors, and pancreatic acinar cell (PAC) tumors.

Butenhoff *et al.* (2012), reporting on a previously conducted study of male and female Sprague-Dawley (SD) rats exposed to dietary levels of 30 and 300 parts per million of PFOA, observed a dose-dependent increase in LC ademonas that was statistically significant at the highest dose.[10] Elevated incidence of hepatic and PAC lesions were also reported in males at 300 ppm, but the authors did not report increases in hepatic or PAC tumors. A subsequent single-dose, dietary study with male CD rats reported LC adenomas, as well as liver and PAC adenomas and combined pancreatic adenomas and carcinomas at 300 ppm (13.6 milligrams per kilogram body weight, or mg/kg, per day).[11] Increased incidences of LC and PAC hyperplasia were also observed. Hepatic ß-oxidation activity was significantly elevated at all times, but cell proliferation in the liver was not.

In the most recent chronic animal study, the National Toxicology Program (NTP) reported liver adenomas in male SD rats and PAC adenomas in male and female rats exposed to PFOA in food.[12] The incidence of LC adenomas was not reported. In the study, male rats were exposed postweaning to 0, 20, 40, and 80 ppm (0, 1.0, 2.2, and 4.6 mg/kg per day) while females were

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9 Chang ET *et al.* A critical review of perfluorooctanoate and prefluorooctanesulfonate exposure and cancer risk in humans. *Crit Rev in Toxicol* 44(51):1–81 (2014).

10 Butenhoff JL *et al.* Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicol* 298(1–3): 1–13 (2012). Target doses for the study were 0, 1.3, and 14.2 mg/kg body weight per day in males and 0, 1.6, and 16.1 mg/kg per day in females.

11 Biegel LB *et al.* Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicol Sci* 60(1): 44–45 (2001).

12 NTP. Technical report on the toxicology and carcinogenesis studies of perfluorooctanoic acid administered in feed to Sprague-Dawley rats. Technical Report 598. Department of Health and Human Services. Research Triangle Park, North Carolina (2019).

exposed to 0, 300, and 1000 ppm (0, 18.2, and 63.4 mg/kg per day).[13] The male rat portion of the study was repeated using significantly lower exposures after "unanticipated toxicity" was observed in male rats exposed to 150 and 300 ppm after 16 weeks. In light of the fact that male SD rats tolerated doses as high as 300 ppm in the previous chronic study by Butenhoff *et al.*, the reports of unanticipated toxicity at comparable levels in the male rats in the NTP study raise concerns about the overall confidence in the study.

In the NTP study there were statistically significant increases in hepatocellular adenomas were reported among the male rats exposed to 2.2 and 4.6 mg/kg per day. Hepatocellular carcinomas were increased at 4.6 mg/kg per day, but the increase was not statistically significant. The study also reported significant increases in hepatocyte cytoplasmic alteration and hypertrophy in the males in all the exposure groups. Significant increases were also observed in hepatocyte single cell death, necrosis, mixed cell foci, inflammation, cystic degeneration, and bile duct hyperplasia.

An increase in PAC adenomas was statistically significantly in male rats in all exposure groups; PAC adenocarcinomas were also increased ion the males but the increase was not statistically significant. The study also noted a significant increase in PAC hyperplasia - a potentially preneoplastic lesion - in all the male groups, including the control group in which hyperplasia was reported in 36 percent of the animals. The high background rate observed in this study is consistent with the historical sensitivity of the Sprague-Dawley rats compared to other rat stains – and more significantly when compared to humans.

Relevance of the Animal Data

A significant amount of genotoxicity and mechanistic data are available to assist in evaluating the results of the epidemiology and animal bioassay results described above. Multiple *in vivo* and *in vitro* assays provide clear evidence that PFOA is not mutagenic and may only cause genotoxicity at toxic concentrations. This finding is corroborated by the results of the NTP study which found that perinatal exposure did not significantly alter the tumor response in exposed animals.[14] Consequently, it is generally agreed that PFOA causes tumors in laboratory animals via a non-genotoxic or epigenetic mechanism.[15]

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[13] The study included groups of animals exposed to PFOA perinatally and postweaning to assess the potential impact of gestational and lactational exposure, but reported very few significant differences between the response in animals exposed postweaning only to those with both perinatal and postweaning exposure.

[14] Anderson LM. Predictive values of traditional animal bioassay studies for human perinatal carcinogenesis risk determination. *Toxicol Appl Pharmacol* 199(2):162-174 (2004).

[15] US Environmental Protection Agency (USEPA). Health Effects Support Document for Perfluorooctanoic Acid (PFOA). EPA 822-R-16-003. Office of Water. Washington, DC. (May 2016).

The tumor types that have been reported consistently in rats exposed to PFOA – liver, LC, and PAC – have been observed with other substances that are PPARα agonists. Because of key toxicodynamic and biological differences in responses between rodents and humans, PPARα activators are considered unlikely to induce liver, LC, and PAC tumors in humans. For liver tumors, this conclusion is based on minimal or no effects observed on growth pathways, hepatocellular proliferation and liver tumors in humans and/or species (*e.g.*, hamsters, guinea pigs and Cynomolgous monkeys) that are more appropriate animal model surrogates than mice and rats.

Several key studies provide support for the key events in the proposed PPARα-activated mode of action (MOA) for rat liver tumors (Table 1). These data are summarized by Klaunig *et al.* (2012) –

> Analysis of gene expression changes elicited following short-term administration of PFOA demonstrated the up regulation of genes characteristic of PPARα activation, including genes involved in fatty acid homeostasis/peroxisomal proliferation as well as those related to cell cycle. In addition, PFOA has been shown to induce peroxisome proliferation in mouse and rat liver, and causes hepatomegaly in mice and rats. While the liver growth caused by PFOA was predominantly attributed to a hypertrophic response, an increase in DNA synthesis following PFOA exposure was observed, and predominated in the periportal regions of the liver lobule. Thus the effect of PFOA on induction of cell cycle gene expression and the increase in DNA synthesis provide evidence in support of both key events 2 and 3 in the proposed MOA for liver tumor induction by PFOA. Empirical evidence also exists in support of the clonal expansion of preneoplastic hepatic lesions by PPARα activators (Step 4). Using an initiation-promotion protocol for induction of liver tumors in Wistar rats, PFOA was shown to increase the incidence of hepatocellular carcinomas in rat liver (33% in PFOA exposed rats vs. 0% in controls).[16]

[16] Klaunig *et al.* Mode of action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and human relevance. *Reprod Toxicol* 33:410-418 (2012).

Table 1. PPARα Mode of Action for PFOA-Induced Liver Tumors in Rats (from Klaunig *et al.*)

Key Event		Support	Key Reference
1	Activation of the PPARα receptor	✓	Maloney & Waxman 1999 Vanden Heuvel *et al.* 2006
2	Induction of cell growth gene expression in the liver	✓	Martin *et al.* 2007 Kennedy *et al.* 2004
3	Cell proliferation	✓	Biegel *et al.* 2001 Martin *et al.* 2007 Thottassery *et al.* 1992
4	Selective clonal expansion of preneoplastic hepatic foci	✓	Abdellatif *et al.* 1190
5	Liver neoplasms	✓	Biegel *et al.* 2001

Klaunig *et al.* also note that the key events in Table 1 appear in a temporal sequence and demonstrate dose-related effects further strengthening the evidence for the PPARα-agonist MOA. Although there are indications that PFOA may also act through PPARα-independent mechanisms[17] in rodents, differences in binding affinity between the rodent and human receptors suggest that it is also unlikely that PFOA induces cancers in humans through the other mechanisms that have been suggested.[18]

The relevance of the liver tumor data from laboratory studies is further called into question as a result of the absence of liver tumors in epidemiology studies (as discussed above) and recent clinical data reported by Convertino *et al.* (2018).[19] In a study of a sensitive subpopulation of cancer patients with normal liver function exposed to weekly PFOA doses as high as 1,200 milligrams (about 16 milligrams/kilogram or mg/kg), Convertino *et al.* reported no differences in clinical hepatic measures.[20] The authors concluded that the disparity between animal and human liver endpoint studies, emphasizing a lack of risk of hepatomegaly, fatty liver, or cirrhosis, are likely due to MOA differences. Increased liver weight due to hepatocellular hypertrophy can often be an adaptive (protective) response in animals due to up-regulation of

[17] Activation of the constitutive activated receptor (CAR) and pregnane X receptor (PXR) by PFOA have been suggested in animal studies.

[18] Hall AP *et al.* Liver Hypertrophy: A Review of Adaptive (Adverse and Non-Adverse) Changes-Conclusions from the 3rd International ESTP Expert Workshop. *Toxicol Pathol* 40:971-994 (2012).

[19] Convertino M *et al.* Stochastic pharmacokinetic-pharmacodynamic modeling for assessing the systematic health risk of perfluorooctanoate (PFOA). *Toxicol Sci* 163(1) 293-306 (2018).

[20] These included triglycerides, urea, glucose, AST, GGT, alkaline phosphatase, total bilirubin, fibrinogen, PTT and aPTT

detoxification enzymes, leading toxicologists to revisit the relevance key liver endpoint studies in animals.[21]

For the induction of rat PAC tumors by PFOA, the available mechanistic data are less robust, but also point to the importance of PPARα activation in the liver. Several factors may contribute to the development of PAC hypertrophy, hyperplasia, and adenomas in the rat, such as testosterone and estradiol levels, growth factor expression (cholecystokinin, or CCK), growth factor receptor overexpression (CCKA receptor), and high fat diet (Klaunig *et al.*).[22] Studies with the compound Wyeth 14,643 suggest that a potent peroxisome proliferator induces PAC tumors by an indirect mechanism. In this study PPARα activation in the liver caused by exposure to Wyeth triggered reduced bile flow and/or changes in bile composition that produced an increase in CCK levels secondary to hepatic cholestasis.[23] As CCK has been shown to act as a growth factor for PACs in rats, a sustained increase in CCK levels would explain the increase in PAC proliferation observed following PFOA exposure and is likely therefore a preneoplastic lesion.

Expression of CCK receptors in humans is much lower as compared to rodents, and the available non-human primate and human data suggest that the CCK pathway is not relevant to human cancer risk. A study with Cynomolgus monkeys exposed to PFOA did not demonstrate an effect on CCK levels or evidence of hepatic cholestasis.[24] A study involving PFOA production workers, moreover, reported a statistically significant negative (inverse) association between mean CCK levels and serum PFOA levels, even after adjusting for potential confounders.[25] The authors also reported no abnormal liver function tests, hypolipidemia, or cholestasis among the workers.

PFOA – Non-Cancer Studies

OEHHA's calculation of an RL for noncancer effects for PFOA is based on a single report of mitochondrial dysfunction observed in female mice livers, the use of a single interspecies dose extrapolation for estimating human equivalent dose (HED), an underestimate of the source

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[21] See for example: Bjork JA *et al.* Multiplicity of nuclear receptor activation by PFOA and PFOS in primary human and rodent hepatocytes. *Toxicol* 288: 8-17 (2011).

[22] Differences in the diets used in the Butenhoff *et al.* and Biegel *et al.* studies have been suggested as the likely reason for the quantitative difference in the PAC lesions observed in the two studies (USEPA 2016).

[23] Obourn JD *et al.* Mechanisms for the pancreatic oncogenic effects of the peroxisome proliferatorWyeth-14,643. *Toxicol Appl Pharmacol* 145:425–36 (1997).

[24] Butenhoff J *et al.* Toxicity of ammonium perfluorooctanoate in male cynomolgus monkeys after oral dosing for 6 months. *Toxicol Sci* 69(1):244–57 (2002).

[25] Olsen GW *et al.* Plasma cholecystokinin and hepatic enzymes, cholesterol and lipoproteins in ammonium perfluorooctanoate production workers. *Drug Chem Toxicol* 23(4):603–20 (2000).

contribution from drinking water, and the unsubstantiated addition of a 3-fold uncertainty factor. These conservative assumptions lead to an RL that is several orders of magnitude lower than is supported by the available data.

The study by Li *et al.* (2017) that OEHHA uses as the basis for the noncancer RL reported proteomic changes in livers of female Balb/c mice exposed to PFOA by gavage for 28 days.[26] The authors reported dose- and gender-dependent changes in hepatic protein expression, primarily related to mitochondria, and suggest that PFOA-induced toxicity is mainly related to mitochondrial dysfunction in mouse liver, rather than through PPARα activation. The gender differences are only seen at the lowest administered dose, however, and PPARα activity is reported in both males and females at the higher doses. It also not clear whether the changes observed in the female mice at the lowest dose resulted in liver toxicity, as the authors did not provide information on the incidence of hypertrophy or apoptosis.

Without further analysis of the significance of the changes in hepatic protein expression reported by Li *et al.*, ACC urges OEHHA not to use these results as a basis for establishing the noncancer RL. And while considerable uncertainty exists about the human relevance of liver effects reported in animal studies,[27] the study by Perkins *et al.* (2004) is the more relevant study to use for this endpoint.[28] Although increases in hepatocellular hypertrophy and liver weight were observed at slightly lower doses in other studies, the animals in the Perkins *et al.* study were exposed to PFOA for a longer period – up to 13 weeks. In addition to *ad libitum* controls, moreover, the study provided pair-fed controls to ensure that effects did not result from differences in food consumption across dose groups. Finally, PPAR-α induction was measured in the Perkins *et al.* study which can provide greater insight into the possible biological basis for the liver effects. A final advantage is that Perkins *et al.* reported a no observed adverse effect level (NOAEL) of 0.06 mg/kg per day. The design of the studies by Li *et al.* and most others did not include dose levels for which a NOAEL could be established. Instead these studies were limited by their design and could only report a lowest observed adverse effect level (LOAEL) which means that further mathematical conversions (safety factors) to derive a NOAEL send the resulting level lower than is scientifically appropriate.

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[26] Li K *et al.* Molecular mechanisms of perfluorooctanoate-induced hepatocyte apoptosis in mice using proteomic techniques. *Environ Sci Tech* 51:11380-11389 (2017).

[27] Darrow LA *et al.* Modeled perfluorooctanoic acid (PFOA) exposure and liver function in a mid-Ohio Valley community. *Environ Health Persp* 124(8):1227-1233 (2016). https://doi.org/10.1093/toxsci/kfy035

[28] Perkins et al. 2004. 13-Week dietary toxicity study of ammonium perfluorooctanoate (APFO) in male rats. Drug Chem Toxicol 27(4):361-378 (2004).

Dosimetric Extrapolation - Use of Pharmacokinetic Data

A key component in extrapolating the dose of PFOA used in the animal studies to doses in humans is estimating the serum elimination half-live of the chemical. Since the elimination half-lives for long-chain PFAS like PFOA have been found to be longer in humans compared with rodents, extrapolation from doses in animals to equivalent doses in humans (the HED) has involved adjustments to account for half-life differences and/or clearance rates.

In its assessment for PFOA, OEHHA estimated the HED by adjusting the serum concentration in rodents measured at the exposure level of interest by the rate of clearance (CL) of the substance from the human body. The CL was calculated using the estimated volume of distribution and serum elimination half-life. Internal dose ratios predicted by the available physiologically-based pharmacokinetic (PBPK) models indicate, however, that the interspecies extrapolations for PFOA and other long-chain PFAS are highly dose dependent, and result from nonlinear toxicokinetics.[29] Furthermore, findings from a large set of 28-day oral gavage studies in rats conducted by the National Toxicology Program (NTP)[30] underscore the differences in dose-response relationships between PFOA across a wide range of endpoints. These findings further suggest that dosimetry scaling is unlikely to be linear across a broad dose range. As a result, a single interspecies extrapolation factor such as that used by OEHHA is not scientifically supportable for PFOA. Instead an approach that uses chemical-specific adjustment factors (CSAFs)[31] derived from the PBPK models better addresses the issue of nonlinear toxicokinetics and the impact on interspecies extrapolation.

Using such an approach, Health Canada compared dose metrics predicted by the various animal PBPK models to calculate a CL ratio between species (CL_A/CL_H).[32] They reasoned that using the model data to derive the CL_A/CL_H allows for a more appropriate comparison of doses of the

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[29] Loccisano AE *et al.* Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. *Reprod Toxicol* 33(4):452-467 (2012). https://doi.org/10.1016/j.reprotox.2011.04.006

[30] NTP. Final reports from the PFAS 28-Day toxicity studies TOX-96 and TOX-97 (2019). https://ntp.niehs.nih.gov/whatwestudy/topics/pfas/index.html

[31] World Health Organization (WHO). Chemical specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. International Programme on Chemical Safety. World Health Organization. Geneva (2005). http://apps.who.int/iris/bitstream/handle/10665/43294/9241546786_eng.pdf;jsessionid=45918ABD3B07EF944ACD546CF50B974F?sequence=1

[32] For each species, the PBPK model was used to predict internal doses for a broad range of oral doses. Model simulations were continued until steady-state conditions or expected lifetimes were reached (Loccisano *et al.* 2012).

same magnitude.[33] Using the CL ratio to estimate exposures, Health Canada's analysis indicates that the approach taken by OEHHA significantly underestimates the human clearance rate and, as a result, leads to estimates of PFOA exposures in humans that are an order of magnitude lower than actual.

Relative Source Contribution

In developing the noncancer RL, OEHHA assumes a relative source contribution (RSC) form drinking water is only of 20 percent. Although 20 percent is often used as a default assumption for the exposure resulting from drinking water, the available evidence suggest that other sources of potential exposure to PFOA have declined dramatically. According to data collected by the Center for Disease Control and Prevention (CDC), mean serum levels of PFOA declined by 60 percent in the US population between 1999 and 2016.[34] (See Figure 1). Given this dramatic decline, it is inappropriate to assume that 80 percent of exposure to these substances comes from sources other than drinking water. While a few other states have assumed an RSC of 50 or 60 percent, it is likely that the contribution of drinking water to overall exposure is even higher – particularly in areas where drinking water contamination has been detected.

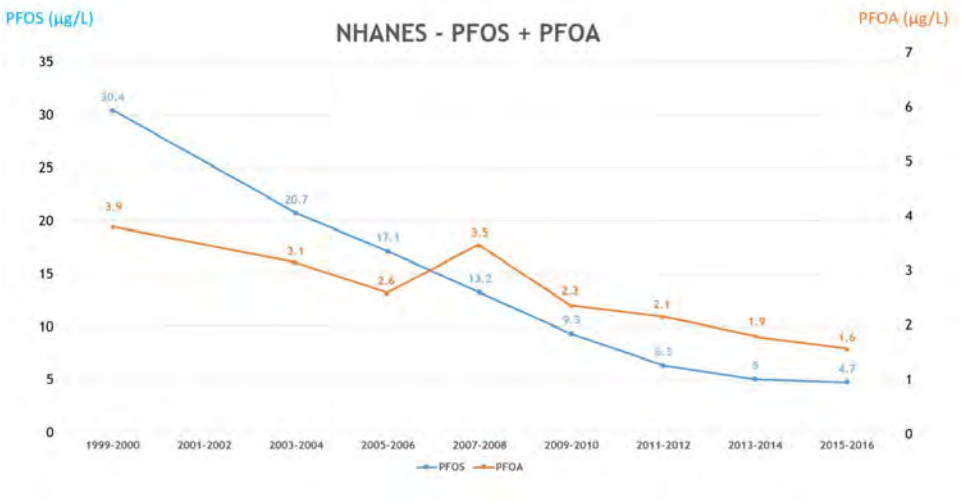

Figure 1. Serum levels of PFOA and PFOS, 1999-2016.[35]

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33 Health Canada. Guidelines for Canadian Drinking Water Quality – Guideline Technical Document – Perfluorooctanoic Acid (PFOA). Ottawa, Ontario (2018).

34 CDC. Fourth national report on human exposure to environmental chemicals, updated tables (January 2019). https://www.cdc.gov/exposurereport/index.html

35 Human exposure monitoring is conducted as part of CDC's National Health and Nutrition Examination Survey (NHANES).

Data Base Uncertainty

OEHHA has added an uncertainty factor of 3 to account for potential for developmental toxicity, based on a study reporting a NOAEL of <0.003 mg/kg per day for reduced pup weight in female offspring of C57BL mice exposed to PFOA during gestation and lactation. [36] These data are inconsistent with earlier studies in CD-1 mice that did not observe body weight changes in female offspring at significantly higher doses (< 3 mg/kg per day).[37] Experiments conducted with PPARα-null mice suggest, moreover, that weight gain in offspring appears to be mediated by PPARα induction.[38] Given the weight of the evidence related to weight gain in perinatally exposed animals, addition of an uncertainty factor is inappropriate.

PFOS – Cancer Studies

The number of epidemiology studies and animal bioassays evaluating the carcinogenic potential of PFOS is limited. Although the epidemiology studies are suggestive of increases in certain cancers, interpretation is limited by small number of cases and potential impact of confounding factors. Only one chronic bioassay has been conducted with PFOS.

Epidemiology Studies

Several analyses of a cohort of 2,083 workers with at least one year of employment at an Alabama PFOS manufacturing plant have been conducted. Exposures was estimated using PFOS serum concentrations in a subset of workers linked to specific jobs and work histories, and were grouped into three categories: non-exposed, low exposure, and high exposure. While the studies suggest an increase in bladder cancer among the high exposure group, the number of cases were small and the authors noted a higher smoking prevalence in the bladder cancer cases for which information was available.[39] A separate analysis of self-reported medical conditions among 1,400 workers at the same facility reported no association between working in a PFOS-exposed job and the risk of any of the surveyed conditions, although the incidence of colon and prostate cancer was elevated.[40]

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[36] van Esterik *et al.* Programming of metabolic effects in C57BL/6JxFVB mice by in utero and lactational exposure to perfluorooctanoic acid. *Arch Toxicol* 90(3):701-715 (2016).

[37] Macon MB *et al.* Prenatal perfluorooctanoic acid exposure in CD-1 mice: Low dose developmental effects and internal dosimetry. *Toxicol Sci* 121(1):134–145 (2011).

[38] Abbott BD *et al.* Perfluorooctanoic acid-induced developmental toxicity in the mouse is dependent on expression of peroxisome proliferator-activated receptor-alpha. *Toxicol Sci* 98:571–581 (2007).

[39] Alexander BH and Olsen GW. Bladder cancer in perfluorooctanesulfonyl fluoride manufacturing workers. *Annals of Epidemiol* 17:471–478 (2007).

[40] Grice M *et al.* Self-reported medical conditions in perfluorooctanesulfonyl fluoride manufacturing workers. *J Occup Environ Med* 49:722–729 (2007).

Four studies have been conducted to investigate the association between PFOS levels in the general population and cancer risk. The first of these examined plasma PFOS concentrations among 1,240 cancer patients reported no association with bladder, pancreatic, or liver cancer, after adjusting for confounders.[41] Although there was some increase in prostate cancer, no dose-response was reported. A subsequent study of 201 patients with newly diagnosed prostate cancer reported no association with serum PFOS levels.[42] In addition, two small studies have investigated breast cancer. Although the first of these studies was suggestive of an association with serum PFOS levels among 31 patients,[43] a larger study of 250 patients failed to confirm the association.[44]

Animal Bioassay

Tumor data were collected as part of chronic study of SD rats exposed to up to 20 ppm PFOS in their diet (0.984 and 1.251 mg/kg per day in males and females respectively).[45] A recovery group was also exposed to 20 ppm for the first 52 weeks and then fed with the control diet for the remainder of the study. An increased incidence of total hepatocellular adenomas, statistically significant only at the highest dose, was observed in both sexes exposed for 2 years, but not for 52 weeks. Thyroid follicular cell adenomas and carcinomas were also reported, but there was no pattern of dose-response or a significant increase compared with controls. While mammary gland tumors were observed in the female rats, the tumors had a relatively comparable incidence across dose groups, including the controls indicating a lack of dose response.

The absence of liver tumors in the recovery group, combined with reports of increased levels of ALT in the male rats, support hepatic tissue damage with compensatory repair as the probable MOA and a likely PPARα mediated cancer mechanism.

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41 Eriksen KT *et al.* Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. *PLOS ONE* 8:e56969 (2013)

42 Hardell E *et al.* Case-control study on perfluorinated alkyl acids (PFAAs) and the risk of prostate cancer. *Environ Intl* 63:35–39 (2014).

43 Bonefeld-Jørgensen EC *et al.* Perfluorinated compounds are related to breast cancer risk in Greenlandic Inuit: A case control study. *Environmental Health* 10:88 (2011).

44 Bonefeld-Jørgensen EC *et al.* Breast cancer risk after exposure to perfluorinated compounds in Danish women: A case-control study nested in the Danish National Birth Cohort. *Cancer Causes Control* 25(11):1439–1448 (2014).

45 Butenhoff JL *et al.* Chronic dietary toxicity and carcinogenicity study with potassium perfluorooctanesulfonate in Sprague Dawley rats. *Toxicol* 293(1-3):1-5 (2012).

Relevance of the Animal Data

Based on negative results of a large series of *in vitro* and *in vivo* short-term tests of genes, chromosomes, or DNA repair, it is generally agreed that PFOS and its salts are not genotoxic. While the data base is not as robust as that for PFOA, there are significant data supporting the role of PPARα in mediating many of the toxic effects observed in animals exposed to PFOS, particularly effects in the liver.[46]

PFOS – Noncancer studies

The immune system effects reported by Dong *et al.* (2009), that are the basis of the MCL recommendation, conflict with the findings reported by other researchers. In addition, the decision to focus on immune effects as the basis for its proposed MCL runs directly counter to the specific concerns expressed about these data by both USEPA and Health Canada.

Several studies have investigated potential effects on the immune system – natural killer (NK) cell activity and plaque forming cell (PFC) response in mice exposed to PFOS. Although the studies reported immune effects, USEPA concluded that the differences in the levels at which effects were reported (and conflicts in the direction of the effects) "highlight the need for additional research to confirm the NOAEL and LOAEL for the immunological endpoints."[47] Health Canada reached a similar conclusion noting that "[f]urther exploration should be performed to address the nearly two orders of magnitude difference in LOAELs in the studies before these endpoints can be reliably considered as a basis for risk assessment."[48] The inconsistency of these study results is detailed below.

Dong *et al.* reported decreased PFC response in male C57BL/6 mice at 0.083 mg/kg per day by gavage.[49] Terminal serum concentrations of PFOS among these mice was 7,132 nanograms per milliliter (ng/ml). A subsequent report by the same group did not observe a PFC response at 0.0167 mg/kg per day (2,360 ng/ml) by gavage. [50] Although a gavage study by Peden-Adams *et*

[46] USEPA. Health Effects Support Document for Perfluorooctane Sulfonate (PFOS). EPA 822-R-16-202 (May 2016).

[47] Ibid.

[48] Health Canada. Guidelines for Canadian Drinking Water Quality – Guideline Technical Document – Perfluorooctane Sulfonate (PFOS). Ottawa, Ontario (2018).

[49] Dong GH *et al.* Chronic effects of perfluorooctanesulfonate (PFOS) exposure on immunotoxicity in adult male C57BL/6 mice. *Arch Toxicol* 83:805–815 (2009)

[50] Dong GH *et al.* Sub-chronic effect of perfluorooctanesulfonate (PFOS) on the balance of type 1 and type 2 cytokine in adult C57BL6 mice. *Arch Toxicol* 85(10): 1235–1244 (2011).

al. (2008)[51] identified decreased PFC response in male B6C3F1 mice exposed to a lower dose than that reported by Dong *et al.*, concerns about the serum levels reported in the mice make interpretation of the data difficult.[52]

In contrast, a dietary study in B6C3F1 mice did not find a change in PFC response in males exposed to 0.25 mg/kg per day for 28 days, resulting in serum PFOS levels of 12,000 ng/ml.[53] In the only study designed to measure immune effects in rats, moreover, the NOAEL was several orders of magnitude higher than some of the LOAELs from mouse studies. [54]

Sensitivity to immunological effects in the animal studies appears to be dependent on several factors – including species (mice vs rat), route of exposure (gavage vs diet), and exposure duration. In addition, a study in PPARα-null 129/Sv mice suggests that immunomodulation in mice is partially dependent on PPARα and rodent-specific.[55] Consequently, USEPA and Health Canada have stressed the need for more research. However, there are no indications that prenatally exposed animals are more sensitive to immunological effects than adults, as changes in PFC response were not observed at ≤1 mg/kg per day in B6F3F1 mice exposed *in utero* on GD 1–17.[56]

Human Immunological Data

Several epidemiology studies have evaluated potential impacts of PFOS exposure on immune suppression, including incidence of infectious disease and vaccine response. As with the animal data, the human data are inconsistent, as noted by Health Canada which concluded that "associations are observed between PFOS levels and decreases in antibodies against some (but not all) illnesses and the influence of PFOS exposure on clinical immunosuppression (i.e.,

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[51] Peden-Adams MM *et al.* Suppression of humoral immunity in mice following exposure to perfluorooctane sulfonate. *Toxicol Sci* 104(1): 144–154 (2008).

[52] Pachkowski B *et al.* The derivation of a reference dose (RfD) for perfluorooctane sulfonate (PFOS) based on immune suppression. *Environ Res* 171:452-469 (2019).

[53] Qazi MR *et al.* 28-day dietary exposure of mice to a low total dose (7 mg/kg) of perfluorooctanesulfonate (PFOS) alters neither the cellular compositions of the thymus and spleen nor humoral immune responses: Does the route of administration play a pivotal role in PFOS-induced immunotoxicity? *Toxicol* 267, 132–139 (2010).

[54] Lefebvre DE *et al.* Immunomodulatory effects of dietary potassium perfluorooctane sulfonate (PFOS) exposure in adult Sprague -Dawley rats. *J Toxicol Environ Health A* 71:1516-1525 (2008).

[55] Qazi MR *et al.* The atrophy and changes in the cellular compositions of the thymus and spleen observed in mice subjected to short-term exposure to perfluorooctane sulfonate are high-dose phenomena mediated in part by peroxisome proliferator-activated receptor-alpha (PPARα). *Toxicol* 260:68–76 (2009)

[56] Keil DE *et al.* Gestational exposure to perfluorooctane sulfonate suppresses immune function in B6C3F1 mice. *Toxicol Sci* 103:77–85 (2008).

incidence of illnesses) appears to be more tenuous."[57] Health Canada further noted that, while the available animal and human data may indicate immune system changes, "it is unclear whether small variations in these measures are sufficient to result in adverse health effects in humans."

A study in children of the Faroe Islands found an inverse relationship in immune response with exposure to perfluorinated alkyl acids, with maternal cord PFOS levels negatively correlated with anti-diphtheria antibody concentration at 5 years. Children in this population demonstrated increased odds of not reaching protective antibody levels for diphtheria after vaccination at 7 years old (Grandjean *et al.* 2012). [58] A subsequent study in a different birth cohort from the same location did not observe a relationship between PFOS exposure and diphtheria antibodies, however.[59]

Increased PFOS exposure was associated with decreased antibodies against rubella in children from a prospective birth cohort of pregnant women in Norway.[60] Prenatal exposure to PFOS was not associated with hospitalizations for infections in a 2010 Danish cohort study,[61] nor with episodes of common cold, gastroenteritis, eczema or asthma in the Norwegian cohort, although an association with infection and fever has been reported in a few other studies. 2013). In a Taiwanese cohort study, the median serum PFOS concentration was significantly higher in asthmatic children,[62] and prenatal exposure to PFOS was positively correlated with cord blood Immunoglobulin E (IgE) levels, particularly in male children. However, Wang *et al.* (2011) [63] found no association with atopic dermatitis. Cord blood IgE levels, food allergy, eczema, wheezing, or otitis media were not associated with maternal PFOS in female infants in a prospective cohort study of pregnant women in Japan.[64]

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57 Health Canada 2018, at 69.

58 Grandjean *et al.* Serum vaccine antibody concentrations in children exposed to perfluorinated compounds. *J Am Med Assoc* 307(4): 391–397.

59 Grandjean P *et al.* Estimated exposures to perfluorinated compounds in infancy predict attenuated vaccine antibody concentrations at age 5-years. *J Immunotoxicol* 14:188–195 (2017).

60 Granum B *et al.* Pre-natal exposure to perfluoroalkyl substances may be associated with altered vaccine antibody levels and immune-related health outcomes in early childhood. *J Immunotox* 10(4): 373–379 (2013).

61 Fei C *et al.* Prenatal exposure to PFOA and PFOS and risk of hospitalization for infectious diseases in early childhood. *Environ Res* 110: 773–777 (2010).

62 Dong GH *et al.* Serum polyfluoroalkyl concentrations, asthma outcomes, and immunological markers in a case–control study of Taiwanese children. *Environ Health Perspect* 121(4): 507–513 (2013).

63 Wang Y *et al.* Modulation of dietary fat on the toxicological effects in thymus and spleen in BALB/c mice exposed to perfluorooctane sulfonate. *Toxicol Lett* 204(2–3): 174–182 (2011).

64 Okada E *et al.* Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. *Environ Res* 112: 118–125 (2012).

Finally, a cohort of 411 adult members of the C8 Health Project in West Virginia was evaluated to determine whether there was an association between serum PFOS levels and antibody response following vaccination with an inactivated trivalent influenza vaccine.[65] Vaccine response, as measured by geometric mean antibody titer rise, was not affected by PFOS exposure. After reviewing the available human data, Health Canada concluded –

> Although some effects on the antibody response have been observed, conflicting results were common in the dataset, which remains relatively small. A low level of consistency was observed across studies, with variations between genders, specific microbial immunoglobins, infections, mother vs. child exposure, and child years, amongst other characteristics. Moreover, the risk of residual confounding, bias, and chance cannot be discarded. These flaws impede concluding on a causative mechanism, and the nature of the association remains unclear.[66]

In considering these data USEPA cautioned that "lack of human dosing information . . . precludes the use of these immunotoxicity data in setting the [reference dose]."[67]

Relevance of Animal Data to Human Risk

The OEHHA analysis suggests that the relevance of reduced PFC response observed in mice to reduced resistance to infection in humans in explaining its rationale for the proposed MCL. Yet, the human studies generally report no increase in infection in children or adults and both USEPA and Health Canada have questioned whether the small variations in the antibodies observed in the available studies are sufficient to result in adverse health effects in humans. As the National Toxicology Program (NTP) noted in its review of PFOS the "effects on diverse endpoints such as suppression of the antibody response and increased hypersensitivity may be unrelated."[68] Moreover, while asserting that the PFC response in mice is "analogous" to decreased vaccine response in humans, OEHHA offers no supporting information and neither USEPA nor Health Canada have reached a similar conclusion.

The 2016 NTP systematic review of the animal data concluded that it cannot be confident in the outcome assessment of the Dong *et al.* 2009 study that is the basis for the

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[65] Looker C *et al.* Influenza vaccine response in adults exposed to perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci* 138: 76–88 (2014).

[66] Health Canada 2018, at 40.

[67] USEPA 2016, at 4-7.

[68] NTP. Monograph on Immunotoxicity Associated with Exposure to Perfluorooctanoic acid (PFOA) or Perfluorooctanoic Sulfonate (PFOS). Office of Health Assessment and Translation. (September 2016).

proposed groundwater criterion.[69] NTP's lack of confidence is supported by the inability of benchmark dose (BMD) modeling of the PFC response data to provide an acceptable fit to any of the dose-response models included in USEPA's BMD software.[70] The inability of BMD modeling to yield a valid POD suggests that the 2009 PFC response data reported by Dong *et al.* are not sufficiently robust.

OEHHA's decision to focus on immune system effects as the basis for its proposed MCL for PFOS runs directly counter to the specific concerns expressed about these data by both USEPA and Health Canada. The analysis provided offers little support for the relevance of the available animal and human data, which NTP is clear to caution may not be related to actual health effects in humans.

Dosimetric Extrapolation - Use of Pharmacokinetic Data

As noted earlier, internal dose ratios predicted by the available PBPK models indicate that the interspecies extrapolations for long-chain PFAS like PFOS are highly dose dependent, and result from nonlinear toxicokinetics.[71] As a result, a single interspecies extrapolation factor such as that used by OEHHA is neither scientifically supportable nor appropriate for PFOS. Instead an approach that uses CSAFs derived from the PBPK models better addresses the issue of nonlinear toxicokinetics and the impact on interspecies extrapolation.

Using the CL ratio between species (CL_A/CL_H) to estimate exposures, Health Canada's analysis indicates that the approach taken by OEHHA significantly underestimates the human clearance rate and, as a result, leads to underestimates of human exposures to PFOS that are up to 500 times lower than actual. [72]

Relative Source Contribution

In developing the noncancer RL for PFOS, OEHHA assumes a relative source contribution (RSC) of 20 percent. Although 20 percent is often used as a default assumption for the exposure resulting from drinking water, the available evidence suggest that other sources of potential exposure to PFOA have declined dramatically. According to data collected by the Center for Disease Control and Prevention (CDC), mean serum levels of PFOS declined by 85 percent in the US population between 1999 and 2016. (See Figure 1). Given this dramatic decline, it is

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69 Ibid, at 133 (Appendix 3. Risk of Bias Heatmaps).

70 NJ Department of Environmental Protection. Technical Support Document: Interim Specific Ground Water Criterion for Perfluorooctane Sulfonate (PFOS). Public Review Draft (January 2019).

71 Loccisano AE *et al.* 2012.

72 Health Canada 2018.

inappropriate to assume that 80 percent of exposure to these substances comes from sources other than drinking water. While a few other states have assumed an RSC of 50 or 60 percent, it is likely that the contribution of drinking water to overall exposure is even higher – particularly in areas where drinking water contamination has been detected.

August 20, 2018

Docket Number ATSDR-2015-0004
Agency for Toxic Substances and Disease Registry
Division of Toxicology and Human Health Sciences
1600 Clifton Road, NE
Mail Stop F-57
Atlanta, GA 30329

Re: Comments on the Draft Toxicological Profile for Perfluoroalkyls, Draft for Public Comment (June 2018)

To Whom It May Concern:

The Chemical Products and Technology Division (CPTD) of the American Chemistry Council (ACC)[1] offers the enclosed comments on the June 2018 draft Toxicological Profile for Perfluoroalkyls. ACC supports the application of the best available science to understanding the potential health effects associated with exposure to per- and poly-fluoroalkyl substances and the effective communication of this information to public health officials. The latest draft of the Toxicological Profile represents a significant departure from the provisional minimum risk levels (MRLs) for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) proposed in the 2015 draft Profile. In addition, the current draft proposes MRLs for perfluorohexane sulfonic acid (PFHxS) and perfluorononanoic acid (PFNA) for the first time and analyzes data available for a number of other perfluoroalkyl substances (PFAs).

The draft Toxicological Profile provides a comprehensive summary of the information available for the PFA substances, but includes a number of questionable assumptions used to develop the MRLs. Among our concerns are the choice of studies used to define adverse effects relevant to humans, the methods used to predict exposures in humans, and the use of uncertainty factors in the evaluation of risk. We urge ATSDR to reconsider these assumptions. Furthermore, because of the significance of the draft Toxicological Profile to the consideration

[1] ACC represents the leading companies engaged in the business of chemistry. ACC members apply the science of chemistry to make innovative products and services that make people's lives better, healthier and safer. ACC is committed to improved environmental, health and safety performance through Responsible Care®, common sense advocacy designed to address major public policy issues, and health and environmental research and product testing. ACC's Chemical Products and Technology Division is composed of a wide range of more than 60 self-funded product and sector groups that are focused on specific chemistries and related technologies. Members participating in these groups include large and small manufacturers, formulators, downstream users, distributors, suppliers and other trade associations.

of the four specific substances and of future PFAs and related substances, it is critical that ATSDR conduct a formal peer review of the document prior to its finalization.

ATSDR notes that MRLs are "intended to serve as screening levels" and are not intended to serve as standards. Unfortunately, these values are often misunderstood by the public which can cause significant confusion and alarm. It is vitally important that, for high-profile substances like PFAs, ATSDR make every effort to ensure that the Toxicological Profiles reflect the best science and avoid the temptation to be overly conservative.

Please feel free to contact me at srisotto@americanchemistry.com or at (202) 249-6727 if you any questions on the enclosed information.

Sincerely,

Steve Risotto

Stephen P. Risotto
Senior Director
Chemical Products and Technology Division

Enclosure

cc: W. Cibulas, Environmental Toxicology Branch

American Chemistry Council
Chemical Products and Technology Division
Comments on the

Agency for Toxic Substances and Disease Registry
Draft Toxicological Profile for Perfluoroalkyls
(June 2018)

I. Executive Summary

In its latest draft of the Toxicological Profile for Perfluoroalkyls, the Agency for Toxic Substances and Disease Registry (ATSDR) incorporates a number of questionable scientific approaches in deriving provisional intermediate, oral minimum risk levels (MRLs) for four perfluoroalkyls (PFA) substances – perfluorooctanoic acid (PFOA), perfluorooctane sulfonic acid (PFOS), perfluorohexane sulfonic acid (PFHxS), and perfluorononanoic acid (PFNA). For all four PFAs, ATSDR bases the MRL derivation on developmental or thyroid effects in rodents while noting that there is "strong evidence" that these effects involve a pathway that is of questionable relevance to humans. In the case of PFOA and PFOS, more relevant data from non-human primates exist that were the basis for ATSDR's 2015 MRL proposals. While the Toxicological Profile suggests that ATSDR conducted a weight-evidence evaluation of the epidemiological literature, only an extensive table summary of epidemiology studies appears to be available for comment and the epidemiology studies play only a minor role in the derivation of the proposed MRLs.

The methodology ATSDR does not account for non-linear toxicokinetics indicated by available pharmacokinetic modeling and significantly overstates the persistence of three of the substances in humans. In developing three proposed MRLs, ATSDR includes an additional modifying factor of 10 for database deficiencies in a manner that is not consistent with Environmental Protection Agency (EPA) guidance on the application of database uncertainty. The draft Toxicological Profile also incorrectly characterizes the proposed MRLs as intermediate values when they are based on an assumption of chronic (≥365 days), not intermediate, exposure in humans.

As a result of numerous questionable and flawed assumptions, the MRLs proposed in the 2018 Toxicological Profile exhibit unprecedented levels of conservatism that directly contradict ATSDR's previous conclusions about these substances and differ considerably with the conclusions of other regulatory authorities including Health Canada[1] and an expert panel convened by the Australian Department of Health.[2] The draft findings are inconsistent with available information on the mechanisms of toxicity and exposure on which the proposed levels

[1] Citations provided later in this report.

[2] Expert Health Panel for Per and Poly-Fluoroalkyl Substances (PFAS). Available at www.health.gov.au/internet/main/publishing.nsf/Content/ohp-pfas-expert-panel.htm

are based. The draft Profile should be withdrawn and subject to a formal peer review, conducted by independent toxicologists and risk assessors, prior to the release of a subsequent draft Profile that addresses the multiple concerns outlined in this comment.

II. Introduction

PFA substances have been in widespread use for many years because of their stability, their ability to impart water and oil repellency, and unique fire-fighting abilities. The two most common PFAs – PFOA and PFOS – are no longer manufactured in the United States, Japan, and Europe in response to concerns about their environmental persistence and potential toxicity. They have been replaced with other per- and poly-fluoroalkyl substances that have improved toxicity and environmental profiles. Although biomonitoring data collected in the late 1990s and early 2000s suggested that levels of PFOA and PFOS were accumulating in humans, data collected by the Center for Disease Control and Prevention (CDC) as part of the National Health and Nutrition Exposure Surveys (NHANES) show a drop of 60 to 80 percent since that time, as a result of the phase out of the production and use of these substances.[3] Despite the past widespread use of PFOA and PFOS, the data from EPA's third Unregulated Contaminant Monitoring Rule (UCMR 3) indicate that the substances were detected in only 2 percent of the nearly 6,000 public water systems (PWS) sampled. PFHxS and PFNA were detected in less than 2 percent of the wells.

ATSDR's June 2018 document is the third draft Toxicological Profile for PFAs. The original draft was released in May 2009; a subsequent draft was issued in August 2015. Like the current version, the 2015 draft reviewed the available literature for all of the PFAs for which monitoring data were available. The 2015 draft, however, only proposed MRLs for PFOA and PFOS since ATSDR concluded that the data on the other substances were insufficient to establish values. The current Profile updates the scientific literature for the PFAs, but largely depends on studies available for the previous draft for the MRL derivation. In developing the current MRLs, however, ATSDR appears to reverse its previous conclusion that rodent data are not appropriate for calculating human health toxicity values based on equivocal data suggesting that developmental effects in rodents may be more relevant than previously thought.

The draft Toxicological Profile represents a comprehensive review of the available information on the 14 PFAs identified in the NHANES or other monitoring studies. Although we have not conducted an extensive review of the available literature, we have identified several key publications that were not considered by ATSDR, however. In addition, the draft does not fully consider the conclusions of some of the referenced studies regarding important aspects of its analysis. Failure to include this information significantly impacts ATSDR's findings.

ATSDR's toxicological profiles, and the MRLs contained within, are used extensively by local public health officials to address community concerns and to assess the need to protect public

[3] Olsen GW *et al.* Per and polyfluoralkyl substances (PFAS) in American Red Cross adult blood donors, 2000-2015. *Environ Res* 157:87-95 (2017).

health. It is important that MRLs are determined to be health protective, but it is equally important that they be firmly grounded in science and subject to rigorous review. Although ATSDR clearly cautions that its MRLs are screening tools for hazardous waste sites and are not intended to define clean-up or action levels, the Agency must recognize that the MRLS often serve as the primary basis for assessing potential health impacts. This is particularly true for PFAs for which considerable confusion exists regarding the most scientifically robust and approach to developing health protective values.

III. Principal Study Selection

Many of the effects observed in the rodent studies, particularly liver and developmental effects, involve the activation of the peroxisome proliferator activated receptor (PPARα) or other nuclear receptors. Activation of the PPARα receptor in rodents initiates a characteristic sequence of morphological and biochemical events, principally, but not exclusively, in the liver (Kennedy *et al.* 2004).[4] The proliferation of peroxisomes has been associated with a variety of effects, including hepatocellular hypertrophy, alterations in lipid metabolism, and decreased pup survival and immune effects. Since humans and non-human primates have been found to be less responsive to PPARα agonists than rodents (Corton *et al.* 2014),[5] the relevance of the rodent findings to humans has been questioned. As a result, ATSDR concluded in its 2015 draft Toxicological Profile that "derivation of MRLs based on rodent data may result in overly conservative values" and instead based the proposed MRLs on studies in non-human primates which ATSDR noted "may be a suitable model for human exposure to PFOA and PFOS."[6]

Both the 2015 and current drafts of the Toxicological Profile describe the available evidence suggesting that PFAs may exert some adverse effects in rodents through mechanisms other than activation of PPARα. Although the current draft provides a minimal amount of new evidence for PPARα-independent mechanisms, ATSDR has apparently concluded that the data are now inexplicably sufficient to reverse its previous conclusion relative to the use of rodent data. As a result, three of the four proposed MRLs are based on developmental effects in rodents. For both PFOA and PFOS, for example, the proposed MRLs are based on a study that ATSDR previously decided not to use. For PFHxS and PFNA, moreover, ATSDR's conclusions ignore evidence that the rodent data used to derive the MRLs may not be relevant to humans.

The approach taken by ATSDR in the draft Toxicological Profile is not consistent with current recent best practices and scientific guidance on systematic review.[7] ATSDR provides a simple

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[4] Kennedy GL *et al.* The toxicology of perfluorooctanoate. *Crit Rev Toxicol* 34(4):351-384 (2004).

[5] Corton JC *et al.* Mode of action framework analysis for receptor-mediated toxicity: the peroxisome proliferator-activated receptor alpha (PPARα) as a case study. *Crit Rev Toxicol* 44(1):1–49 (2014).

[6] ATSDR. Draft toxicological profile for perfluoroalkyls. Department of Health and Human Services, Atlanta, GA (August 2015), at 32. (ATSDR 2015)

[7] National Toxicology Program. OHAT Risk of Bias Tool for Human and Animal Studies. Office of Health Assessment and Translation. Research Triangle Park, NC: Division of the National Toxicology Program, National

review of its literature review framework (Appendix B) – rather than an assessment of the risk of bias, key features, and data confidence of the available studies that are a part of accepted systematic review practices and that are included in other recent Toxicological Profiles.[8] Given the heightened public interest in PFAs and related substances, it is perplexing that ATSDR would not feel compelled to "show its work" in assessing the available studies.

a. PFOA

The proposed MRL is based on reports of altered activity and skeletal effects in the adult offspring of mice exposed to PFOA through gestation. Both studies include a single-dose group which greatly limits their value as critical studies for evaluating low doses because of the absence of a dose-response relationship. In the study by Onishchenko *et al.* (2011), mild sex-related differences in exploratory behavior patterns were reported after 5 weeks of age. PFOA-exposed males were more active, while PFOA-exposed females were less active, than their respective controls.

In the second principal study identified by ATSDR, Koskela *et al.* (2016) reported mild alterations in bone morphometry and mineral density of femurs and tibias in mice while noting that the biomechanical properties of the bones were not affected. Based on the absence of an impact on mechanical function, the biological significance of bone geometry and mineral density alterations is uncertain and may not be a suitable basis for the MRL calculation. Notably, no increases in the occurrence of malformations/variations were observed in similar studies conducted in rats.[9,10] Koskela *et al.* also appear to have conducted their statistical analysis on a per-fetus basis, rather than per-litter as advised by EPA's guidelines for assessing developmental toxicity which has been widely critiqued as a study deficiency in the past.[11] If ATSDR were to conduct the expanded systematic review tables and weigh the strengths and weaknesses of candidate primary studies alongside one another in the Appendix B tables requested in the section above, this would become readily apparent.

Lau *et al.* (2006) also reported skeletal effects in the offspring of mice exposed to PFOA, but the effects did not increase in a dose-related manner. Consequently, the effects noted by Lau *et al.*

Institute of Environmental Health Sciences (2015). https://ntp.niehs.nih.gov/pubhealth/hat/review/index-2.html.

8 ATSDR. Draft toxicological profile for molybdenum. Department of Health and Human Services, Atlanta, GA (April 2017).

9 Staples *et al.* The embryo-fetal toxicity and teratogenic potential of ammonium perfluorooctanoate (APFO) in the rat. *Fundam Appl Toxicol* 4(3 Pt 1): 429–440 (1984).

10 Butenhoff *et al.* The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. *Toxicol* 196(1–2):95–116 (2004).

11 EPA. Guidelines for developmental toxicity risk assessment. Risk Assessment Forum. EPA/600/FR-91/001(December 1991). (EPA Guidelines 1991). https://www.epa.gov/risk/guidelines-developmental-toxicity-risk-assessment

would generally not be considered biological significant.[12] In noting the striking difference between their results and the minor effects reported in the two-generation study in rats by Butenhoff *et al.* (2004), the authors suggest that they are most likely related to pharmacokinetic differences between the two species.

In its 2015 assessment of PFOA, ATSDR chose the increase in liver weights in *Cynomolgus* monkeys reported by Butenhoff *et al.* (2002) as the basis for its proposed MRLs. In the current assessment, ATSDR concludes that the small number of animals examined in the Butenhoff *et al.* study and the early deaths at several dose levels preclude using the non-human primate data for the MRL calculation. Given that the effects seen in the non-human primates are consistent with those reported by Butenhoff *et al.* (2012) in rats, and that there is evidence of histological hepatic effects in rats coupled with increased liver weight and hypertrophy that provide an indication that the effects are adverse – rather than adaptive[13] – it may be appropriate to use evidence of adverse histotological effects in the rat liver as the basis for the MRL.[14]

b. PFOS

The current draft of the Toxicological Profile proposes an MRL for PFOS based on a two-generation study by Luebker *et al.* (2005) reporting delayed eye opening and decreased pup weight in rats. These effects appear to occur independent of PPARα activation and, consequently, should be considered relevant to humans. In its MRL derivation, however, ATSDR has ignored the conclusions of the authors regarding the relevant dose resulting in the adverse effects.

In the case of pup weight, the decreases noted in the second generation (F2) offspring at 0.4 milligrams per kilogram body weight per day (mg/kg) were transient, disappearing by the end of lactation. Reduced body weights were not reported in the F1 pups from the 0.4 mg/kg dose group. For both F1 and F2 offspring, body weight was reduced in the 1.6 mg/kg group. As a result the authors identified 0.4 mg/kg as a no-observed-adverse-effect level (NOAEL) and 1.6 mg/kg as a lowest-observable-adverse-effect level (LOAEL). ATSDR, in contrast, inappropriately considers the LOAEL to be 0.4 mg/kg without explanation.

Similarly Luebker *et al.* conclude that the slight delay in eye opening observed in the F1 pups from the 0.4 mg/kg dose group should not be considered an adverse effect, and identify 0.4

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[12] EPA Guidelines 1991, at 13. The 1991 guidelines note that a dose-related increase in variations in skeletal ossification is interpreted as an adverse developmental effect, but assessing the biological significance of the variation must take into account what is known about the developmental stage.

[13] Hall AP *et al.* Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes—conclusions from the 3rd International ESTP Expert Workshop. *Toxicol Pathol* 40(7): 971–994 (2012).

[14] Health Canada. Perfluorooctanoic acid (PFOA) in drinking water. Document for public consultation (2016a). https://www.canada.ca/en/health-canada/programs/consultation-perfluorooctanoic-acid-pfoa-in-drinking-water/document.html

mg/kg as the NOAEL. This finding is consistent with the results from the other studies in rats and mice referenced in the draft Profile which report NOAELs of 1.0 mg/kg or more.

ATSDR's decision to consider 0.4 mg/kg as a LOAEL, rather than NOAEL, has a significant impact on the MRL calculation. Correcting the calculation to consider 0.4 mg/kg as a NOAEL makes the MRL calculation more consistent with the "intermediate" oral PFOS MRL proposed in the 2015 draft Toxicological Profile which was based on non-human primate data.

c. PFHxS

Very few studies exist that could be used as a basis for calculating an MRL for PFHxS. As noted in the draft Profile, the hypertrophy and other hepatic effects reported by Butenhoff *et al.* (2009) and Bijland *et al.* (2013) are not considered relevant for human risk assessment, based on the criteria described by Hall *et al.* Although Butenhoff *et al.* also report thyroid follicular cell damage, they note that that the observed changes in rats "are consistent with the known effects of inducers of microsomal enzymes where the hepatocellular hypertrophy results in a compensatory hypertrophy and hyperplasia of the thyroid." Because of the possible link to PPARα activation in the liver and the significant differences in thyroid function between rodents and humans,[15] the rat data are not appropriate for use in human risk assessment.

Based on the strong likelihood that both the available hepatic and thyroid effects data from animal studies are not relevant to humans, ATSDR should withdraw its proposed MRL for PFHxS.

d. PFNA

ATSDR identifies only three animal studies available for in the draft Toxicological Profile. Significantly, one of these studies (Wolf *et al.* 2010) reported that the developmental effects in offspring of mice exposed to PFNA required PPARα activation that is of questionable relevance to humans.[16] The decreased body weight gain and development delays reported in the offspring of mice administered PFNA via gavage on GDs 1-17 in Das *et al.* 2015 occurred concomitant with maternal toxicity and therefore, should not be used as the critical effect. Moreover, Wolf et al. (2010) in PPARα knockout mice did not find alterations in pup body weight or postnatal development at 2 mg/kg-day, suggesting that these effects are rodent-specific responses to PFNA. Since developmental concerns were also not identified from the PFNA epidemiology literature, it is not clear what rational ATSDR would have to conclude that the decreased pup body weight and developmental delays from Das *et al.* are appropriate endpoints for evaluating human health risk from exposure to PFNA.

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[15] Capen CC *et al.* Species differences in thyroid, kidney, and urinary bladder carcinogenesis. *IARC Scientific Publications* 147:1-14 (1999).

[16] Although the authors did not rule out the possibility of PPARα relevance to a human response to PFNA, they noted the lower number of these receptors in the liver of humans versus mice.

As is the case for PFHxS, the available data do not support the development of an MRL for PFNA.

IV. Available Epidemiology

A number of epidemiology studies have evaluated potential health outcomes associated with occupational, drinking water, and population exposures to PFAs, particularly PFOA and PFOS. Most of the studies lack exposure monitoring data; most provide only a single measurement which does not provide information on historical exposure. The lack of historical exposure data is a particular limitation of the occupational and community population studies where past exposures were typically higher than current exposures. Although several studies have reported statistically significant associations, the findings are not consistent across studies. Observed dose-response patterns in some studies are also less clear when evaluated across multiple studies. Using a weight-of-evidence approach, the draft Toxicological Profile identifies several potential hazard associated with PFA exposure.[17] The details of the WOE analysis of the epidemiology studies should be made available to the peer reviewers and the public for comment on its scientific merits.

While ATSDR suggests that its approach to developing MRLs is to identify sensitive endpoints from epidemiology studies, the human studies appear to play only a minimal role in the proposed MRLs for the PFAs. Most of the endpoints chosen as the basis for the proposed MRL from animal studies do not align with endpoints identified in the epidemiological studies. This is particularly true for PFHxS and PFNA and supports a conclusion that the data are not sufficient for developing an MRL for these two PFAs.

Notably a recent Expert Health Panel commissioned by the Australian Department of Health reviewed the epidemiology data for PFOA and PFOS and found limited or no evidence for any causal link with any human disease. [18]

V. Estimating Human Exposures

Large pharmacokinetic differences exist between humans and animals for the PFAs considered by ATSDR, with lower clearance (i.e., higher half-life values) reported for humans than for rats, mice, and non-human primates. These differences result in higher target tissue doses in humans when exposed to the same external doses as laboratory animals. Consequently, default approaches for interspecies extrapolation (e.g., using an interspecies uncertainty factor of 10 or allometric scaling) are not considered to be sufficiently predictive. To better account for these interspecies toxicokinetic differences, the World Health Organization (WHO) developed an approach using chemical-specific adjustment factors (CSAF). [19] Consistent with

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[18] www.health.gov.au/internet/main/publishing.nsf/Content/ohp-pfas-expert-panel.htm

[19] WHO. Chemical specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment. International Programme on Chemical Safety. World Health Organization. Geneva (2005).

this approach, ATSDR calculates a human equivalent dose (HED) for the PFAs by adjusting the serum concentration in rodents measured at the drinking water exposure by the rate of clearance (CL) of the substance from the human body in developing the proposed MRLs. The CL was calculated using the estimated volume of distribution and serum elimination half-life.[20]

Internal dose ratios predicted by the available physiologically-based pharmacokinetic (PBPK) models indicate, however, that the interspecies extrapolations for PFOA and PFOS are highly dose dependent, and result from nonlinear toxicokinetics. [21] As a result, a single interspecies extrapolation factor such as that used by ATSDR is not scientifically supportable for either PFOA or PFOS. Instead an approach that uses CSAF values derived from the PBPK models better addresses the issue of nonlinear toxicokinetics and its impact on interspecies extrapolation. Using such an approach, Health Canada compared dose metrics predicted by the various animal PBPK models to calculate a CL ratio between species (CL_A/CL_H).[22] They reasoned that using the model data to derive the CL_A/CL_H allows for a more appropriate comparison of doses of the same magnitude.[23] Using the CL ratio to estimate exposures, Health Canada's analysis indicates that the approach taken by ATSDR significantly underestimates the human clearance rate and, as a result, ATSDR calculates HED values that are 10 to 500 times lower than actual. Human biomonitoring declines reported in the last decade support this point.

As described, the risk assessment calculations by both ATSDR and Health Canada are highly dependent on the estimate of the elimination half-life in humans. Reported half-life estimates in humans range considerably and appear to show a gender differences for at least some PFAs. Estimates of the mean half-life for PFOA vary from 2.3 years in a study of a general population exposed via drinking water[24] to 3.8 years in an occupationally-exposed cohort. [25] For PFOS, a

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http://apps.who.int/iris/bitstream/handle/10665/43294/9241546786_eng.pdf;jsessionid=45918ABD3B07EF944ACD546CF50B974F?sequence=1

[20] The volume of distribution is defined as the volume of blood (in milliliters per kilogram) in which the amount of a chemical would need to be uniformly distributed to produce the observed blood concentration. Half-life is a measure of the time (in days) required to eliminate one half of a quantity of a chemical from the body.

[21] Loccisano AE *et al.* Comparison and evaluation of pharmacokinetics of PFOA and PFOS in the adult rat using a physiologically based pharmacokinetic model. *Reprod Toxicol* 33(4):452–467 (2012).

[22] For each species, the PBPK model was used to predict internal doses for a broad range of oral doses. Model simulations were continued until steady-state conditions or expected lifetimes were reached (Loccisano *et al.* 2012).

[23] Health Canada 2016a; Health Canada. Perfluorooctane Sulfonate in Drinking Water. Document for public consultation. Prepared by the Federal-Provincial-Territorial Committee on Drinking Water (2016b). https://www.canada.ca/en/health-canada/programs/consultation-perfluorooctane-sulfonate-pfos-in-drinking-water/document.html

[24] Bartell SM *et al.* Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. *Environ Health Perspect* 118(2):222-228 (2010).

[25] Olsen GW *et al.* Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Persp* 115:1298–1305 (2007).

recent analysis of data from the NHANES[26] from 1999-2000 through 2013-2014 estimated a serum elimination half-life of PFOS of 3.8 years in males and 3.4 years in females. [27] Similarly, data from a community in Sweden exposed to PFAs via a contaminated water supply following installation of a treatment system suggested a serum elimination half-life for PFOS of 3.4 years for 106 residents aged 4 to 83.[28] An earlier study of occupational exposures, on the other hand, suggested a half-life of 5.4 years for PFOS among retired workers.

Although there are less data available for PFHxS, the information that exists suggests a similar pattern of a longer half-life estimate for occupational studies than for those in the general population. While the 2007 study of retired workers suggested a half-life of 7.3 to 8.5 years,[29] a recent analysis in a population exposed to contaminated drinking water suggests a half-life of 5.3 years.[30] For PFNA, ACC/CPTD is aware of only one study that estimates half-life based on analysis in urine collected from a general population sample.[31]

As ATSDR notes, human elimination half-lives estimates are most applicable to the serum levels of the study population from which they were derived, which are several orders of magnitude higher in workers compared to the general population. While the occupational estimates generate more conservative estimates, half-life values derived from general population data are the most relevant to exposures considered by ATSDR.[32] Reducing the half-life estimate from 3.8 to 2.9 years for PFOA and from 5.4 to 3.4 years for PFOS significantly affects ATSDR's estimate of human serum levels associated with exposure from drinking water. For PFOS, for example, assuming a half-life of 3.4 years increases the exposure required to achieve human serum concentrations equivalent to those in the rodent studies by a factor of 10.

VI. Application of Uncertainty and Modifying Factors

ATSDR has proposed to adjust the Human Equivalent Dose by a factor of 300 to generate the MRL for each of the PFA to account for uncertainties in the available data base. In each case, the Agency includes a factor of 3 as a dosimetric adjustment to extrapolate from animal to humans and a factor of 10 for human variability. For PFOA, ATSDR adds an additional factor of

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[26] More information on NHANES is available at https://www.cdc.gov/exposurereport/index.html.

[27] Gomis MI *et al*. Historical human exposure to perfluoroalkyl acids in the United States and Australia reconstructed from biomonitoring data using population-based pharmacokinetic modelling. *Environ Int.* 108: 92-102 (2017).

[28] Li Y *et al.* Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. *Occup Environ Med.* 75:46-51 (2018). Li *et al.* 2018

[29] Olsen *et al.* 2007.

[30] Li *et al.* 2018.

[31] Zhang Y *et al.* Biomonitoring of perfluoroalkyl acids in human urine and estimates of biological half-life. *Environ Sci Tech* 47 (18):10619-10627 (2013).

[32] EPA. Health effects support document for perfluorooctanoic acid (PFOA). EPA 822-R-16-003 (May 2016), at 4-12. https://www.epa.gov/sites/production/files/2016-05/documents/pfoa_hesd_final_508.pdf

10 to account for the use of a LOAEL, instead of a no-effect level. For the other three, a modifying factor of 10 is added for data base uncertainty, based on "concern that immunotoxicity may be a more sensitive endpoint" than the developmental/thyroid effects chosen as the basis for the MRL.

As discussed earlier, the two principal PFOA studies identified by ATSDR are single-dose studies which greatly limits their value in evaluating low doses. Both studies report only mild effects of questionable biological significance. The results of skeletal defects in mice, moreover, contradict the findings of two studies in rats. Consequently, the two principal studies represent poor choices for generating the proposed MRL and seem to be selected because they generate the lowest value, particularly with the inclusion of a 10-fold LOAEL/NOAEL adjustment. Given the significant number of available animal studies, including a non-human primate study, ATSDR should select an alternative basis for the MRL that can provide a more scientifically robust and defensible result.

The addition of a modifying factor for PFOS, PFHxS, and PFNA is equally problematic. While ATSDR provides no guidance on how to apply a modifying factor based on data base uncertainty, EPA's guidance explains that a database uncertainty factor (UF_D) is applied when reproductive and developmental toxicity studies are missing since they have been found to provide useful information for establishing the lowest no adverse effect level.[33] The EPA guidance notes that, for a reference dose (RfD) 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.[34] In deciding whether to apply an UF_D, EPA advises that the assessor should consider both the data lacking and the data available for particular organ systems as well as life stages.[35] In the case of both PFHxS and PFNA, for which little data exist for any endpoint and no chemical-specific data exist to suggest immunotoxicity, it is unclear what scientific basis ATSDR uses to conclude a modifying factor is appropriate. In fact, the discussion of a modifying factor is the first time that immunotoxicity is mentioned in the draft Profile in the context of either of these two substances.

As for PFOS, the reproductive and development data base is robust and does not suggest the need to account for an incomplete characterization of toxicity. Similarly, the potential immunotoxic effects of PFOS have been studied in both laboratory animals and humans. The

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[33] Ibid, at 4-45.

[34] Dourson ML *et al.* (1996) Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul Toxicol Pharmacol* 24:108–120 (1996).

[35] EPA Risk Assessment Forum. A review of the reference dose and reference concentration processes. EPA/630/P-02/002F (December 2002). https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

results of these studies are inconsistent and both EPA[36] and Health Canada[37] have questioned the relevance of immune system effects observed in mice and the small antibody variations seen in epidemiology studies to adverse health effects in humans. It is inappropriate, therefore, to conclude that immunotoxic effects represent a more sensitive health effect such that a modifying factor of 10 should be included.

VI. Exposure Duration

ATSDR's characterization of the proposed MRLs as intermediate (15 to 364 days) is inconsistent with their derivation, as outlined above. While the levels are based on data collected in non-chronic studies, the dosimetric modeling is based on serum levels in humans reaching steady-state.[38] Based on pharmacokinetic modeling, the time to reach steady state in human serum for PFOA and PFOS requires more than 364 days.[39] This is likely to also be true for PFHxS and PFNA with estimated half-lives of 3,100 and 900 days, respectively. Consequently, the proposed MRLs are based on an assumption of chronic (≥365 days), not intermediate, exposure in humans.

VIII. Impact on MRL Calculation

The above comments outline several assumptions in the ATSDR analysis that significantly impact the derivation of the proposed MRLs.[40] Based on our analysis, these assumptions add a level of conservatism of 1000-fold or more to the proposed MRLs for PFOA and PFOS – assuming no change in the principal studies. The proposed MRL for PFOA-oral, intermediate is 10,000 times lower than the point of departure (POD) based on the LOAEL for mice and 100,000 times lower than the POD for non-human primates. This seems inappropriate given that rodents are more sensitive to PFOA than humans in terms of PPAR-mediated responses, and thus are not quite representative of humans.

Given the significant number of questionable assumptions that ATSDR has made, and the impact of these assumptions on the proposed MRLs, it is vitally important that the approach taken in the draft Toxicological Profile be reevaluated and that the draft be subject to formal peer review before the MRLs are used in any fashion.

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[36] EPA. Health effects support document for perfluorooctane sulfonate (PFOS). EPA 822-R-16-002 (May 2016). https://www.epa.gov/sites/production/files/2016-05/documents/pfos_hesd_final_508.pdf

[37] Health Canada 2016b.

[38] Wambaugh *et al.* Dosimetric anchoring of *in vivo* and *in vitro* studies for perfluorooctanoate and perfluorooctanesulfonate. *Toxicol Sci* 136(2):308-327 (2013).

[39] Loccisano *et al.* 2012.

[40] Given the multi-step derivation of the proposed MRLs, including the application of prediction models, a quantitative uncertainty analysis should be performed. The results of this analysis should be presented with the proposed MRLs to ensure accurate, science-based risk communication.