Response to Peer Review Comments on the 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"

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CONTENTS

ACRONYMS

Please note that acronyms are not consistently defined throughout this document, as much of the text was extracted in its original format from charge questions and reviewer comments. Please refer to the table below for acronym meanings, when needed.

BMD	Benchmark dose
BMDL	Benchmark dose lower limit
BMR	Benchmark response
CASRN	Chemical Abstracts Service Registry Number
DAF	Dosimetric adjustment factor
EPA/USEPA	U.S. Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GLP	Good Laboratory Practices
HED	Human equivalent dose
HERO	Health & Environmental Research Online
HFPO	Hexafluoropropylene oxide
hL-FABP	Human liver fatty acid-binding protein
ICR	Institute of Cancer Research
LOAEL	Lowest-observed-adverse-effect level
mg/kg/day	Milligrams per kilogram per day
MOA	Mode of action
NOAEL	No-observed-adverse-effect level
OECD	Organization for Economic Cooperation and Development
OPPT	Office of Pollution Prevention and Toxics
PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
POD	Point of departure
POD_{HED}	Point of departure human equivalent dose
PPARα	Peroxisome proliferator-activated receptor alpha
RfD	Reference dose
$T_{1/2}$	Half-life
TG	Test guideline
TSCA	Toxic Substances Control Act
TSCATS1	Toxic Substances Control Act Test Submissions 1
UF	Uncertainty factor(s)
UF_A	Interspecies uncertainty factor
UF_D	Database uncertainty factor
UF_H	Intraspecies uncertainty factor
UF_L	LOAEL to NOAEL extrapolation uncertainty factor
UF_S	Extrapolation from subchronic to a chronic exposure duration uncertainty factor
WOE	Weight of evidence
WOS	Web of Science

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INTRODUCTION

This document was prepared under the EPA Contract No. EP-C-17-017, Task Order 0008 with Eastern Research Group, Inc. Five independent external peer reviewers reviewed the draft assessment, and their comments are presented with the EPA's responses. Appendix A includes the full comments from the reviewers.

The EPA is issuing draft subchronic and chronic oral toxicity values (i.e., RfDs) for HFPO dimer acid and its ammonium salt (CASRN 13252-13-6 and CASRN 62037-80-3)—or "GenX chemicals' for public comment. The EPA is publishing these toxicity values to facilitate future decision-making by the Agency's Programmatic, Regional, and/or State partners associated with contamination concerns in a variety of exposure scenarios when they are finalized. The EPA developed this toxicity assessment to provide the health effects information used as the basis for derivation of these RfDs for GenX chemicals.

The database used to derive these RfDs for HFPO dimer acid and its ammonium salt includes oral animal toxicity studies of acute, short-term, subchronic, and chronic duration in rats and mice. Available information identifying health effects from inhalation or dermal exposures to GenX chemicals in animals is limited. Repeated-dose toxicity data are available for oral exposure, but not for the other exposure routes (inhalation and dermal exposures). Thus, this assessment applies only to the oral route of exposure. Two studies were available: one oral reproductive and developmental toxicity study in mice and one prenatal developmental toxicity study in rats. These studies report liver toxicity (increased relative liver weight, hepatocellular hypertrophy, and single-cell necrosis), kidney toxicity (increased relative kidney weight), immune effects (antibody suppression), developmental effects (an increased number of early deliveries and delays in genital development), and cancer (liver and pancreatic tumors). Overall, the available toxicity studies demonstrate that the liver is particularly sensitive to toxicity induced by HFPO dimer acid and its ammonium salt.

The critical study the EPA chose for determining the subchronic and chronic RfDs for HFPO dimer acid and/or its ammonium salt is the oral reproductive/developmental toxicity study in mice, with a NOAEL of 0.1 mg/kg/day based on liver effects (single-cell necrosis in males) (DuPont-18405-1037, 2010). Following EPA guidance, the resulting POD_{HED} is 0.023 mg/kg/day. UF applied include a 10 for intraspecies variability, 3 for interspecies differences, and 3 for database deficiencies, including immune effects and additional developmental studies, to yield a subchronic RfD of 0.0002 mg/kg/day. In addition to those UF, a UF of 3 was also applied for extrapolation from a subchronic to a chronic duration in the derivation of the chronic RfD of 0.00008 mg/kg/day.

Overall, the peer reviewers agreed with the EPA's decisions regarding the:

- Choice of critical study
- Choice of critical effect
- BMD modeling
- Determination of a HED dose using body weight scaling
- UF application
- Cancer classification

The peer reviewers made several clarifying comments. Specifically, they asked for additional clarification on the:

- Justification of the UF_A
- Selection of studies for dose-response modeling
- Blood effects as a potential critical adverse endpoint
- Kidney hazard identification and additional clinically relevant endpoints
- Chronic study reanalysis and female liver tumors
- Systematic review procedures

Minor comments and editorial suggestions were reviewed and are addressed directly in this document. Specific responses to major comments are provided under each respective section/question.

SECTION I: TECHNICAL CHARGE TO EXTERNAL REVIEWERS

Technical Charge to External Peer Reviewers
Contract No. EP-C-17-017
Task Order 0008
June 2018

External Peer Review of EPA's Draft Human Health Toxicity Assessment for GenX Chemicals

BACKGROUND

The EPA is issuing subchronic and chronic toxicity values (i.e., RfDs) for 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid (CASRN 13252-13-6)—or HFPO dimer acid—and 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt. HFPO dimer acid and its ammonium salt are collectively referred to as "GenX chemicals" because they are the two major chemicals associated with the GenX processing aid technology.

The EPA developed toxicity assessment of GenX chemicals to provide the health effects information used as the basis for derivation of RfDs. The toxicity assessment identifies and characterizes the health hazards of these chemicals and includes information to address the first two steps of the human health risk assessment paradigm: hazard identification and dose-response assessment. The EPA is publishing these toxicity values to facilitate decision-making by the Agency's Programmatic, Regional, and/or State partners associated with contamination concerns in a variety of exposure scenarios.

This document was developed by building on previously completed assessments. Specifically, the EPA assessed the toxicity of HFPO dimer acid and its ammonium salt in 2008 under the TSCA. Most of the available data were submitted to the EPA by DuPont/Chemours, the manufacturer of these chemicals, under TSCA, as required pursuant to a consent order (USEPA, 2009) or as required under TSCA reporting requirements (e.g., section 8(e)). The EPA evaluated all toxicokinetic and repeat-dose toxicity studies of 28 days and longer against the EPA OPPT's *Application of Systematic Review in TSCA Risk Evaluations* processes.

The submitted studies and literature identified by the search of publicly available sources are available through the EPA's HERO website[1]: https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627. Please see the instructions for requesting access to HERO if you do not have an active account.

[1] Access to full-text references was provided to external peer-reviewers through the HERONet website, an internal database of bibliographic information and scientific studies. Due to copyright laws/regulations, access to the copyright protected materials that are stored in the HERONet database are prohibited from public dissemination. However, a list of all citations and access to publicly available references is provided via the public HERO website (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627).

REVIEW MATERIALS PROVIDED BY THE EPA

- 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"* (USEPA, 2018)
- References and supporting documentation (also see appendix A)
    - *Benchmark Dose Technical Guidance* (USEPA, 2012).
    - Background references are available on the EPA's HERONet. If you do not have an active account for HERO, please see the attached instructions for access. Once your account is active, the URL above will take you directly to the references needed.

CHARGE QUESTIONS

HFPO dimer acid and its ammonium salt or GenX Chemicals

1. The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

2. For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?

    a. If so, please explain your justification.
    b. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.

3. EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?

4. Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).

    a. Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.
    b. Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

5. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for GenX chemicals.

    a. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.
    b. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

6. The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.

    a. Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.
    b. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.
    c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

7. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.

8. **Editorial or Additional Comments:** Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.

REFERENCES

DuPont-18405-1037: E.I. du Pont de Nemours and Company. 2010. *An Oral (Gavage) Reproduction/ Developmental Toxicity Screening Study of H-28548 in Mice*. USEPA OPPTS 870.3550; OECD Test Guideline 421. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: December 29, 2010), Ashland, OH.

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.

USEPA (U.S. Environmental Protection Agency). 2005. *Guidelines for Carcinogen Risk Assessment*. EPA/630/P-03/001B. USEPA, Risk Assessment Forum, Washington, DC. Accessed May 2018. https://www.epa.gov/risk/guidelines-carcinogen-risk-assessment.

USEPA (U.S. Environmental Protection Agency). 2009. *Consent Order and Determinations Supporting Consent Order for Premanufacture Notice Numbers: P-08-508 and P-08-509*. USEPA, Office of Pollution Prevention and Toxics, Washington, DC. Accessed May 2018. https://chemview.epa.gov/chemview/proxy?filename=sanitized_consent_order_p_08_0508c.pdf.

USEPA (U.S. Environmental Protection Agency). 2011. *Recommended Use of Body Weight $^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose*. EPA/100/R11/0001. USEPA, Office of the Science Advisor, Risk Assessment Forum, Washington, DC. Accessed May 2018. https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf.

USEPA (U.S. Environmental Protection Agency). 2012. *Benchmark Dose Technical Guidance*. EPA/100/R-12/ 001. USEPA, Risk Assessment Forum, Washington, DC. Accessed May 2018. https://www.epa.gov/risk/benchmark-dose-technical-guidance.

USEPA (U.S. Environmental Protection Agency). 2018. 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."* USEPA, Office of Water, Washington, DC.

SECTION II: REVIEWER COMMENTS ORGANIZED BY CHARGE QUESTION

CHARGE QUESTION 1

The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.

Chou

The literature search strategy and study evaluation considerations are clearly described and appear to be appropriate.

The reviewer suggests an additional article by Rakhshandehroo, M., Knoch, B., Muller, M., and Kersten, S. (2010). Peroxisome Proliferator-Activated Receptor Alpha Target Genes. *PPAR Research*, 2010, 1-20.

Using primary hepatocytes culture from mice and human, this study demonstrated that human hepatocytes are sensitive to enzymatic induction by PPARalpha agonists. Of the 208 PPARα targeted genes in mouse hepatocytes, 85 (41%) are also targeted in human hepatocytes. In addition, 12 genes targeted by PPARα in human hepatocytes are not in mouse hepatocytes. Six of the human-specific genes are in the biotransformation pathways and the other six are in the lipid metabolism pathways, including the pathways of peroxisomal β-oxidation, microsomal ω-hydroxylation, lipogenesis, plasma triglyceride metabolism, and cholesterol/bile transport & metabolism. The potential manifestation of the effects on these human-specific target genes should be considered when determining UF_A.

> **EPA Response**: Thank you for providing the reference to the review paper by Rakhshandehroo et al. (2010). This review paper provides an overview of PPARα target genes involved in different biological processes in the liver and the literature evaluating differences and similarities between the genes impacted in mice and humans. The authors conclude that based on their review of the literature, the function and specific target genes of PPARα are generally well conserved between mouse and human specifically for lipid metabolism. Only a subset of those genes impacted in mice and humans, however, are linked to the PPARα response element, and none of those identified as human fell only in that category. In addition, the review paper does not specifically address GenX chemicals or other perfluorinated compounds (Rakhshandehroo et al., 2010).
>
> As described in section 4.7, findings consistent with PPARα agonists were observed (e.g., increases in liver weight, hepatocellular hypertrophy, and increased β-oxidation activity) for GenX chemicals; however, data gaps exist for key events and other MOAs might be involved (Rakhshandehroo et al., 2010). Therefore, the EPA concluded that the MOA for GenX chemicals is not clearly understood and cannot be solely attributed to PPARα.
>
> The commenter noted that several genes involved in biotransformation and lipid metabolism are human-specific and recommended the potential effects resulting from regulation of these human-specific PPARα target genes should be considered when determining UF_A. As pointed out above, none of those human genes were directly linked to the PPARα response element by the authors (Rakhshandehroo et al., 2010). The UF_A is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include

both toxicokinetic (i.e., absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. The toxicokinetic portion of the UF_A was accounted for by application of the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* in the determination of the POD for derivation of the RfD (USEPA, 2011). Uncertainty related to toxicodynamic processes (i.e., MOA), however, still exists; thus, the EPA applied a UF_A of 3 along with a UF_D of 3 for data gaps. Given the lack of specificity of the review paper to GenX chemicals, this publication was not added to the document (Rakhshandehroo et al., 2010). The EPA updated the UF_A discussion, however, to highlight that this addresses uncertainty related to toxicodynamic processes such as MOA.

Kamendulis

Yes, the literature search strategy, study selection and evaluation considerations were very well presented and sufficiently clear. The process used was described well and was a very thorough and transparent approach to systematically evaluate each of the available scientific studies that described the health effects of GenX chemicals.

I am unaware of other peer-reviewed studies that should be included in this assessment.

EPA Response: Thank you for your response.

Leung

The draft report describes the systematic approach taken toward the identification and selection of pertinent studies on this topic. The search strategy is overall easy to understand and transparent, although there are some concerns and several areas which could be better addressed:

a. The most important issue is that the search results highlight the overall relative scarcity of available data regarding the potential risks of Gex chemicals. Almost all of the literature used to base the current assessments are animal studies conducted by Dupont submitted as part of the TSCA. The HERO database shows 119 included references, from which only 29 are from non-Dupont sources (and not all, albeit most, are necessarily from peer-reviewed journals.) The lack of rigorous studies published in well-regarded journals spanning the animal, human, and epidemiologic literature is a substantial limitation to interpreting the health impacts of exposure to these chemicals.

EPA Response: In section 3.3.1, the EPA describes the limited number of publicly available studies. Section 1.1 (History of Assessment of GenX Chemicals at EPA) is included in the assessment to provide the reader with the background information necessary to understand the origin of the data used for this assessment. Additionally, throughout the document, the data have been well characterized as either published, peer-reviewed studies or studies submitted by DuPont/Chemours. All of the available DuPont/Chemours studies considered in the assessment have also been made publicly available through HERO at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627. Despite the limited number of publicly available and peer-reviewed studies, the studies that are available for assessing GenX chemicals toxicity are robust (i.e., full study reports containing individual animal data) and adhere to good laboratory practices and EPA and international (i.e., OECD) TGs.

Specifically, and as noted in section 3.3.2 of the assessment, all of the TSCA-submitted studies considered for derivation of the RfDs were conducted according to OECD TGs and/or EPA health effects TGs for pesticides and toxic substances, which "are generally intended to meet testing requirements for human health impacts of chemical substances under the FIFRA and TSCA." All available studies were considered for inclusion (see appendices A and B), and most of the studies selected for inclusion in this assessment adhered to the Principles of Good Laboratory Practice, and full study reports, including individual animal data, were submitted for Agency review (see section 3.3.2). It should also be noted that two of the DuPont studies used in this assessment were published in peer-review journals:

- Hoke, R.A., B.D. Ferrell, T.L. Sloman, R.C. Buck, and L.W. Buxton. 2016. Aquatic hazard, bioaccumulation and screening risk assessment for ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate. *Chemosphere* 149:336–342.

- Rae, J.M., L. Craig, T.W. Slone, S.R. Frame, L.W. Buxton, and G.L. Kennedy. 2015. Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-/propanoate in Sprague-Dawley rats. *Toxicology Reports* 2:939–949.

b. One concern regarding the search strategy, as shown in Appendix A (Table A-5), is that studies for which only an abstract was available were excluded. What does this signify, and were there any further attempts done to assess the relevance of these excluded studies (which are not shown)?

EPA Response: The GenX chemicals literature search resulted in just one study for which only an abstract was available within the cited journal.[1] This study was related to immunotoxicity of HFPO dimer acid and its ammonium salt. The reference lacked information with which to evaluate it using the EPA's criteria. In fact, only the title was available online when conducting the literature search. The literature search and systematic review process did not include contacting authors to obtain more details for abstract-only references. The EPA, however, did not deem this a critical limitation as a more recent report describing an immunotoxicity in mice (Rushing et al., 2017), by the same authors as the abstract, was also identified and is summarized in the draft document.

c. The draft report demonstrates the approach used to rank the relevance and usefulness of included studies. Studies were then scored by various EPA OPPT criteria quite rigorously (Appendix B) in an objective a manner as possible by several reviewers. It would be relevant to state the number of reviewers, whose aggregate assessment was reported as weighted scores, involved in this process, as well as their professional areas of expertise.

EPA Response: The study reviews were conducted using established EPA OPPT protocols. Specifically, the GenX chemicals study reviews were conducted by EPA staff with expertise in toxicology, biology, pharmacokinetics/modeling, and related fields with understanding of OECD TGs and risk assessment. Each expert reviewed 1–2 studies. Following individual

[1]Rushing, B.R., and J. Dewitt. 2013. Immunotoxic effects of undecafluoro-2-methyl-3-oxahexanoic acid in mouse models. *Abstracts of Papers - American Chemical Society* Vol 245.

study reviews, a separate staff scientist performed a final review of all reviewers' evaluations to provide a level of consistency across the evaluations.

d. One additional comment regarding the scoring system is regarding Metric #22, which assessed "Health outcomes thought to be unrelated to exposure". It does not appear that are any further details regarding what these outcomes may have been, although there are occasional comments when such health outcomes were not reported in the study. Further detail regarding these health outcomes would provide greater context to the conclusions posed by this draft report.

EPA Response: Thank you for the comment. All health effects that demonstrated a dose response and were significantly different from the control are reported in the assessment; this includes effects that were categorized as "outcomes unrelated to exposure" by study authors. Therefore, these details are already covered in the body of the assessment and will not be added to the systematic review sheet tables. The EPA will consider this input, however, as EPA OPPT implements its systematic review procedures and in any updates in the future.

Slitt

The literature search strategy was appropriate and thorough. It was well described and included clear criteria for the inclusion and exclusion of studies. The databases utilized (i.e. PubMed, WOS, Toxline, and TSCATS1) are appropriate and the search terms were comprehensive in nature. The methods in section B used to evaluate study quality were systematic and thorough. The metrics and criteria applied for Animal and in vitro toxicity studies were exceedingly thorough and well defined. The weighting and relative important used for weighting the criteria was appropriate. Overall, this semi-quantitative approach in evaluating the data/studies to be included is considered to be is appropriate and thorough.

A minor comment is that exposure for in vitro studies on page A-9, Table A-5 should be described. It is assumed that it is via addition of chemical to media, however it should be described for what criteria would be constitute inclusion/exclusion for in vitro studies. For example, solubility and vehicle could be mentioned.

EPA Response: The EPA will add the following bullet in under exposure in Table A-5:

- Exposure is via cells in culture or subcellular matrices.

Warren

Sections 3.3.1 (Literature Search Strategy and Results) and 3.3.2 (Study Screening Process and Study Evaluation) succinctly describe a laborious task that requires considerable skill if done well. I have refrained from using the phrase "done correctly" in recognition of the subjective judgments inherent to the process. The search of public literature is clearly comprehensive, as I was able to locate only one additional study that might be worthy of inclusion in the toxicity assessment (see below). Admittedly, I am unsure as to whether it meets the inclusion-exclusion criteria in Table A-5. Based on its title and abstract, it might be appropriate to include in Section 1.3 (Occurrence). As for the screening process and evaluation of manufacturer-submitted studies, like the toxicity assessment, I too acknowledge the importance of adherence to OECD/EPA TGs and GLP. And while I find the scoring system and qualitative rating process a bit arbitrary, the evaluation domains and metrics within them make for a transparent means in the pursuit of consistency and ultimately, validity in

toxicity values. Therefore, I support the study evaluation process while encouraging its refinement over time.

Additional Peer-Reviewed Study

1. WA Gebbink, L van Asseldonk, and SPJ van Leeuwen. Presence of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in River and Drinking Water near a Fluorochemical Production Plant in the Netherlands. *Environ. Sci. Technol.*, 51(19):11057-11065, October 3, 2017.

The above study investigated the presence of legacy and emerging PFAS (including GenX) in river water collected in 2016 up- and downstream from a fluorochemical production plant in The Netherlands. Additionally, drinking water samples were collected from municipalities in the vicinity of the production plant, and like the river water, were positive for GenX.

> **EPA Response**: Thank you for the suggested reference. EPA has added a more inclusive list of international occurrence references to section 1.3 (Occurrence), including this reference.

CHARGE QUESTION 2

For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?

a. **If so, please explain your justification.**
b. **If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.**

Chou

The selection of liver effects as the critical effect for derivation of oral subchronic and chronic RfDs, using the oral reproductive/developmental toxicity screening study in adult mice, is well justified. It is justified based on increases in liver weight, relatively high number of animals (22-25) per treatment group for liver endpoints, and consistent adverse effects corroborated in other studies presented in the Draft. The weaknesses of other studies are well described in the draft on p. 51-52.

> **EPA Response**: Thank you for your response.

Kamendulis

a. **If so, please explain your justification.**

I agree with the selection of the critical study selected for deriving RfD's for GenX chemicals (DuPont-18405-1037 2010). This oral reproductive/developmental toxicity study in mice identified liver effects, specifically single-cell necrosis in males, as the critical effect and was used to derive the subchronic and chronic RfDs for GenX chemicals. As noted, this study utilized a larger sample size (n=24 / dose) and provided the most health protective POD_{HED}. Further, this study was an 84-85

day study compared to the other mouse study (28 days). In addition, several other studies provide support for the selection of liver necrosis as the critical effect following oral exposure to GenX chemicals (DuPont-24459 2008; DuPont-24447 2008; DuPont-18405-1307 2010; DuPont-18405-1238 2013; DuPont-18405-841 2010). Liver effects (changes in liver enzyme levels, histopathological lesions, and tumors) were observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals.

EPA Response: Thank you for your response.

b. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.

N/A

EPA Response: No response required.

Leung

This study in which the subchronic and chronic RfDs is based upon consisted of 150 mice, in which histopathologic changes and increased liver weights were seen in all three dose groups. However, as summarized in the draft report, these findings have also largely been supported by at least seven other studies, including one from the peer-reviewed published literature, although only one was considered chronic (i.e., >90 days duration). Evidence of hepatic damage is also supported by the very significant increases in serum transaminase levels among mice exposed to the higher doses. Overall, availability of more than one study assessing *chronic* exposure would be relevant toward the hepatic and other potential clinical endpoints.

The draft report describes that the other organ systems and endpoints in which adverse effects have been found were hematology, renal, development/reproduction, immunology, and carcinogenesis (Section 5). No differences in survival at a planned 12-month necropsy timepoint were found in the one available chronic study. Regarding these other organ systems:

a. It is described that the maximum decrement of hemoglobin/hematocrit levels in male mice and in rats was 12% from several studies assessing doses up to 100 mg/kg/day lasting up to 180 days. This may be clinical significant, and consideration of this endpoint in greater detail may be relevant. Additionally, in the one chronic study, female mice and rats had up to a 24% decrease in hemoglobin/hematocrit levels, further supporting this as a possible critical effect.

EPA Response: We agree with the commenter regarding the potential clinical significance of the decreased hemoglobin/hematocrit endpoint as described in section 6.1. As outlined in the document, however, the hematological effects were inconsistently observed in both mice and rats, especially as study duration increased. The limitations in the database related to blood effects could be addressed with additional studies. The EPA has added the need for additional research to the UF_D discussion. Additionally, the EPA highlighted these inconsistencies in the observed hematological endpoints to the uncertainty discussion (section 7.0).

b. Regarding renal effects, the primary endpoints that have been assessed are weight of the kidneys, microscopic changes of the kidney, and blood urea nitrogen concentrations. The draft report states that given the limited changes in these parameters, renal toxicity is unlikely a critical effect. Animal physiology is not my area of expertise, but there certainly other factors important in the assessment of human renal function like serum creatinine concentrations and filtration rate that are also clinically important.

EPA Response: As discussed in section 3.3.4, the EPA reported potential adverse effects that were statistically significantly different from the control and/or had a dose-response. Therefore, the EPA did not report every endpoint that was measured in each individual DuPont/Chemours report. The increases in kidney weight and blood urea nitrogen concentrations as well as the microscopic changes of the kidney were statistically significantly changed from the control group. Serum creatinine levels were measured in many of the toxicity studies (DuPont-24447, 2008; DuPont-24459, 2008; DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1238; 2013), but none of the treated groups in these studies had creatinine levels that were statistically significantly different from the control. Filtration rate was not measured in any of the studies.

c. It is unfortunate that the potential developmental/reproductive toxicities are based only on two animal studies. From the description of these, these toxicities are rare and may not be clinically significant. However, only F1 mice were studied and there are no data on potential effects spanning more than two generations.

EPA Response: The developmental effects observed as a result of *in utero* exposure to GenX chemicals (e.g., decreased birth weight and altered puberty as evidence by vaginal patency and balanopreputial separation) are considered adverse effects (USEPA, 1991, 1996). These are common developmental endpoints measured in developmental toxicity studies. The lack of a full two-generation reproductive toxicity study evaluating exposures during early organogenesis (i.e., gestation day 0 to gestation day 6) and studies evaluating additional developmental endpoints observed following exposure to other PFAS (i.e., skeletal development in mice and altered puberty in mice (USEPA, 2016a, 2016b)) is identified as a database deficiency. Please see the discussion in section 6.4.2 for more information.

d. The data for immunotoxicity are even more limited, but suggest some adverse effects among female mice. In the absence of more data, it is difficult to ascertain whether these preliminary findings should be considered critical effects.

EPA Response: Data on the potential for GenX chemicals to impact aspects of immune function beyond immunosuppression are lacking. Additional studies would be useful to support a more conclusive determination of immunotoxic potential. Please see the UF_D discussion in section 6.4.2 for more information.

e. I agree with the summary in Section 5.6, which describes the inability to determine overall carcinogenicity based on the substantially limited available data. However, the observation of increased hepatocellular carcinomas in female dosed rats should not be discounted.

EPA Response: In section 4.4, the EPA concluded that the increased incidence of liver tumors in females at 500 mg/kg/day is treatment-related. The EPA does not discount these data but found the existing evidence from this single chronic study inadequate to justify a

quantitative assessment for either males or females. The EPA has added the lack of additional chronic studies in the most sensitive species to the UF_D discussion in section 6.4.2.

Slitt

Yes, a survey of the data included in the report points to liver effects in adult male is appropriate and defensible. The study meets the criteria listed in almost all elements for being considered of high quality. It meets every metric as high or medium, such as test substance, test setup, exposure characterization, etc. The critical effect of single cell necrosis in male mice is based on n=24-25 F0 males, which a large "n". The selection of this study is scientifically justifiable based on it sufficiently meeting the review criteria. The selection of single-cell necrosis is a reasonable measure to use as a critical effect. This measure has been used previously for PFOA and PFOS, in which rodent studies that have demonstrated hepatotoxicity demonstrate concordance with studies in human populations illustrating slight elevation serum in liver enzymes.

It should be noted that there was PFOA contamination for the test substance for DuPont 18405-1037, 2010 lists that purity of the test article at 84%. Another study listed in the document that meets the evaluation criteria with high confidence (DuPont -24459, 2008) lists a slightly higher purity of the test article (88% purity). This 28-day oral dosing study that evaluated 0.1, 3 and 30 mg/kg/day did not observe any statistically significant increases in liver single cell necrosis at 0.1 mg/kg, but did observe significant elevation of serum liver enzymes, liver weight, and single cell necrosis in males at 3 mg/kg. Given that the purity of the test article was slightly higher, this study should also be considered along with DuPont 18405-1037, 2010 for considering 0.1 mg/kg in male mice for the RfD.

EPA Response: The EPA agrees with the reviewer that the 28-day oral dosing study in mice (DuPont-24459, 2008) is an important study, which is why it was considered as a candidate POD and selected for BMD modeling alongside the oral reproductive/developmental toxicity screening study (DuPont-18405-1037, 2010). As described in section 6.2 and appendix E of the assessment, the results of the BMD modeling (presented in Table 8) demonstrate that DuPont-18405-1037 (2010) yielded the most health protective POD_{HED}. Moreover, this study used a larger sample size (n=24 / dose versus n=10 / dose). Finally, DuPont-18405-1037 (2010) was of a longer duration (84/85 days) than DuPont-24459 (28 days). For these reasons, the EPA selected DuPont-18405-1037 (2010) as the critical study for the derivation of RfDs for GenX chemicals.

Additionally, section 7.2 (Effects Characterization) further describes purity as a consideration in study selection.

Warren

Selection of the gavage study of reproduction/developmental toxicity in mice (DuPont 18405-1037, 2010) and single cell necrosis for toxicity value derivation are scientifically justified and well defended in the toxicity assessment. However, the dismissal of studies from further consideration as the critical study on the basis of NOAEL alone (i.e., having a NOAEL > 0.1 mg/kg/day) is a questionable practice. This is especially true when a dismissed study's NOAEL is but an order of magnitude greater than that of the critical study (i.e., 1 mg/kg/day), a difference dwarfed by the total UF applied to toxicity value derivation. Nonetheless, Section 6.1 makes a convincing case for

selection of critical study and effect, especially with regard to preference for liver necrosis over a hematological or immunological effect and use of a subchronic study for chronic RfD derivation in lieu of a chronic toxicity/oncogenicity study reporting the same critical effect, albeit at a considerably higher dose in a less sensitive species.

EPA Response: Section 6.1 has been revised to further discuss selection of critical studies and effects, specifically addressing why those with NOAELs higher than 0.1 mg/kg/day were considered no further for modeling.

CHARGE QUESTION 3

EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?

Chou

The reviewer agrees with the selection of BMDL10 from Table E-2 and Table E-4. The modeling approach, selection of benchmark response level, and the selected model used to identify the PODs are well justified and defensible.

EPA Response: Thank you for your response.

Kamendulis

Yes, I agree with the approach used. As there are no biologically based dose-response models available for GenX chemicals, benchmark dose modeling was used, and was consistent with EPA's guidance document (USEPA 2012). The BMD and the BMDL were estimated using a BMR of 10% extra risk for dichotomous data, and candidate PODs were estimated from all 3 doses (plus control) for the critical study.

EPA Response: Thank you for your response.

Leung

BMD modeling is not within my area of expertise. However, it appears that the draft report makes a reasonable effort to identify clinically-relevant endpoints required for the modeling from the available limited data. I defer to the other reviewers regarding whether the translation of a POD to a human dose equivalent, the determination of uncertainty factors (UFs), and the mathematical calculations of the corresponding subchronic and chronic RfDs, are appropriate.

EPA Response: Thank you for your response.

Slitt

This is out of my area of expertise, so I decline responding to this question.

EPA Response: No response required.

Warren

Application of BMD modeling to data from two of three studies having NOAELs of 0.1 mg/kg/day was appropriate for POD identification, as neither sex of rats in the third study (DuPont-17751-1026 2009) exhibited liver necrosis. The modeling approach appears consistent with USEPA's *Benchmark Dose Technical Guidance* document, including use of dichotomous models, selection of BMR, and inclusion of data from all dose groups given an adequate model fit when none are omitted. Criteria for model selection appears to be largely consistent with Agency guidance, as well. For example, model selection considerations included those with goodness of fit p-values > 0.1, lowest Akaike's Information Criterion (provided BMDLs are "sufficiently" close), sufficiently small BMD:BMDL ratios, and lowest scaled residuals for doses near the BMD/BMDL. In addition, fitted curves of incidence rate as a function of dose allowed for an assessment of visual fit. Finally, selection was made of the lowest BMDL (0.15 mg/kg/day), which was one-half that of the alternative derived from the 28-day gavage study in mice. As such, the toxicity assessment's BMD modeling results were obtained using time-tested and widely-accepted methods and model choice decision logic, making it scientifically justified and defensible. It is noteworthy that such methods stand in stark contrast to the means by which North Carolina derived a drinking water equivalent level on the basis of an RfD derived by application of a total UF of 1,000 to the NOAEL of 0.1 mg/kg/day for single cell necrosis in the liver.

EPA Response: Thank you for your response.

CHARGE QUESTION 4

Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).

a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**
b. **Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?**

Chou

a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**

The reviewer agrees to the use of ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs in the draft assessments.

EPA Response: Thank you for your response.

b. Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

Considering the differences of enzymes targeted by PPARα activation between the mouse and the human (see comments for Item 1), the UF_H and UF_D values used in this draft are appropriate; any values less than 3 would not be acceptable.

EPA Response: Please see response to Charge Question 1:

Thank you for providing the reference to the review paper by Rakhshandehroo et al. (2010). This review paper provides an overview of PPARα target genes involved in different biological processes in the liver and the literature evaluating differences and similarities between the genes impacted in mice and humans. The authors conclude that based on their review of the literature, the function and specific target genes of PPARα are generally well conserved between mouse and human specifically for lipid metabolism. Only a subset of those genes impacted in mice and humans, however, are linked to the PPARα response element and none of those identified as human fell only in that category. In addition, the review paper does not specifically address GenX chemicals or other perfluorinated compounds (Rakhshandehroo et al. 2010).

As described in section 4.7, findings consistent with PPARα agonists were observed (e.g., increases in liver weight, hepatocellular hypertrophy, and increased β-oxidation activity) for GenX chemicals; however, data gaps exist for key events and other MOAs might be involved (Rakhshandehroo et al., 2010). Therefore, the EPA concluded that the MOA for GenX chemicals is not clearly understood and cannot be solely attributed to PPAR.

The commenter noted that several genes involved in biotransformation and lipid metabolism are human specific and recommended the potential effects resulting from regulation of these human-specific PPARα target genes should be considered when determining UF_A. As pointed out above, none of those human genes were directly linked to the PPARα response element by the authors. The UF_A is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both toxicokinetic (i.e., absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. The toxicokinetic portion of the UF_A was accounted for by application of the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* in the determination of the POD for derivation of the RfD (USEPA, 2011). Uncertainty related to toxicodynamic processes (i.e., MOA), however, still exists; thus, the EPA applied a UF_A of 3 along with a UF_D of 3 for data gaps. Given the lack of specificity of the review paper to GenX chemicals, this publication was not added to the document (Rakhshandehroo et al., 2010).

Kamendulis

a. Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.

Yes, the application of ¾ allometric scaling was justified and adequately presented. Any uncertainty in using this approach is accounted for by application of uncertainty factors (see answers to question 5 below).

EPA Response: Thank you for your response.

b. Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

Yes, the methods that were used to derive the RfD's for GenX chemicals took into consideration toxicokinetic differences between animals and humans. Further, any uncertainties are accounted for by the application of uncertainty factors (see question 5 below).

EPA Response: Thank you for your response.

Leung

This is not my area of expertise, thus I defer to the other reviewers.

EPA Response: No response required.

Slitt

a. Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.

This is not within my expertise to respond.

EPA Response: No response required.

b. Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

Yes, the methods used do account for the appropriate uncertainties.

EPA Response: Thank you for your response.

Warren

Yes, use of the default dosimetric adjustment factor (DAF) of bw to the ¾ is scientifically justified and defensible given the lack of a PBPK model for GenX (possibly excluding that of Gomis et al., 2018), paucity of toxicokinetic data in experimental animals and their absence in humans, and little mechanistic information to inform the issue of how internal dose relates to the nature, magnitude, and time-course of biological effects. As noted in the toxicity assessment, its use is also justified given GenX's lack of metabolism, relatively short clearance time compared to longer-chain PFAS, and application to adult mice as opposed to those in earlier life stages. Furthermore, the default approach is consistent with the hierarchy of approaches for interspecies extrapolation clearly expressed by USEPA in multiple documents, including *Recommended Use of Body Weight3/4 as the*

Default Method in Derivation of the Oral Reference Dose and *Harmonization in Interspecies Extrapolation: Use of BW3/4 as Default Method in Derivation of the Oral RfD*. The toxicity assessment's use of a UF of 3 for interspecies differences is also consistent with Agency guidance, as the default DAF appropriately addresses some, but not all, of the considerable cross-species uncertainties in both GenX toxicokinetics and toxicodynamics. Lastly, it is encouraging to see the use of an updated body weight for adult humans in the DAF equation (i.e., 80 kg), a minor change, but one that increases confidence that the toxicity assessment reflects the state-of-the-science.

EPA Response: Thank you for your response.

CHARGE QUESTION 5

EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UF_H), interspecies differences (UF_A), database limitations (UF_D), duration (UF_S), and LOAEL-to-NOAEL extrapolation (UF_L) for GenX chemicals.

a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**
b. **Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.**

Chou

a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**

Please see response to Question 4b.

EPA Response: No response required.

b. **Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.**

The draft provided appropriate scientific rationale for the selection of the UF values. Additional justification for UF_A may be used. See comments for Question 1.

EPA Response: Please see response to Charge Question 1:

Thank you for providing the reference to the review paper by Rakhshandehroo et al. (2010). This review paper provides an overview of PPARα target genes involved in different biological processes in the liver and the literature evaluating differences and similarities between the genes impacted in mice and humans. The authors conclude that based on their review of the literature, the function and specific target genes of PPARα are generally well conserved between mouse and human specifically for lipid metabolism. Only a subset of those genes impacted in mice and humans, however, are linked to the PPARα response element and none of those identified as human only fell in that category. In addition, the review paper does not specifically address GenX chemicals or other perfluorinated compounds (Rakhshandehroo et al., 2010).

As described in section 4.7, findings consistent with PPARα agonists were observed (e.g., increases in liver weight, hepatocellular hypertrophy, and increased beta-oxidation activity) for GenX chemicals; however, data gaps exist for key events and other MOAs may be involved (Rakhshandehroo et al., 2010). Therefore, the EPA concluded that the MOA for GenX chemicals is not clearly understood and cannot be solely attributed to PPARα.

The commenter noted that several genes involved in biotransformation and lipid metabolism are human specific and recommended the potential effects resulting from regulation of these human-specific PPARα target genes should be considered when determining UF_A. As pointed out above, none of those human genes were directly linked to the PPARα response element by the authors (Rakhshandehroo et al., 2010). The UF_A is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both toxicokinetic (i.e., absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. The toxicokinetic portion of the UF_A was accounted for by application of EPA's *Recommended Use of Body Weight3/4 as the Default Method in Derivation of the Oral Reference Dose* in the determination of the POD for derivation of the RfD (USEPA, 2011). However, uncertainty related to toxicodynamic processes (i.e., MOA), however, still exists; thus, the EPA applied a UF_A of 3 along with a UF_D of 3 for data gaps. Given the lack of specificity of the review paper to GenX chemicals, this publication was not added to the document (Rakhshandehroo et al., 2010).

Kamendulis

a. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Yes, uncertainty has been adequately accounted for in the derivation of RfDs for GenX chemicals. An interspecies uncertainty factor of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans. A factor of 3 was used in lieu of a 10 since a POD_{HED} was derived from the BMDL as specified in EPA's guidance document (USEPA 2011b). As the allometric scaling accounts for some aspects of species extrapolation, some uncertainty remains. Thus, the application of an UF of 3 appears appropriate.

An intraspecies uncertainty factor (UF_H) of 10 is assigned to account for variability in the responses within the human populations. This is also appropriate.

The BMD approach was utilized, therefore application of a UF for using a LOAEL to NOAEL is not needed.

For the chronic RfD, although a chronic bioassay is available (in rats), a UF of 3 was applied for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 3. The 2-year study identified a NOAEL of 1 mg/kg-day for liver effects (increased liver enzyme levels and centrilobular hepatocellular hypertrophy and cystic focal degeneration in males and centrilobular necrosis in both sexes), consistent with the effects observed in the oral reproductive/developmental study in mice, however, at a much higher NOAEL. As data suggests that rats appear to be less sensitive than mice, a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. This appears appropriate.

While only a limited number of studies have been conducted for GenX chemicals, a database uncertainty factor (UF_D) of 3 was applied. This is due to the quality of the available studies – which include acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity studies in mice and rats with dosing durations of up to 2 years, and one reproductive and developmental toxicity study in mice and one prenatal and developmental toxicity study in rats. Many of these studies were conducted under GLP conditions, with full study reports available and reviewed. Thus, the application of a UF_D of 3 appears appropriate.

EPA Response: Thank you for your response.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

I have addressed this comment in my responses to 5a above - the application of the UF's used to derive RfD's for GenX chemicals was scientifically based and well described.

EPA Response: Thank you for your response.

Leung

This is not my area of expertise, thus I defer to the other reviewers.

EPA Response: No response required.

Slitt

a. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Yes. The use of uncertainty factors was used according to EPA guidance (USEPA, 2011b). The authors were unable to find any human epidemiological studies, so an UF of 10 was used appropriately. The application of a UF of 3 for interspecies differences is appropriate as well because the chronic cancer bioassays were performed in rat and not mouse, with the several studies herein demonstrating that rats appear to be a less sensitive species than mouse. Lastly, a UFD of 3 is in agreement with the guidance, as there are many knowledge gaps for GenX. Lastly, uncertainty for intraspecies variability and database limitations must be included. Currently there are no studies to address whether developmental or immunotoxicity is observed at exposures lower than the 0.1 mg/kg RfD that was observed for liver effects in male mice.

EPA Response: Thank you for your response.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

Yes, Section 7 did outline and provide sufficient rationales for the application of the selected uncertainty factors. See response in part a.

EPA Response: Thank you for your response.

Warren

Yes, the considerable uncertainty surrounding the risk of GenX exposure has been adequately accounted for in the toxicity assessment. Section 6.4.2 does a good job of explaining the rationale behind each individual UF value and I agree with the total UFs of 100 and 300 used for RfD derivation.

EPA Response: Thank you for your response.

CHARGE QUESTION 6

The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.

a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**
b. **Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.**
c. **Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.**

Chou

a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**

Yes, the draft clearly synthesized the toxicological effects.

EPA Response: Thank you for your response.

b. **Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.**

Yes, the weight of evidence for hazard identification has been clearly described, and scientifically justified to meet the definition of "adverse".

EPA Response: Thank you for your response.

c. **Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.**

Yes, the conclusions are supported by scientific evidences. Please also see comments above.

EPA Response: Thank you for your response.

Kamendulis

a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**

I agree that the available data were adequately and clearly described.

EPA Response: Thank you for your response.

b. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.

Yes, I feel that the weight of evidence for effects on the liver, as well as other endpoints (hematological and immune systems, and reproductive and developmental) was scientifically evaluated and justified.

EPA Response: Thank you for your response.

c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

The liver effects observed following exposure to GenX chemicals were consistently seen across several studies including both male and female rats and mice, for short-term and chronic exposures. Thus, the conclusions drawn are scientifically supported and adequately described in the document.

EPA Response: Thank you for your response.

Leung

The Hall 2012 reference helps delineates the differences between physiologic adaptive responses vs pathologic toxic effects resulting in hepatocellular hypertrophy. Using this framework, the draft report summarizes the available studies with hepatic endpoints, which did show increased proportions of dosed animals with increased liver weights, abnormal serum liver function tests, hepatocellular hypertrophy, and hepatocellular necrosis. These studies were also the basis for the observation that mice may be more sensitive to toxic hepatic effects than rats, given the development of the adverse findings at comparably higher doses in rats. The report does not comment on whether steatosis was assessed and/or observed in these studies.

The hepatic toxicologic effects are consistent and reproducible, as seen from the eight studies of either mice or rats and in both genders. Altogether, the conclusions regarding adversity in the draft report are appropriately summarized.

EPA Response: Thank you for your response. Additional text has been added to section 4.7 (Mode of Action) that characterizes the steatosis data. Steatosis is not assessed in any of the DuPont/Chemours studies. Wang et al. (2016) is the only available study to qualitatively mention observing steatosis in mouse liver samples but does not provide quantitative measurements.

Slitt

a. Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.

Yes, the review agrees with the overall conclusion of the authors in section 4.7 for the mode of action. The conclusion from the document is that the findings are not adequate to definitely conclude that a PPARα mechanism of action (MOA) exists for HFPO dimeric acid and/or ammonium salt. There is a lack of publications that have evaluated the mechanisms of action for GenX and the authors cannot draw firm conclusions regarding PPARα involvement with limited findings. This is

based on a study by Wang et al., 2016 that administered 1 mg/kg/day via oral gavage for 28 days to male ICR mice that was not considered to be of high qualitative determination because it lacked sufficient quality in multiple elements. The finding from this study, point to treatment with the HFPO dimeric acid ammonium salt impacting pathways in liver for PPARα signaling, retinol catabolism, and fatty acid degradation, however the lack of rigor in the experimental design limits the interpretation. The authors do cite Dupont-24459 2008 and Dupont-24447 2008 with some potential endpoints that are consistent with peroxisome proliferation (i.e. hepatic β-oxidation), however they do not identify PPARα as the cause for the observed effects. Thus, the conclusion that the findings are not adequate to definitely that a PPARα mechanism of action (MOA) exists for HFPO dimeric acid and/or ammonium salt is appropriate.

EPA Response: Thank you for your response.

b. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.

Yes. The weight of evidence (WOE) for hazard identification has been clearly described and scientifically justified in this document. The WOE is based on the liver being a target organ for toxicity from the oral exposure to HFPO dimeric acid. The document cites liver effects for both male and female mice and rats, with male mice being the most sensitive species. This conclusion is based on several studies carried out in which the test methods and study design meet criteria as being "high" in quality. These studies assessed the impact of GenX in numerous endpoints (i.e. gross measures such as food consumption, appearance, serum clinical markers of injury, gross organ changes, and tissue pathology). These studies did evaluate relative liver weight and hepatocellular hypertrophy in the context of evidence of hepatic necrosis, as measured by serum enzyme levels and evidence of cellular necrosis.

EPA Response: Thank you for your response.

c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

Yes, the conclusions regarding adversity are scientifically supported and clearly described. There were no human epidemiology studies for GenX to base adversity, so rodent studies were used as a basis for the RfD. The conclusion is that 0.1 mg/kg/day causes liver effects that are observed as single cell necrosis in males (DuPont-18405-1037 2010). Other studies presented in the document (refer to Table 7) also support liver effects being considered the adverse effect for GenX at similar NOAELS (Dupont-24459, DuPont-18405-1238). Because these studies evaluated necrosis and liver weight and found evidence for cytotoxicity, they meet the concern for adversity as outlined by Hall et al. 2012). In addition, because there is a lack of substantive information regarding the mechanism of action for GenX in rodent and cell-based studies, the role of PPARα as being the cause for the observed liver effects, such as liver weight and single-cell necrosis, cannot be concluded.

EPA Response: Thank you for your response.

Warren

The toxicity assessment, particularly Section 5.1, does an admirable job of synthesizing the available data on liver effects and making the case (using a weight-of-evidence approach) for GenX exposure

as a potential hazard to the human liver provided a threshold dose is met. Concern that the liver may not be the most appropriate target organ for RfD derivation is minimal at best, as four manufacturer-submitted studies reported liver effects at the LOAEL. In these studies, effects were seen in both sexes of mice and rats under conditions of varying exposure magnitude and duration. Furthermore, the critical effect of single-cell necrosis typically co-occurred with liver hypertrophy, increased relative liver weight, and/or elevated liver enzymes, not to mention liver tumors in chronically-treated, high dose, female rats. As for the issue of whether hepatic hypertrophy and increased relative liver weight are "adverse", the toxicity assessment clearly warns against the use of such an unqualified label in the event such effects are PPARα-mediated. This is consistent with the consensus opinion of Hall and colleagues (an expert panel of workshop attendees) that hepatic hypertrophy, in the absence of histologic or clinical pathology, should be considered adaptive or non-adverse. Given the weight ascribed to the Hall et al. (2012) publication by the toxicology community, and deservedly so, it is appropriate that USEPA share the same opinion in the absence of evidence to the contrary.

EPA Response: Thank you for your response.

CHARGE QUESTION 7

The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.

Chou

Currently, there is no sufficient evidence to determined that the adenomas and adenocarcinoma observed in rodent liver are not relevant in humans. Nonetheless, the evidence in rodent adenocarcinoma present in the existing studies is not very strong. The reviewer agrees to the descriptor "there is suggestive evidence of carcinogenic potential for GenX chemicals."

EPA Response: Thank you for your response.

Kamendulis

There are no dermal or inhalation studies available that evaluated evaluating cancer, however, one oral exposure bioassay for HFPO dimer acid ammonium salt in rats showed an increase in liver tumors (females) and pancreatic acinar adenomas and carcinomas (combined, males). While there were increases in tumors at the high dose, a dose-response pattern was not observed. Further, data suggests that mice may be more sensitive to GenX chemicals compared to rats. As such, based on the available data, I agree with EPA's Guidelines for Carcinogen Assessment that classifies GenX chemicals as *Suggestive Evidence of Carcinogenic Potential* following oral exposure only.

EPA Response: Thank you for your response.

Leung

From my understanding of Section 4.4, the EPA had requested that studies be repeated to exclude both control and dosed animals when it was observed that early deaths were found in both groups that were not necessarily associated with incident tumors (thus presuming that the deaths may have

been unrelated to the intervention). The reanalysis was performed, but only in male rats and only to assess for testicular hyperplasia/tumors. Thus, it is unclear why the reanalyses were not comprehensively performed for both male and female rats, and also systematically for other potential tumors.

From the available information that did not reflect this exclusion, there were statistically significantly increased proportions of hepatocellular carcinomas in female dosed rats and a trend toward increased pancreatic carcinomas in male dosed rats, although both occurred only at single high doses; a typical dose-response pattern was not observed. I agree with the report that mice should also be studied, as it was reported that mice may be more susceptible toward GenX carcinogenicity. Altogether, given these points, I agree with the summary in Section 5.6, which describes the inability to determine overall carcinogenicity based on the substantially limited available data. However, the observation of increased hepatocellular carcinomas in female dosed rats should not be discounted.

> **EPA Response**: The EPA does not discount the increased hepatocellular carcinomas in female dosed rats and classifies this effect as adverse and related to HFPO dimer acid ammonium salt exposure in sections 4.4 and 5.6. Review of the initial results indicated the increased incidences of liver tumors and pancreatic acinar tumors were significant despite the inclusion of early deaths, thus the EPA agreed to limit the reanalysis to testicular hyperplasia and tumors in male rats only.
>
> Sections 4.4 and 5.6 have been edited to provide additional details regarding the reanalysis. The EPA has identified the lack of a chronic study in the most sensitive species as an uncertainty.

Slitt

The conclusion is based on evidence that the liver is the target organ for toxicity and primary organ for tumor development. The lack of cancer bioassay data using mice, which are the more sensitive species, limits the conclusion. The conclusion is based on findings from a study that meets the review criteria as being of high consideration and is in line with the EPA's Guidelines for Carcinogenic Potential for Carcinogen Risk Assessment (USEPA, 2005). Based on this document, is appropriate to make this conclusion based on the literature collected and studies that met criteria for consideration. Moreover, the lack of substantive information regarding PPARα activation does not allow one to rule out the potential for PPARα-independent activation contributing to the observed liver effects.

> **EPA Response**: The EPA has identified the lack of a chronic study in the most sensitive species as an uncertainty.

Warren

I agree that the one chronic toxicity/oncogenicity study is suggestive of carcinogenic potential for GenX, and in the absence of information that the observed tumors were rat-specific, believe the weight-of-evidence descriptor should apply to humans exposed via the oral route. Sections 4.4 and to a greater extent, 5.6, speak to the considerable weaknesses in the cancer bioassay data. Nonetheless, liver and pancreatic tumors in GenX-exposed female and male rats, respectively, were significantly elevated over controls. As rats have been shown to be considerably less sensitive than mice to many

non-cancer effects of GenX, a chronic bioassay in the more sensitive species would be a logical next step. While the toxicity assessment provides an informative overview of mode of action possibilities (e.g., cytotoxicity followed by reparative proliferation, genotoxicity though the data are mixed, and promotion mediated by PPARα), data are insufficient to select one over the others. Obviously, if the observed liver tumors in rats were PPARα-mediated, humans would be resistant to tumor induction via this mechanism.

> **EPA Response**: The EPA has identified the lack of a chronic study in the most sensitive species as an uncertainty.

SECTION III: REVIEWER ADDITIONAL AND EDITORIAL COMMENTS

****All comments/suggestions/edits offered by reviewers in this section have been carefully considered and addressed appropriately in the revised draft assessment****

Chou

P. 5, Line 8: Please clarify. In this paragraph the first sentence states that the salt form of HEPO dimer acid is solid. Then, on Line 8: "Both compounds are liquids at room temperature, …". Do you mean "When in water, both compounds are …"?

> **EPA Response**: Thank you for your comment. The following revision was made in section 2.1:
>
> "Both compounds can volatilize from water to air, where they will dissolve in aerosolized water droplets or bind to suspended particulate matter."

P. 6: Melting point of -21.0 C must be a mistake if it is solid at room temperature. Please make correction. The reviewer now notices that DuPont's 24637 (2008) study was conducted with 86% of the substance, which is in a liquid form. The physical and chemical property values and descriptions of the substance were based on a mixture of impurities. These values need to be either corrected to be CAS no. specific or the purity of the test substance needs to be specified.

> **Response**: Values have been corrected in the table and text in section 2.0.

P. 16, Line 10 (excluding lines in table): should the Table 2 Table "3"?

> **EPA Response**: Yes. Change made. Thank you.

P. 16, Line 26 (excluding lines in table): should the Table 2 Table "3"?

> **EPA Response**: Yes. Change made. Thank you.

P. 30, Line 26: This is a recovery study (DuPont-24459). Please modify the study description.

> **EPA Response**: DuPont-24459 (2008) included an assessment of endpoints at both the 28-day completion of dosing and after a 28-day recovery period. Because the derived reference values assume that exposure is continuous, the assessment of endpoints after the 28-day recovery period were not considered. Only endpoints assessed after the completion of the 28-day continuous dosing period were considered for RfD derivation.

References used by the reviewer:

Rakhshandehroo, M., Knoch, B., Müller, M., & Kersten, S. (2010). Peroxisome Proliferator-Activated Receptor Alpha Target Genes. PPAR Research, 2010, 1–20. https://doi.org/10.1155/2010/612089

> **EPA Response**: Please see response to Charge Question 1:
>
> Thank you for providing the reference to the review paper by Rakhshandehroo et al. (2010). This review paper provides an overview of PPARα target genes involved in different biological processes in the liver and the literature evaluating differences and similarities between the genes impacted in mice and humans. The authors conclude that based on their review of the literature, the function and specific target genes of PPARα are generally well conserved between mouse and human specifically for lipid metabolism. However, only a subset of those genes impacted in mice and humans are linked to the PPARα response

element and none of those identified as human only fell in that category. In addition, the review paper does not specifically address GenX chemicals or other perfluorinated compounds (Rakhshandehroo et al., 2010).

As described in section 4.7, findings consistent with PPARα agonists were observed (e.g., increases in liver weight, hepatocellular hypertrophy, and increased beta-oxidation activity) for GenX chemicals; however, data gaps exist for key events and other MOAs may be involved (Rakhshandehroo et al., 2010). Therefore, the EPA concluded that the MOA for GenX chemicals is not clearly understood and cannot be solely attributed to PPARα.

The commenter noted that several genes involved in biotransformation and lipid metabolism are human specific and recommended the potential effects resulting from regulation of these human-specific PPARα target genes should be considered when determining UF_A. As pointed out above, none of those human genes were directly linked to the PPAR***α*** response element by the authors (Rakhshandehroo et al., 2010). The UF_A is applied to account for the extrapolation of laboratory animal data to humans, and it generally is presumed to include both toxicokinetic (i.e, absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. The toxicokinetic portion of the UF_A was accounted for by application of EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* in the determination of the POD for derivation of the RfD (USEPA, 2011). However, uncertainty related to toxicodynamic processes (i.e., MOA) still exists; thus, EPA applied a UF_A of 3 along with a UF_D of 3 for data gaps. Given the lack of specificity of the review paper to GenX chemicals, this publication was not added to the document (Rakhshandehroo et al., 2010).

Kamendulis

In general, the document was very thorough and well written. However, in many places, particularly in section 2, the document was very redundant, and the sentence structure was a bit cumbersome in places. Section 5 was a summary of 4 and was also a bit redundant.

EPA Response: Section 2 has been revised to reduce redundancy.

Section 4 provides a study-by-study summary of the available data for GenX chemicals. Given the extensive nature of the reporting in the DuPont/Chemours studies, this section serves as a more succinct presentation of the relevant data. Section 5 is an organ system-specific synthesis of the weight of the evidence to determine potential hazards of GenX chemicals exposure.

P7 – section 2.2 the second sentence does not appear complete "The degradation data suggest that the substances will be very (i.e., half-life [$T_{1/2}$] > 6 months) in air, water, soil, and sediments".

EPA Response: Thank you for the comment. The following revision was made in section 2.2:

"The degradation data suggest that the substances will be very persistent (i.e., half-life [$T_{1/2}$] > 6 months) in air, water, soil, and sediments."

P16 – in 2 instances, references to table 2 are made in the text, however, I believe they are referring to table 3.

EPA Response: Yes. Change made. Thank you.

P40 – second paragraph – the study data in discussed in relation to PFOA effects. Up to this point, no other study descriptions contained references to PFOA effects. Should this information be included in the study description?

EPA Response: Thank you for the comment. The text was edited to remove PFOA from section 4.6.1.

P51 – last sentence of the first paragraph does not appear complete "While considered adverse, the hematological effects are inconsistently observed, especially as study duration increases, thus EPA did not hematotoxicity as the critical effect".

EPA Response: Thank you for the comment. The following revision was made 6.1:

"Furthermore, while considered adverse, the hematological effects were inconsistently observed, especially as study duration increased."

P61 – second to last paragraph, last sentence – "Differences in toxicokinetics in rodents could result in sex-specific differences in the toxicity studies of them." – it is unclear what is meant by this statement.

EPA Response: Thank you for the comment. The following revision was made in section 7.6:

"The observed sex-specific toxicokinetic differences in rodents likely contribute to the observed sex-specific differences in toxic response."

Leung

The draft report is well-written and provides a clear summary on what is currently known regarding the potential adverse health effects of GenX chemicals.

a. However, I would suggest a more comprehensive "summary of health hazards" in Section 5, by better utilizing the information presented in the toxicity studies of Section 4 and perhaps the inclusion of a corresponding table. There are multiple organ systems and health effects which are not summarized (e.g., abnormalities in endocrine systems [glucose and triglycerides concentrations, adrenal cortex hypertrophy], abnormalities in serum clinical chemistries), differences in weight, differences in hair growth, non-neoplastic effects, etc.). These may have not been statistically significant, but it would still be helpful to place in context with the other organ systems mentioned in Section 5.

EPA Response: As described in section 3.3.4, the EPA focused its review primarily on statistically significant adverse effects (see section 5). Section 4 provides a study-by-study summary of the available data for GenX chemicals. Given the extensive nature of the reporting in the DuPont/Chemours studies, this section serves as a more succinct presentation of the relevant data. Section 5 is an organ system-specific synthesis of the weight of the evidence to determine potential hazards of GenX chemicals exposure. Additionally, the EPA has made all the DuPont/Chemours study data (including individual animal data) available on its HERO website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

b. As such, the studies presented in Section 4 are important but could be better and more systematically organized. Suggest the use of additional subheadings in Sections 4.2 to 4.5 to guide this long narrative text.

EPA Response: Subheadings denoting study author and date have been added in sections 4.2 to 4.5.

Slitt

The document reads well and summarizes the findings appropriately.

EPA Response: Thank you for your response.

Warren

The toxicity assessment is well written, though there are numerous minor errors in syntax, subject-verb agreement, and punctuation, none of which detract significantly from the effort. Overall, the toxicity assessment should be characterized as a high-quality work product typical of USEPA. See several recommendations for minor editorial changes below.

1) The toxicity assessment might want to note that Pan et al. (2017) not only examined blood, liver and muscle of common carp, but also found detectable levels in the sera of Chinese residents residing near the fluoropolymer production plant

EPA Response: The EPA acknowledges this information is provided in the reference cited and has cited this reference in the updated occurrence section (1.3).

2) On p. 13, "fetus mice" might be better expressed as "fetal mice"

EPA Response: Edit accepted. Thank you.

3) Table 2 at the end of the 1st two paragraphs on p. 16 should be Table 3

EPA Response: Edit accepted. Thank you.

4) the last sentence on p. 19 should read 28 and 95, not 28 and 90

EPA Response: Edit accepted. Thank you.

5) change lines 6 and 7 on p. 20 to read …(e.g., effects such as liver toxicity), and populations at risk of exposure to HFPO….;

EPA Response: Edit accepted. Thank you.

6) change "undermined" to "undetermined" toward the bottom of p. 31

EPA Response: Edit accepted. Thank you.

7) place a period after (0%-8.3%) on p. 36 and begin a new sentence

EPA Response: Edit accepted. Thank you.

8) insert the word "study" after toxicity in the 1st line of the last paragraph on p. 38

EPA Response: Edit accepted. Thank you.

9) consider rewriting the paragraph immediately before section 4.6 to read as follows: The NOAEL for this prenatal and developmental toxicity study is 10 mg/kg-day based on an increase in early deliveries, decreases in gravid uterine weight, and decreased fetal weights for both sexes, all having a LOAEL of 100 mg/kg-day

EPA Response: Edit accepted. Thank you.

10) should the "to" in the fourth sentence on p. 41 be changed to "did"

EPA Response: Thank you for your comment. The following edit was made in section 4.6.1:

Ultimately, this study found that HFPO dimer acid ammonium salt exhibited a weaker binding affinity and bound differently to hL-FABP than PFOA and PFOS (Sheng et al., 2018).

11) delete the word "increasing" from the 8th line of paragraph 2 on p. 43

EPA Response: Edit accepted. Thank you.

12) consider an alternative to the phrase "suggestive of hazard" on p. 45

EPA Response: Edit accepted. Thank you.

13) delete the word "female" in the 2nd line of the 2nd paragraph of Section 5.5 on p. 46

EPA Response: Edit accepted. Thank you.

14) insert the word "consider" or "select" after the word "not" in line 7 of p. 51

EPA Response: Edit accepted. Thank you.

15) consider changing the first sentence on p. 52 to read as follows: Additionally, there were increases in serum liver proteins at 0.5 mg/kg-day in males, though they did not statistically significantly differ from control

EPA Response: Clarifying edit added to text.

16) delete "by" or "via" from the last sentence of the 1st paragraph on p. 53

EPA Response: Edit accepted. Thank you.

17) consider changing the phrase "liver as a hazard" at the end of the 1st paragraph on p. 58

EPA Response: Edit accepted. Thank you.

18) insert the word "studies" after "other" in line 4 of the 2nd paragraph on p. B-1

EPA Response: Edit accepted. Thank you.

19) change nominator to numerator on p. B-11

EPA Response: Edit accepted. Thank you.

APPENDIX A: INDIVIDUAL REVIEWER COMMENTS

COMMENTS SUBMITTED BY

Karen Chou, PhD
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Michigan State University
East Lansing, Michigan

External Peer Review of EPA's Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX Chemicals)

1. **The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.**

   The literature search strategy and study evaluation considerations are clearly described and appear to be appropriate.

   The reviewer suggests an additional article by Rakhshandehroo, M., Knoch, B., Muller, M., and Kersten, S. (2010). Peroxisome Proliferator-Activated Receptor Alpha Target Genes. *PPAR Research*, 2010, 1-20.

   Using primary hepatocytes culture from mice and human, this study demonstrated that human hepatocytes are sensitive to enzymatic induction by PPARalpha agonists. Of the 208 PPARα targeted genes in mouse hepatocytes, 85 (41%) are also targeted in human hepatocytes. In addition, 12 genes targeted by PPARα in human hepatocytes are not in mouse hepatocytes. Six of the human- specific genes are in the biotransformation pathways and the other six are in the lipid metabolism pathways, including the pathways of peroxisomal β-oxidation, microsomal ω-hydroxylation, lipogenesis, plasma triglyceride metabolism, and cholesterol/bile transport & metabolism. The potential manifestation of the effects on these human-specific target genes should be considered when determining UF_H.

2. **For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?**

   a. **If so, please explain your justification.**
   b. **If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.**

   The selection of liver effects as the critical effect for derivation of oral subchronic and chronic RfDs, using the oral reproductive/developmental toxicity screening study in adult mice, is well justified. It is justified based on increases in liver weight, relatively high number of animals (22-25) per treatment group for liver endpoints, and consistent adverse effects corroborated in other studies presented in the Draft. The weaknesses of other studies are well described in the draft on p. 51-52.

3. **EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?**

The reviewer agrees with the selection of BMDL10 from Table E-2 and Table E-4. The modeling approach, selection of benchmark response level, and the selected model used to identify the PODs are well justified and defensible.

4. **Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).**

    a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**

The reviewer agrees to the use of ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs in the draft assessments.

    b. **Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?**

Considering the differences of enzymes targeted by PPARα activation between the mouse and the human (see comments for Item 1), the UF_H and UF_D values used in this draft are appropriate; any values less than 3 would not be acceptable.

5. **EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for GenX chemicals.**

    a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**

Please see response to Question 4b.

    b. **Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.**

The draft provided appropriate scientific rationale for the selection of the UF values. Additional justification for UF_A may be used. See comments for Question 1.

6. **The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.**

    a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**

Yes, the draft clearly synthesized the toxicological effects.

    b. **Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.**

Yes, the weight of evidence for hazard identification has been clearly described, and scientifically justified to meet the definition of "adverse".

c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

Yes, the conclusions are supported by scientific evidences. Please also see comments above.

7. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.

Currently, there is no sufficient evidence to determined that the adenomas and adenocarcinoma observed in rodent liver are not relevant in humans. Nonetheless, the evidence in rodent adenocarcinoma present in the existing studies is not very strong. The reviewer agrees to the descriptor "there is suggestive evidence of carcinogenic potential for GenX chemicals."

8. Editorial or Additional Comments: Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.

P. 5, Line 8: Please clarify. In this paragraph the first sentence states that the salt form of HEPO dimer acid is solid. Then, on Line 8: "Both compounds are liquids at room temperature, …". Do you mean "When in water, both compounds are …"?

p. 6: Melting point of -21.0 C must be a mistake if it is solid at room temperature. Please make correction. The reviewer now notices that DuPont's 24637 (2008) study was conducted with 86% of the substance, which is in a liquid form. The physical and chemical property values and descriptions of the substance were based on a mixture of impurities. These values need to be either corrected to be CAS no. specific or the purity of the test substance needs to be specified.

p. 16, Line 10 (excluding lines in table): should the Table 2 Table "3"?

p. 16, Line 26 (excluding lines in table): should the Table 2 Table "3"?

p. 30, Line 26: This is a recovery study (DuPont-24459). Please modify the study description.

References used by the reviewer:

Rakhshandehroo, M., Knoch, B., Müller, M., & Kersten, S. (2010). Peroxisome Proliferator-Activated Receptor Alpha Target Genes. *PPAR Research*, *2010*, 1–20. https://doi.org/10.1155/2010/612089

COMMENTS SUBMITTED BY

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Associate Professor
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Bloomington, Indiana

External Peer Review of EPA's Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX Chemicals)

1. **The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.**

   Yes, the literature search strategy, study selection and evaluation considerations were very well presented and sufficiently clear. The process used was described well and was a very thorough and transparent approach to systematically evaluate each of the available scientific studies that described the health effects of GenX chemicals.

   I am unaware of other peer-reviewed studies that should be included in this assessment.

2. **For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?**

   a. **If so, please explain your justification.**

   I agree with the selection of the critical study selected for deriving RfD's for GenX chemicals (DuPont-18405-1037 2010). This oral reproductive/developmental toxicity study in mice identified liver effects, specifically single-cell necrosis in males, as the critical effect and was used to derive the subchronic and chronic RfDs for GenX chemicals. As noted, this study utilized a larger sample size (n=24 / dose) and provided the most health protective POD_{HED}. Further, this study was an 84/85 day study compared to the other mouse study (28 days). In addition, several other studies provide support for the selection of liver necrosis as the critical effect following oral exposure to GenX chemicals (DuPont-24459 2008; DuPont-24447 2008; DuPont-18405-1307 2010; DuPont-18405-1238 2013; DuPont-18405-841 2010). Liver effects (changes in liver enzyme levels, histopathological lesions, and tumors) were observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals.

   b. **If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.**

   N/A

3. **EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?**

   Yes, I agree with the approach used. As there are no biologically based dose-response models available for GenX chemicals, benchmark dose modeling was used, and was consistent with EPA's guidance document (USEPA 2012). The BMD and the BMDL were estimated using a

BMR of 10% extra risk for dichotomous data, and candidate PODs were estimated from all 3 doses (plus control) for the critical study.

4. **Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).**

    a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**

Yes, the application of ¾ allometric scaling was justified and adequately presented. Any uncertainty in using this approach is accounted for by application of uncertainty factors (see answers to question 5 below).

    b. **Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?**

Yes, the methods that were used to derive the RfD's for GenX chemicals took into consideration toxicokinetic differences between animals and humans. Further, any uncertainties are accounted for by the application of uncertainty factors (see question 5 below).

5. **EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for GenX chemicals.**

    a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**

Yes, uncertainty has been adequately accounted for in the derivation of RfDs for GenX chemicals. An interspecies uncertainty factor of 3 was applied to account for uncertainty in extrapolating from laboratory animals to humans. A factor of 3 was used in lieu of a 10 since a POD_{HED} was derived from the BMDL as specified in EPA's guidance document (USEPA 2011b). As the allometric scaling accounts for some aspects of species extrapolation, some uncertainty remains. Thus, the application of an UF of 3 appears appropriate.

An intraspecies uncertainty factor (UF_H) of 10 is assigned to account for variability in the responses within the human populations. This is also appropriate.

The BMD approach was utilized, therefore application of a UF for using a LOAEL to NOAEL is not needed.

For the chronic RfD, although a chronic bioassay is available (in rats), a UF of 3 was applied for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 3. The 2-year study identified a NOAEL of 1 mg/kg-day for liver effects (increased liver enzyme levels and centrilobular hepatocellular hypertrophy and cystic focal degeneration in males and centrilobular necrosis in both sexes), consistent with the effects observed in the oral reproductive/developmental study in mice, however, at a much higher NOAEL. As data suggests

that rats appear to be less sensitive than mice, a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. This appears appropriate.

While only a limited number of studies have been conducted for GenX chemicals, a database uncertainty factor (UF_D) of 3 was applied. This is due to the quality of the available studies – which include acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity studies in mice and rats with dosing durations of up to 2 years, and one reproductive and developmental toxicity study in mice and one prenatal and developmental toxicity study in rats. Many of these studies were conducted under GLP conditions, with full study reports available and reviewed. Thus, the application of a UF_D of 3 appears appropriate.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

I have addressed this comment in my responses to 5a above - the application of the UF's used to derive RfD's for GenX chemicals was scientifically based and well described.

6. The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.

a. Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.

I agree that the available data were adequately and clearly described.

b. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.

Yes, I feel that the weight of evidence for effects on the liver, as well as other endpoints (hematological and immune systems, and reproductive and developmental) was scientifically evaluated and justified.

c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

The liver effects observed following exposure to GenX chemicals were consistently seen across several studies including both male and female rats and mice, for short-term and chronic exposures. Thus, the conclusions drawn are scientifically supported and adequately described in the document.

7. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.

There are no dermal or inhalation studies available that evaluated evaluating cancer, however, one oral exposure bioassay for HFPO dimer acid ammonium salt in rats showed an increase in liver tumors (females) and pancreatic acinar adenomas and carcinomas (combined, males). While there were increases in tumors at the high dose, a dose-response pattern was not observed. Further, data suggests that mice may be more sensitive to GenX chemicals compared to rats. As

such, based on the available data, I agree with EPA's Guidelines for Carcinogen Assessment that classifies GenX chemicals as *Suggestive Evidence of Carcinogenic Potential* following oral exposure only.

8. Editorial or Additional Comments: Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.

In general, the document was very thorough and well written. However, in many places, particularly in section 2, the document was very redundant, and the sentence structure was a bit cumbersome in places. Section 5 was a summary of 4 and was also a bit redundant.

P7 – section 2.2 the second sentence does not appear complete "The degradation data suggest that the substances will be very (i.e., half-life [$T_{1/2}$] > 6 months) in air, water, soil, and sediments".

P16 – in 2 instances, references to table 2 are made in the text, however, I believe they are referring to table 3.

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P51 – last sentence of the first paragraph does not appear complete "While considered adverse, the hematological effects are inconsistently observed, especially as study duration increases, thus EPA did not hematotoxicity as the critical effect".

P61 – second to last paragraph, last sentence – "Differences in toxicokinetics in rodents could result in sex-specific differences in the toxicity studies of them." – it is unclear what is meant by this statement.

COMMENTS SUBMITTED BY

Angela M. Leung, MD
Health Sciences Clinical Assistant Professor of Medicine
David Geffen School of Medicine
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External Peer Review of EPA's Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX Chemicals)

1. **The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.**

   The draft report describes the systematic approach taken toward the identification and selection of pertinent studies on this topic. The search strategy is overall easy to understand and transparent, although there are some concerns and several areas which could be better addressed:

   a. The most important issue is that the search results highlight the overall relative scarcity of available data regarding the potential risks of Gex chemicals. Almost all of the literature used to base the current assessments are animal studies conducted by Dupont submitted as part of the TSCA. The HERO database shows 119 included references, from which only 29 are from non-Dupont sources (and not all, albeit most, are necessarily from peer-reviewed journals.) The lack of rigorous studies published in well-regarded journals spanning the animal, human, and epidemiologic literature is a substantial limitation to interpreting the health impacts of exposure to these chemicals.

   b. One concern regarding the search strategy, as shown in Appendix A (Table A-5), is that studies for which only an abstract was available were excluded. What does this signify, and were there any further attempts done to assess the relevance of these excluded studies (which are not shown)?

   c. The draft report demonstrates the approach used to rank the relevance and usefulness of included studies. Studies were then scored by various EPA OPPT criteria quite rigorously (Appendix B) in an objective a manner as possible by several reviewers. It would be relevant to state the number of reviewers, whose aggregate assessment was reported as weighted scores, involved in this process, as well as their professional areas of expertise.

   d. One additional comment regarding the scoring system is regarding Metric #22, which assessed "Health outcomes thought to be unrelated to exposure". It does not appear that are any further details regarding what these outcomes may have been, although there are occasional comments when such health outcomes were not reported in the study. Further detail regarding these health outcomes would provide greater context to the conclusions posed by this draft report.

2. **For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?**

   a. If so, please explain your justification.

b. If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.

This study in which the subchronic and chronic RfDs is based upon consisted of 150 mice, in which histopathologic changes and increased liver weights were seen in all three dose groups. However, as summarized in the draft report, these findings have also largely been supported by at least seven other studies, including one from the peer-reviewed published literature, although only one was considered chronic (i.e. >90 days duration). Evidence of hepatic damage is also supported by the very significant increases in serum transaminase levels among mice exposed to the higher doses. Overall, availability of more than one study assessing *chronic* exposure would be relevant toward the hepatic and other potential clinical endpoints.

The draft report describes that the other organ systems and endpoints in which adverse effects have been found were hematology, renal, development/reproduction, immunology, and carcinogenesis (Section 5). No differences in survival at a planned 12-month necropsy timepoint were found in the one available chronic study. Regarding these other organ systems:

a. It is described that the maximum decrement of hemoglobin/hematocrit levels in male mice and in rats was 12% from several studies assessing doses up to 100 mg/kg/day lasting up to 180 days. This may be clinical significant, and consideration of this endpoint in greater detail may be relevant. Additionally, in the one chronic study, female mice and rats had up to a 24% decrease in hemoglobin/hematocrit levels, further supporting this as a possible critical effect.

b. Regarding renal effects, the primary endpoints that have been assessed are weight of the kidneys, microscopic changes of the kidney, and blood urea nitrogen concentrations. The draft report states that given the limited changes in these parameters, renal toxicity is unlikely a critical effect. Animal physiology is not my area of expertise, but there certainly other factors important in the assessment of human renal function like serum creatinine concentrations and filtration rate that are also clinically important.

c. It is unfortunate that the potential developmental/reproductive toxicities are based only on two animal studies. From the description of these, these toxicities are rare and may not be clinically significant. However, only F1 mice were studied and there are no data on potential effects spanning more than two generations.

d. The data for immunotoxicity are even more limited, but suggest some adverse effects among female mice. In the absence of more data, it is difficult to ascertain whether these preliminary findings should be considered critical effects.

e. I agree with the summary in Section 5.6, which describes the inability to determine overall carcinogenicity based on the substantially limited available data. However, the observation of increased hepatocellular carcinomas in female dosed rats should not be discounted.

3. EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?

BMD modeling is not within my area of expertise. However, it appears that the draft report makes a reasonable effort to identify clinically-relevant endpoints required for the modeling from the available limited data. I defer to the other reviewers regarding whether the translation of a POD to a human dose equivalent, the determination of uncertainty factors (UFs), and the mathematical calculations of the corresponding subchronic and chronic RfDs, are appropriate.

4. **Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).**

    a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**
    b. **Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?**

    This is not my area of expertise, thus I defer to the other reviewers.

5. **EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for GenX chemicals.**

    a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**
    b. **Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.**

    This is not my area of expertise, thus I defer to the other reviewers.

6. **The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.**

    a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**
    b. **Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.**
    c. **Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.**

    The Hall 2012 reference helps delineates the differences between physiologic adaptive responses vs pathologic toxic effects resulting in hepatocellular hypertrophy. Using this framework, the draft report summarizes the available studies with hepatic endpoints, which did show increased proportions of dosed animals with increased liver weights, abnormal serum liver function tests, hepatocellular hypertrophy, and hepatocellular necrosis. These studies were also the basis for the observation that mice may be more sensitive to toxic hepatic effects than rats, given the development of the adverse findings at comparably higher doses in rats. The report does not comment on whether steatosis was assessed and/or observed in these studies.

The hepatic toxicologic effects are consistent and reproducible, as seen from the eight studies of either mice or rats and in both genders. Altogether, the conclusions regarding adversity in the draft report are appropriately summarized.

7. **The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.**

   From my understanding of Section 4.4, the EPA had requested that studies be repeated to exclude both control and dosed animals when it was observed that early deaths were found in both groups that were not necessarily associated with incident tumors (thus presuming that the deaths may have been unrelated to the intervention). The reanalysis was performed, but only in male rats and only to assess for testicular hyperplasia/tumors. Thus, it is unclear why the reanalyses were not comprehensively performed for both male and female rats, and also systematically for other potential tumors.

   From the available information that did not reflect this exclusion, there were statistically significantly increased proportions of hepatocellular carcinomas in female dosed rats and a trend toward increased pancreatic carcinomas in male dosed rats, although both occurred only at single high doses; a typical dose-response pattern was not observed. I agree with the report that mice should also be studied, as it was reported that mice may be more susceptible toward GenX carcinogenicity. Altogether, given these points, I agree with the summary in Section 5.6, which describes the inability to determine overall carcinogenicity based on the substantially limited available data. However, the observation of increased hepatocellular carcinomas in female dosed rats should not be discounted.

8. **Editorial or Additional Comments: Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.**

   The draft report is well-written and provides a clear summary on what is currently known regarding the potential adverse health effects of GenX chemicals.

   a. However, I would suggest a more comprehensive "summary of health hazards" in Section 5, by better utilizing the information presented in the toxicity studies of Section 4 and perhaps the inclusion of a corresponding table. There are multiple organ systems and health effects which are not summarized (e.g., abnormalities in endocrine systems [glucose and triglycerides concentrations, adrenal cortex hypertrophy], abnormalities in serum clinical chemistries), differences in weight, differences in hair growth, non-neoplastic effects, etc.). These may have not been statistically significant, but it would still be helpful to place in context with the other organ systems mentioned in Section 5.

   b. As such, the studies presented in Section 4 are important but could be better and more systematically organized. Suggest the use of additional subheadings in Sections 4.2 to 4.5 to guide this long narrative text.

COMMENTS SUBMITTED BY

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and
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Kingston, Rhode Island

External Peer Review of EPA's Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX Chemicals)

1. **The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.**

The literature search strategy was appropriate and thorough. It was well described and included clear criteria for the inclusion and exclusion of studies. The databases utilized (i.e. PubMed, WOS, Toxline, and TSCATS1) are appropriate and the search terms were comprehensive in nature. The methods in section B used to evaluate study quality were systematic and thorough. The metrics and criteria applied for Animal and *in vitro* toxicity studies were exceedingly thorough and well defined. The weighting and relative important used for weighting the criteria was appropriate. Overall, this semi-quantitative approach in evaluating the data/studies to be included is considered to be is appropriate and thorough.

A minor comment is that exposure for *in vitro* studies on page A-9, Table A-5 should be described. It is assumed that it is via addition of chemical to media, however it should be described for what criteria would be constitute inclusion/exclusion for in vitro studies. For example, solubility and vehicle could be mentioned.

2. **For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?**

    a. **If so, please explain your justification.**

Yes, a survey of the data included in the report points to liver effects in adult male is appropriate and defensible. The study meets the criteria listed in almost all elements for being considered of high quality. It meets every metric as high or medium, such as test substance, test setup, exposure characterization, etc. The critical effect of single cell necrosis in male mice is based on n=24-25 F0 males, which a large "n". The selection of this study is scientifically justifiable based on it sufficiently meeting the review criteria. The selection of single-cell necrosis is a reasonable measure to use as a critical effect. This measure has been used previously for PFOA and PFOS, in which rodent studies that have demonstrated hepatotoxicity demonstrate concordance with studies in human populations illustrating slight elevation serum in liver enzymes.

It should be noted that there was PFOA contamination for the test substance for DuPont 18405-1037, 2010 lists that purity of the test article at 84%. Another study listed in the document that meets the evaluation criteria with high confidence (DuPont -24459, 2008) lists a slightly higher purity of the test article (88% purity). This 28-day oral dosing study that evaluated 0.1, 3 and 30 mg/kg/day did not observe any statistically significant increases in liver single cell necrosis at 0.1 mg/kg, but did observe significant elevation of serum liver enzymes, liver weight, and single cell necrosis in males at 3 mg/kg. Given that the purity of the test article was slightly higher, this study should also be

considered along with DuPont 18405-1037, 2010 for considering 0.1 mg/kg in male mice for the RfD.

3. EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?

This is out of my area of expertise, so I decline responding to this question.

4. Given what is known and not known about the interspecie differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).

a. Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.

This is not within my expertise to respond.

b. Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?

Yes, the methods used do account for the appropriate uncertainties.

5. EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for GenX chemicals.

a. Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.

Yes. The use of uncertainty factors was used according to EPA guidance (USEPA, 2011b). The authors were unable to find any human epidemiological studies, so an UF of 10 was used appropriately. The application of a UF of 3 for interspecies differences is appropriate as well because the chronic cancer bioassays were performed in rat and not mouse, with the several studies herein demonstrating that rats appear to be a less sensitive species than mouse. Lastly, a UFD of 3 is in agreement with the guidance, as there are many knowledge gaps for GenX. Lastly, uncertainty for intraspecies variability and database limitations must be included. Currently there are no studies to address whether developmental or immunotoxicity is observed at exposures lower than the 0.1 mg/kg RfD that was observed for liver effects in male mice.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.

Yes, Section 7 did outline and provide sufficient rationales for the application of the selected uncertainty factors. See response in part a.

6. The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.

a. Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.

Yes, the review agrees with the overall conclusion of the authors in section 4.7 for the mode of action. The conclusion from the document is that the findings are not adequate to definitely conclude that a PPARα mechanism of action (MOA) exists for HFPO dimeric acid and/or ammonium salt. There is a lack of publications that have evaluated the mechanisms of action for GenX and the authors cannot draw firm conclusions regarding PPARα involvement with limited findings. This is based on a study by Wang et al., 2016 that administered 1 mg/kg/day via oral gavage for 28 days to male ICR mice that was not considered to be of high qualitative determination because it lacked sufficient quality in multiple elements. The finding from this study, point to treatment with the HFPO dimeric acid ammonium salt impacting pathways in liver for PPARα signaling, retinol catabolism, and fatty acid degradation, however the lack of rigor in the experimental design limits the interpretation. The authors do cite Dupont-24459 2008 and Dupont-24447 2008 with some potential endpoints that are consistent with peroxisome proliferation (i.e. hepatic β-oxidation), however they do not identify PPARα as the cause for the observed effects. Thus, the conclusion that the findings are not adequate to definitely that a PPARα mechanism of action (MOA) exists for HFPO dimeric acid and/or ammonium salt is appropriate.

b. Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.

Yes. The weight of evidence (WOE) for hazard identification has been clearly described and scientifically justified in this document. The WOE is based on the liver being a target organ for toxicity from the oral exposure to HFPO dimeric acid. The document cites liver effects for both male and female mice and rats, with male mice being the most sensitive species. This conclusion is based on several studies carried out in which the test methods and study design meet criteria as being "high" in quality. These studies assessed the impact of GenX in numerous endpoints (i.e. gross measures such as food consumption, appearance, serum clinical markers of injury, gross organ changes, and tissue pathology). These studies did evaluate relative liver weight and hepatocellular hypertrophy in the context of evidence of hepatic necrosis, as measured by serum enzyme levels and evidence of cellular necrosis.

c. Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.

Yes, the conclusions regarding adversity are scientifically supported and clearly described. There were no human epidemiology studies for GenX to base adversity, so rodent studies were used as a basis for the RfD. The conclusion is that 0.1 mg/kg/day causes liver effects that are observed as single cell necrosis in males (DuPont-18405-1037 2010). Other studies presented in the document (refer to Table 7) also support liver effects being considered the adverse effect for GenX at similar NOAELS (Dupont-24459, DuPont-18405-1238). Because these studies evaluated necrosis and liver weight and found evidence for cytotoxicity, they meet the concern for adversity as outlined by Hall et al. 2012). In addition, because there is a lack of substantive information regarding the mechanism

of action for GenX in rodent and cell-based studies, the role of PPARα as being the cause for the observed liver effects, such as liver weight and single-cell necrosis, cannot be concluded.

7. **The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.**

The conclusion is based on evidence that the liver is the target organ for toxicity and primary organ for tumor development. The lack of cancer bioassay data using mice, which are the more sensitive species, limits the conclusion. The conclusion is based on findings from a study that meets the review criteria as being of high consideration and is in line with the EPA's Guidelines for Carcinogenic Potential for Carcinogen Risk Assessment (USEPA, 2005). Based on this document, is appropriate to make this conclusion based on the literature collected and studies that met criteria for consideration. Moreover, the lack of substantive information regarding PPARα activation does not allow one to rule out the potential for PPARα-independent activation contributing to the observed liver effects.

8. **Editorial or Additional Comments: Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.**

The document reads well and summarizes the findings appropriately.

COMMENTS SUBMITTED BY

David Alan Warren, MPH, PhD
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University of South Carolina Beaufort
Beaufort, South Carolina

External Peer Review of EPA's Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and its Ammonium Salt (GenX Chemicals)

1. **The document describes the process for identifying and selecting pertinent studies. Please comment on whether the literature search strategy, study selection considerations, and study evaluation considerations are appropriate and clearly described. Please identify additional peer-reviewed studies that the assessment should consider.**

   Sections 3.3.1 (Literature Search Strategy and Results) and 3.3.2 (Study Screening Process and Study Evaluation) succinctly describe a laborious task that requires considerable skill if done well. I have refrained from using the phrase "done correctly" in recognition of the subjective judgments inherent to the process. The search of public literature is clearly comprehensive, as I was able to locate only one additional study that might be worthy of inclusion in the toxicity assessment (see below). Admittedly, I am unsure as to whether it meets the inclusion-exclusion criteria in Table A-5. Based on its title and abstract, it might be appropriate to include in Section 1.3 (Occurrence). As for the screening process and evaluation of manufacturer-submitted studies, like the toxicity assessment, I too acknowledge the importance of adherence to OECD/EPA TGs and GLP. And while I find the scoring system and qualitative rating process a bit arbitrary, the evaluation domains and metrics within them make for a transparent means in the pursuit of consistency and ultimately, validity in toxicity values. Therefore, I support the study evaluation process while encouraging its refinement over time.

   Additional Peer-Reviewed Study

   1. WA Gebbink, L van Asseldonk, and SPJ van Leeuwen. Presence of Emerging Per- and Polyfluoroalkyl Substances (PFASs) in River and Drinking Water near a Fluorochemical Production Plant in the Netherlands. *Environ. Sci. Technol.*, 51(19):11057-11065, October 3, 2017.

   The above study investigated the presence of legacy and emerging PFAS (including GenX) in river water collected in 2016 up- and downstream from a fluorochemical production plant in The Netherlands. Additionally, drinking water samples were collected from municipalities in the vicinity of the production plant, and like the river water, were positive for GenX.

2. **For GenX chemicals the critical study chosen for determining the subchronic and chronic RfDs is the oral reproductive/developmental toxicity screening study in adult mice (DuPont 18405-1037, 2010) and the critical effect is liver effects (single cell necrosis) in adult males. Is the selection of the critical study and critical effect for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and defensible?**

   a. **If so, please explain your justification.**
   b. **If not, please provide your rationale and detail an alternative critical study and/or critical effect you would select to support the derivation of the subchronic and chronic RfDs.**

   Selection of the gavage study of reproduction/developmental toxicity in mice (DuPont 18405-1037, 2010) and single cell necrosis for toxicity value derivation are scientifically justified and well defended in the toxicity assessment. However, the dismissal of studies from further

consideration as the critical study on the basis of NOAEL alone (i.e., having a NOAEL > 0.1 mg/kg/day) is a questionable practice. This is especially true when a dismissed study's NOAEL is but an order of magnitude greater than that of the critical study (i.e., 1 mg/kg/day), a difference dwarfed by the total UF applied to toxicity value derivation. Nonetheless, Section 6.1 makes a convincing case for selection of critical study and effect, especially with regard to preference for liver necrosis over a hematological or immunological effect and use of a subchronic study for chronic RfD derivation in lieu of a chronic toxicity/oncogenicity study reporting the same critical effect, albeit at a considerably higher dose in a less sensitive species.

3. **EPA employed benchmark dose modeling in the identification of a point-of-departure (POD) for GenX chemicals (USEPA 2012). Is the modeling approach, selection of benchmark response level, and the selected model used to identify the POD for derivation of the RfD scientifically justified and defensible?**

   Application of BMD modeling to data from two of three studies having NOAELs of 0.1 mg/kg/day was appropriate for POD identification, as neither sex of rats in the third study (DuPont-17751-1026 2009) exhibited liver necrosis. The modeling approach appears consistent with USEPA's *Benchmark Dose Technical Guidance* document, including use of dichotomous models, selection of BMR, and inclusion of data from all dose groups given an adequate model fit when none are omitted. Criteria for model selection appears to be largely consistent with Agency guidance, as well. For example, model selection considerations included those with goodness of fit p-values > 0.1, lowest Akaike's Information Criterion (provided BMDLs are "sufficiently" close), sufficiently small BMD:BMDL ratios, and lowest scaled residuals for doses near the BMD/BMDL. In addition, fitted curves of incidence rate as a function of dose allowed for an assessment of visual fit. Finally, selection was made of the lowest BMDL (0.15 mg/kg/day), which was one-half that of the alternative derived from the 28-day gavage study in mice. As such, the toxicity assessment's BMD modeling results were obtained using time-tested and widely-accepted methods and model choice decision logic, making it scientifically justified and defensible. It is noteworthy that such methods stand in stark contrast to the means by which North Carolina derived a drinking water equivalent level on the basis of an RfD derived by application of a total UF of 1,000 to the NOAEL of 0.1 mg/kg/day for single cell necrosis in the liver.

4. **Given what is known and not known about the interspecies differences in toxicokinetics of GenX chemicals, EPA applied body weight to the ¾ allometric scaling to adjust the POD to estimate a human equivalent dose (HED) in the derivation of the respective RfDs (USEPA 2011).**

   a. **Is applying the body weight to the ¾ for GenX chemicals scientifically justified and defensible? If not, please provide your rationale and detail the alternative approach you would use.**
   b. **Do the methods used to derive the RfDs for GenX chemicals appropriately account for uncertainties in evaluating the toxicokinetic differences between the experimental animal data and humans?**

   Yes, use of the default dosimetric adjustment factor (DAF) of bw to the ¾ is scientifically justified and defensible given the lack of a PBPK model for GenX (possibly excluding that of Gomis et al., 2018), paucity of toxicokinetic data in experimental animals and their absence in humans, and little mechanistic information to inform the issue of how internal dose relates to the

nature, magnitude, and time-course of biological effects. As noted in the toxicity assessment, its use is also justified given GenX's lack of metabolism, relatively short clearance time compared to longer-chain PFAS, and application to adult mice as opposed to those in earlier life stages. Furthermore, the default approach is consistent with the hierarchy of approaches for interspecies extrapolation clearly expressed by USEPA in multiple documents, including *Recommended Use of Body Weight3/4 as the Default Method in Derivation of the Oral Reference Dose* and *Harmonization in Interspecies Extrapolation: Use of BW3/4 as Default Method in Derivation of the Oral RfD*. The toxicity assessment's use of a UF of 3 for interspecies differences is also consistent with Agency guidance, as the default DAF appropriately addresses some, but not all, of the considerable cross-species uncertainties in both GenX toxicokinetics and toxicodynamics. Lastly, it is encouraging to see the use of an updated body weight for adult humans in the DAF equation (i.e., 80 kg), a minor change, but one that increases confidence that the toxicity assessment reflects the state-of-the-science.

5. **EPA has evaluated and applied where appropriate uncertainty factors to account for intraspecies variability (UFH), interspecies differences (UFA), database limitations (UFD), duration (UFS), and LOAEL-to-NOAEL extrapolation (UFL) for GenX chemicals.**

    a. **Has uncertainty been adequately accounted for in the derivation of the RfDs? Please describe and provide suggestions, if needed.**
    b. **Does the provided scientific rationale support the application of the selected uncertainty factors? Please explain.**

    Yes, the considerable uncertainty surrounding the risk of GenX exposure has been adequately accounted for in the toxicity assessment. Section 6.4.2 does a good job of explaining the rationale behind each individual UF value and I agree with the total UFs of 100 and 300 used for RfD derivation.

6. **The draft assessment for GenX chemicals identifies liver effects as a potential human hazard. EPA evaluated the available evidence for liver effects, including the potential role of PPARα, using Hall et al. (2012) criteria for adversity.**

    a. **Please comment on whether the available data have been clearly and appropriately synthesized for these toxicological effects.**
    b. **Please comment on whether the weight of evidence for hazard identification has been clearly described and scientifically justified.**
    c. **Please comment on whether the conclusions regarding adversity are scientifically supported and clearly described.**

    The toxicity assessment, particularly Section 5.1, does an admirable job of synthesizing the available data on liver effects and making the case (using a weight-of-evidence approach) for GenX exposure as a potential hazard to the human liver provided a threshold dose is met. Concern that the liver may not be the most appropriate target organ for RfD derivation is minimal at best, as four manufacturer-submitted studies reported liver effects at the LOAEL. In these studies, effects were seen in both sexes of mice and rats under conditions of varying exposure magnitude and duration. Furthermore, the critical effect of single-cell necrosis typically co-occurred with liver hypertrophy, increased relative liver weight, and/or elevated liver enzymes, not to mention liver tumors in chronically-treated, high dose, female rats. As for the issue of whether hepatic hypertrophy and increased relative liver weight are "adverse", the

toxicity assessment clearly warns against the use of such an unqualified label in the event such effects are PPARα-mediated. This is consistent with the consensus opinion of Hall and colleagues (an expert panel of workshop attendees) that hepatic hypertrophy, in the absence of histologic or clinical pathology, should be considered adaptive or non-adverse. Given the weight ascribed to the Hall et al. (2012) publication by the toxicology community, and deservedly so, it is appropriate that USEPA share the same opinion in the absence of evidence to the contrary.

7. The draft assessment concludes that there is suggestive evidence of carcinogenic potential for GenX chemicals and that this descriptor applies to oral routes of human exposure (USEPA 2005). Please comment on whether the available animal and mechanistic studies support this conclusion.

I agree that the one chronic toxicity/oncogenicity study is suggestive of carcinogenic potential for GenX, and in the absence of information that the observed tumors were rat-specific, believe the weight-of-evidence descriptor should apply to humans exposed via the oral route. Sections 4.4 and to a greater extent, 5.6, speak to the considerable weaknesses in the cancer bioassay data. Nonetheless, liver and pancreatic tumors in GenX-exposed female and male rats, respectively, were significantly elevated over controls. As rats have been shown to be considerably less sensitive than mice to many non-cancer effects of GenX, a chronic bioassay in the more sensitive species would be a logical next step. While the toxicity assessment provides an informative overview of mode of action possibilities (e.g., cytotoxicity followed by reparative proliferation, genotoxicity though the data are mixed, and promotion mediated by PPARα), data are insufficient to select one over the others. Obviously, if the observed liver tumors in rats were PPARα-mediated, humans would be resistant to tumor induction via this mechanism.

8. Editorial or Additional Comments: Please provide any editorial or additional comments you would like to make here. These should be any comments that are not in direct response to the technical charge questions above.

The toxicity assessment is well written, though there are numerous minor errors in syntax, subject-verb agreement, and punctuation, none of which detract significantly from the effort. Overall, the toxicity assessment should be characterized as a high-quality work product typical of USEPA. See several recommendations for minor editorial changes below.

1. the toxicity assessment might want to note that Pan et al. (2017) not only examined blood, liver and muscle of common carp, but also found detectable levels in the sera of Chinese residents residing near the fluoropolymer production plant
2. on p. 13, “fetus mice” might be better expressed as “fetal mice”
3. Table 2 at the end of the 1st two paragraphs on p. 16 should be Table 3
4. the last sentence on p. 19 should read 28 and 95, not 28 and 90
5. change lines 6 and 7 on p. 20 to read …(e.g., effects such as liver toxicity), and populations at risk of exposure to HFPO….;
6. change “undermined” to “undetermined” toward the bottom of p. 31
7. place a period after (0%-8.3%) on p. 36 and begin a new sentence

8. insert the word “study” after toxicity in the 1st line of the last paragraph on p. 38
9. consider rewriting the paragraph immediately before section 4.6 to read as follows: The NOAEL for this prenatal and developmental toxicity study is 10 mg/kg-day based on an increase in early deliveries, decreases in gravid uterine weight, and decreased fetal weights for both sexes, all having a LOAEL of 100 mg/kg-day
10. should the “to” in the fourth sentence on p. 41 be changed to “did”
11. delete the word “increasing” from the 8th line of paragraph 2 on p. 43
12. consider an alternative to the phrase “suggestive of hazard” on p. 45
13. delete the word “female” in the 2nd line of the 2nd paragraph of Section 5.5 on p. 46
14. insert the word “consider” or ”select” after the word “not” in line 7 of p. 51
15. consider changing the first sentence on p. 52 to read as follows: Additionally, there were increases in serum liver proteins at 0.5 mg/kg-day in males, though they did not statistically significantly differ from control
16. delete “by” or “via” from the last sentence of the 1st paragraph on p. 53
17. consider changing the phrase “liver as a hazard” at the end of the 1st paragraph on p. 58
18. insert the word “studies” after “other” in line 4 of the 2nd paragraph on p. B-1
19. change nominator to numerator on p. B-11