RESPONSE TO ADDITIONAL FOCUSED EXTERNAL PEER REVIEW OF DRAFT HUMAN HEALTH TOXICITY VALUES FOR HEXAFLUOROPROPYLENE OXIDE (HFPO) DIMER ACID AND ITS AMMONIUM SALT (GENX CHEMICALS)

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ACRONYMS AND ABBREVIATIONS

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.

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate transaminase
ATPase	adenosine triphosphatase
BCF	bioconcentration factor
BCF_{SS}	bioconcentration factor, steady state
BCRP	breast cancer resistance protein
BMDL	benchmark dose lower limit
CASRN	Chemical Abstracts Service Registry Number
DMSO	dimethyl sulfoxide
E	embryonic day
EPA	U.S. Environmental Protection Agency
F_0	parent generation
F_1	offspring of the F_0 generation
GD	gestation day
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
GWG	gestational weight gain
H&E	hematoxylin and eosin
HED	human equivalent dose
HFPO	hexafluoropropylene oxide
HFPO-DA	hexafluoropropylene oxide dimer acid
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
LD	lactation day
LOAEL	lowest-observed-adverse-effect level
mg/kg	milligram per kilogram
mg/kg/day	milligrams per kilogram per day
MOA	mode of action
NA	not applicable
ND	not detected
NOAEL	no-observed-adverse-effect level
NLM	National Library of Medicine
NTP	National Toxicology Program
P-gp	P-glycoprotein

PECO	population, exposure, comparator, and outcomes
PFAS	per- and polyfluoroalkyl substances
PFBS	perfluorobutanesulfonic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctanesulfonic acid
PND	postnatal day
POD	point of departure
PODHED	point of departure human equivalent dose
PPAR	peroxisome proliferator-activated receptor
PPARα	peroxisome proliferator-activated receptor alpha
PWG	Pathology Working Group
RfD	reference dose
T3	triiodothyronine
T4	thyroxine
TSCATS1	Toxic Substances Control Act Test Submissions 1
UF	uncertainty factor(s)
UFD	database uncertainty factor
UF_S	extrapolation from subchronic to a chronic exposure duration uncertainty factor
WOS	Web of Science

SECTION I: TECHNICAL CHARGE TO EXTERNAL REVIEWERS

Technical Charge to External Peer Reviewers

Contract No. EP-C-17-017
Task Order 68HERH20F0097 (ERG Task Order 37)
April 2021

Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

BACKGROUND

In November 2018, the U.S. Environmental Protection Agency (EPA) published the Draft Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6 and CASRN 62037-80-3) for public comment. EPA has revised the draft assessment in response to these public comments (https://www.regulations.gov/docket?D=EPA-HQ-OW-2018-0614) and incorporated relevant literature identified through literature searches completed in February 2019, October 2019, and March 3, 2020. EPA is requesting a second external peer review of the substantive changes made in response to this new information.

In the draft assessment released for public comment, EPA selected the reproductive and developmental toxicity study from DuPont (DuPont 18405-1037, 2010) and hepatocellular single cell necrosis in parental males as the critical study and effect. In this revised draft, EPA considered relevant data published since the public comment period and the results of a National Toxicology Program (NTP) Pathology Working Group (PWG) review of liver lesions observed in two pertinent studies (DuPont 18405-1037, 2010; DuPont-18405-1307, 2010). The purpose of this review was to reevaluate slides from these two studies according to the more recent International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) Organ Working Group's diagnostic criteria that describes how pathologists can distinguish between apoptosis and single cell necrosis in standard hematoxylin and eosin (H&E) stained liver tissue sections (Elmore et al., 2016). Other liver effects were classified according to the INHAND document containing standardized terminology of the liver (Thoolen et al., 2010). The NTP review also identified differences in effects between parental males and females observed in the pathology slides. Based on the NTP PWG review, a change was made to the selected critical effect in the revised draft.

Recent publications on the reproductive/developmental toxicity of GenX chemicals raised additional concern related to impacts on pregnancy that may lead to additional effects later in life. These new findings led EPA to reexamine uncertainty factors (UFs) used in the toxicity assessment.

Please review the accompanying revised Toxicity Assessment and provide detailed responses to the charge questions below.

CHARGE QUESTIONS

1. Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.

2. In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and

the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.

In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

a. Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?

    i. If so, please explain your reasoning.

    ii. If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.

    iii. Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

   EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

   a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

   b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

4. EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

   The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

   Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

   a. Given the evidence provided above and EPA's guidance on selection of uncertainty factors (EPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?

   b. Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.

5. **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*. U.S. EPA OPPTS 870.3550; OECD Test Guideline 421. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: December 29, 2010), Ashland, OH.

DuPont-18405-1307: E.I. du Pont de Nemours and Company. 2010. *H-28548: Subchronic Toxicity 90-Day Gavage Study in Mice*. OECD Test Guideline 408. Study conducted by E.I. du Pont de Nemours and Company (Study Completion Date: February 19, 2010), Newark, DE.

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

EPA (Environmental Protection Agency). 2002. *A Review of the Reference Dose and Reference Concentration Processes*. EPA/630/P-02/0002F. EPA, Risk Assessment Forum, Washington, DC. Accessed May 2018. https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf.

Thoolen, B., R.R. Maronpot, T. Harada, A. Nyska, C. Rousseaux, T. Nolte, D.E. Malarkey, W. Kaufmann, K. Kuttler, U. Deschl, D. Nakae, R. Gregson, M.P. Vinlove, A.E. Brix, B. Sing, F. Belpoggi, and J.M. Ward. 2010. Proliferative and nonproliferative lesions of the rat and mouse hepatobiliary system. *Toxicologic Pathology* 38(7_suppl):5S-81S. doi:10.1177/0192623310386499.

SECTION II: REVIEWER COMMENTS ORGANIZED BY CHARGE QUESTION

Charge Question 1

Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.

Chou

The review does not know any recent pertinent literature for GenX chemicals that is not included in this document.

EPA Response: Thank you for your response.

Faustman

This reviewer is not aware of additional relevant literature. Note that only Pub med via NLM was searched and although there is a tremendous increase in perflorinated compound associated literature this reviewer's search did not identify any highly relevant publications that would have modified their comments on the draft EPA document.

EPA Response: Thank you for your response.

Kamendulis

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

EPA Response: Thank you for your response.

Leung

Several literature searches and search updates were performed of submitted DuPont/Chemours studies and of publicly-available scientific published studies between July 2017-March 2020 on this topic. Inclusion criteria of retrieved studies were conducted in accordance with PECO criteria for systematic reviews. The references studies appear to be complete; I am not aware of any other available literature that may be pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals.

EPA Response: Thank you for your response.

Salmon

I am not aware of any recent literature which was not identified in the Toxicity Assessment document.

EPA Response: Thank you for your response.

Slitt

I am not aware of any additional studies to include. The most recent PubMed search I performed a PubMed was on May 20, 2021 and did not retrieve any additional publications that would quantitatively impact the RfDs.

Overall, 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 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 importance used for weighting the criteria was appropriate. Overall, this semi-quantitative approach in evaluating the data/studies is considered appropriate and thorough.

EPA Response: Thank you for your response.

Warren

It appears that the six publications listed below only became available, even electronically, after the last literature search update on March 3, 2020. Even so, the authors of the GenX toxicity assessment were likely aware of their existence before finalization and distribution of the draft for external peer review (e.g., Conley et al. (2021) is referred to on p. 94, yet does not appear in the reference list). As such, I hesitate to mention them, though all are informative in one way or another. Of the six, there is one *in vivo* (a) and two *in vitro* (b, c) toxicity studies, two studies that address the occurrence of PFAS in North Carolina (d, e) and an informative review (f). Conley et al. is not a viable candidate for principal study with its relatively high doses and short exposure durations. However, like the two *in vitro* studies and Blake and Fenton review, it increases concern for developmental effects yet to be fully characterized, and in so doing, lends support for a full database uncertainty factor (UF_D) of 10. Interestingly, despite detecting elevated levels of several legacy PFAS in the blood of Wilmington, NC residents, Kotlarz et al. failed to detect GenX above analytical reporting limits.

a. Conley JM, Lambright CS, Evans N, et al. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. https://doi.org/10.1016/j.envint.2020.106204.

b. Coperchini F, Croce L, Denegri M, et al. Adverse effects of in vitro GenX exposure on rat thyroid cell viability, DNA integrity and thyroid-related gene expression. https://doi.org/10.1016/j.envpol.2020.114778.

c. Bangma J, Szilagyi J, Blake, BE, et al. An assessment of serum-dependent impacts on intracellular accumulation and genomic response of per- and polyfluoroalkyl substances in a placental trophoblast model. https://doi.org/10.1002/tox.23004.

d. Petre M-A, Genereux DP, Koropeckyi-Cox L, et al. Per- and Polyfluoroalkyl Substance (PFAS) Transport from Groundwater to Streams near a PFAS Manufacturing Facility in North Carolina, USA. https://doi.org/10.1021/acs.est.0c07978.

e. Kotlarz N, McCord J, Collier D, et al. Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. https://doi.org/10.1289/EHP6837.

f. Blake, RE and Fenton SE. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects. https://doi.org/10.1016/j.tox.2020.152565.

EPA Response: Thank you for providing these references. See below for a response to each publication.

a. Conley JM, Lambright CS, Evans N, et al. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. https://doi.org/10.1016/j.envint.2020.106204.

EPA Response: As the commenter noted, this publication was included in the draft toxicity assessment. A study summary is provided in section 4.5 and it was considered for dose-response. It was missing from the reference list and has now been added to the references.

b. Coperchini F, Croce L, Denegri M, et al. Adverse effects of in vitro GenX exposure on rat thyroid cell viability, DNA integrity and thyroid-related gene expression. https://doi.org/10.1016/j.envpol.2020.114778.

EPA Response: This mechanistic *in vitro* study was not added to the assessment because in vivo data exist demonstrating effects on thyroid hormones in response to GenX chemicals exposure. While the study's mechanistic information for thyroid hormone effects is interesting, including this information is not necessary for selecting the critical effect or determining the reference doses (RfDs) which were based on liver toxicity endpoints.

c. Bangma J, Szilagyi J, Blake, BE, et al. An assessment of serum-dependent impacts on intracellular accumulation and genomic response of per- and polyfluoroalkyl substances in a placental trophoblast model. https://doi.org/10.1002/tox.23004.

EPA Response: This mechanistic *in vitro* study was not added to the assessment because in vivo data exist demonstrating effects on the placenta in response to GenX chemicals exposure. While the study's mechanistic information for placental effects is interesting, including this information is not necessary for selecting the critical effect or determining the RfDs which were based on liver toxicity endpoints.

d. Petre M-A, Genereux DP, Koropeckyi-Cox L, et al. Per- and Polyfluoroalkyl Substance (PFAS) Transport from Groundwater to Streams near a PFAS Manufacturing Facility in North Carolina, USA. https://doi.org/10.1021/acs.est.0c07978.

EPA Response: Thank you for providing this study. It has been added to the Occurrence section of the assessment (1.3).

e. Kotlarz N, McCord J, Collier D, et al. Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. https://doi.org/10.1289/EHP6837.

EPA Response: Thank you for providing this study. It has been added to the Occurrence section of the assessment (1.3).

f. Blake, RE and Fenton SE. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects. https://doi.org/10.1016/j.tox.2020.152565.

EPA Response: EPA is aware of this review and used it to identify primary references for GenX chemicals that are cited in this assessment. The reference was not added to the assessment because it is a review article and, therefore, does not provide new primary data.

Charge Question 2

In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.

In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**
    i. **If so, please explain your reasoning.**
    ii. **If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.**
    iii. **Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.**

Chou

The reviewer agrees to the constellation approach of applying hepatic lesions as one lesion and the consequent selection of female hepatic lesion as the critical effect.

The two recent studies by Conley et al. (2019) and Blake et al. (2020) have provided additional information to strengthen the hypothesis that the MOA of GenX is associated with the disruption of lipid and carbohydrate metabolism. Collectively, existing data of GenX toxicity indicate that liver lesion is the most sensitive and the earliest observable (i.e. with shortest latency) and measurable target after exposure. It also makes sense that pregnant animals are a sensitive model in the principal study because pregnancy is associated with extra metabolic demands; its synthetic, metabolic, and excretory functions are

physiologically tuned into elevated gears to meet the anabolic challenges during early gestation, followed by catabolic challenges in advanced pregnancy. It is therefore not surprising that liver damage caused by GenX appeared sooner and more apparent when compared with non-pregnant animals. The selection of the critical effect is also in agreement with the known mechanisms of toxicity, specifically, disturbance of lipid and carbohydrate metabolism. The draft document has already provided sufficient evidence to support the reviewer's comments. In case there is a need for a reference on the role of liver in lipid and carbohydrate metabolism during pregnancy, the reviewer provides a recent publication by Lu et al. (2021).

EPA Response: Thank you for your response.

Faustman

i. This reviewer fully supports the use of the DuPont -18405-1037,2010 oral reproductive/developmental toxicity study in mice for the use of deriving the subchronic and chronic RfDs for Gen X chemicals. This reviewer found the detailed discussion of this study in the Draft GenX Chemicals Toxicity Assessment to be very well supported and that the discussion of other possible alternative assays for setting this RfD to be fully supported. The discussion of the study findings across specific organ effects included a thorough discussion of dose-response, histology and adversity. In fact, given the challenges that the assessment of the perflorinated compounds pose to regulators, this internal draft could be used as an excellent example of how toxicological signals across multiple organs, species, sex and dose is evaluated in a systems-based approach.

iii. EPA provides a discussion within their review of the DuPont-18405-1037, 2010 study that demonstrates that they were very interested in determining the species differences, the dose response differences and the consistency or inconsistency of this study in relationship to the larger literature that discusses the types of endpoints occurring following PPAR activation. EPA provided evidence and re-assessment of the liver histology to ensure detailed histological analysis was conducted (see NTP reassessment and use of the INHAND criteria) that was focused on clarifying apoptotic and necrotic histological manifestation and further clarification of adverse histological and cytology impacts. This information is provided in the draft and a thorough discussion of mechanistic studies available is also currently included. Another area of study that can inform the interpretation of the oral reproductive/developmental toxicity study are the mechanistic studies that look in detail at molecular responses across doses. The draft report developed by EPA does an excellent job of reviewing these diverse array of studies and both highlighting their strengths, context for informing the overall assessment of in vivo studies and identifying critical data gaps and limitations in studies (see for example comments on use of DMSO as a confounder in many of the mechanistic studies). Although the MOA for the HFPO dimer acid and salt is plausible, there is also plausibility in a MOA that looks beyond just PPAR alpha. These studies have helped to support the choice of the critical endpoint of liver effects from the reproductive and developmental study by Dupont for the derivation of the RfD.

EPA Response: Thank you for your response.

Kamendulis

I agree with the selection of the critical study selected for deriving RfD's for GenX chemicals (DuPont-18405-1037 2010), and that the derivation of RfDs for GenX chemicals is scientifically justified and clearly detailed

in the document. The oral reproductive/developmental toxicity study in mice identified liver effects as the critical effect and was used to derive the subchronic and chronic RfDs for GenX chemicals. Further, an independent review (re-analysis) of the pathology slides from the 2010 DuPont study was performed by an NTP PWG (2019), who confirmed that the study NOAEL for DuPont-18405-1037, 2010 is 0.1 mg/kg/day and the LOAEL is 0.5 mg/kg/day based on liver effects classified under current INHAND diagnostic criteria (cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) in male and female mice.

EPA Response: Thank you for your response.

Leung

i. Following the application of PECO inclusion criteria to the retrieved studies, there remain limited dose-response data for GenX chemicals (11 animal studies, all via the oral route; no human studies), from which mainly hepatic, hematologic, reproductive/developmental, renal, and immune effects were evaluated. These available data (all scored to be high quality) provide evidence that hepatic changes, as seen in both male and female mice and rats, at varying doses (0.5-1000 mg/kg/day), and of varying durations of exposure (15 days to 2 years), appear to be the most sensitive adverse health effects from GenX oral exposure. Hepatic damage included increased liver enzymes, increased liver weight, and the increase in a constellation of liver lesions by pathology (cytoplasmic alteration, single-cell necrosis, focal necrosis, and hepatocellular apoptosis) from several studies. From these, the DuPont-18405-1037, 2010 study and its pathologic demonstration of liver lesions (as observed among female mice) were selected for the derivation of the subchronic and chronic RfDs for GenX chemicals. I agree that this study appears to provide the best available evidence of the most sensitive adverse effects, and its selection as the critical study for the purposes of RfD derivation is scientifically justified.

iii. There does not appear to be sufficient evidence from the available GenX studies to support other adverse health effects that would be more sensitive and/or provide more robust data supporting its RfD derivations.

EPA Response: Thank you for your response.

Salmon

The selection of DuPont-18405-1037 (2010), the oral reproductive/developmental toxicity study in mice, as the critical study for derivation of the RfDs is thoroughly described and justified in the document, along with detailed review of other candidate studies and various endpoints. The endpoint chosen in the critical study appears consistently in other similar studies, but the critical study selected is the most sensitive, following standard risk assessment guidelines. Other possible endpoints are either less sensitive, of uncertain biological significance, or do not show consistent dose response and time dependence. The duration of the critical study is directly appropriate for determination of the subchronic RfD, but for the chronic RfD the only chronic study available is in the rat, which is shown in the subchronic studies to be less sensitive to the effects of GenX chemicals than the mouse. Use of DuPont-18405-1037 (2010) as the critical study for the chronic RfD, with the appropriate subchronic-to chronic uncertainty factor, is therefore justified.

EPA Response: Thank you for your response.

Slitt

i. The scientific justification for the selection of DuPont-18405-1037, 2010 for the derivation of subchronic and chronic RfDs for GenX is based on a search that yielded 75 studies as of March 2020. Of those 75 studies, rigorous criteria and metrics were applied to weight each study for quality of the study and ultimately whether the study was able to demonstrate a change in health outcome, if the outcome was more likely than not attributable to test article exposure, and the dose at which the change was observed. From these, ten studies in rats or mice were identified to determine NOAEL and LOAELs, with mice being a more sensitive species. Of these studies both sub-chronic and chronic studies, four describe liver effects, that include increased liver weight, single-cell necrosis, and cytoplasmic alterations. The DuPont-18405-1037, 2010 study meets the criteria listed in almost all elements for being considered of high quality. In addition, livers from this study were re-analyzed by a panel of eight NTP pathologists (NTP PWG, 2019) and concluded that the NOAEL in the F0 generation was 0.1 mg/kg and 0.5 mg/kg was the LOEAL. The study meets every metric as high or medium, such as test substance, test setup, exposure characterization, etc. The critical effect of single cell necrosis is based on a large n=24-25. The selection of this study is scientifically justifiable based on it sufficiently meeting the review criteria. The selection of liver weight and cytotoxicity is a reasonable measure to use as a critical effect and meets the Hall criteria. This measure has been used previously for other perfluoroalkyl substances, such as PFOA and PFOS, in which rodent studies that have demonstrated hepatotoxicity in rodents is concordant with studies in human populations that describe adverse liver effects, such as increased serum ALT and AST enzyme activity. In addition, the DuPont-18405-1037, 2010 describes developmental effects to the F1 generation (i.e. decreased birth weight) at a NOAEL of 0.5 mg/kg and LOAEL of 5 mg/kg.

ii. I do not disagree with the selected critical study. Other studies included have similar limitations, like test article purity – which is either low (84%) or not described.

iii. 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 higher, this study should could be considered an alternative to DuPont 18405-1037, 2010 for considering 0.1 mg/kg in male mice for the RfD.

EPA Response: The liver effects noted in the 28-day oral toxicity study in mice (DuPont-24459, 2008) were not considered as a potential point of departure (POD) in support of the derivation of the RfD. Although the purity of the test substance was greater in this study, the dose range was not optimized for the identification of low-dose effects in the 28-day mouse study (0, 0.1, 3, and 30 mg/kg/day-dose groups) compared to the 90-day mouse and reproductive/developmental mouse toxicity studies (0, 0.1, 0.5 and 5 mg/kg/day-dose groups). For example, in DuPont-18405-1037 (2010), the lowest-observed-adverse-effect-level (LOAEL) (i.e., the lowest dose at which an adverse effect is observed) of 0.5 mg/kg/day falls between the low and mid-doses of the dosing design used in DuPont-24459 (2008). Additionally, the 90-day mouse and reproduction/developmental mouse toxicity studies accounted for the lower purity by adjusting the dose formulations by a factor of 1.19. Finally, given the availability of longer duration studies

demonstrating effects at low doses, the 28-day oral toxicity study in mice was not included in the NTP PWG review. For the reasons outlined above, EPA did not consider the 28-day oral toxicity study in mice as a potential POD in support of the derivation of the RfD.

Warren

Selection of Dupont-18405-1037, 2010 for RfD derivation is scientifically justified and clearly described in the current toxicity assessment, as it was in 2018. Confidence in the study is only increased by the NTP PWG's review of liver pathology, as it is essentially confirmatory of the original findings. Also, despite an adverse response (i.e., critical effect) being defined by a constellation of lesions rather than a single one, the resulting POD_{HED} changed very little from 2018 (i.e., it was reduced from 0.023 to 0.01 mg/kg/day). It is also noteworthy that the reduction in POD_{HED} remained minor despite the change from male to female mice as its basis. Though I support the use of Dupont's reproductive/developmental study for RfD derivation, the new definition of adversity raises an issue for dose-response modeling. Based on the slide review worksheets in Appendix E, several mice were diagnosed by the NTP PWG with varying degrees of cytoplasmic alteration only, or in some cases, cytoplasmic alteration accompanied by mixed cell infiltration. In the absence of necrosis or apoptosis, it would seem appropriate to consider these diagnoses as adaptive and non-adverse (as was done in Table 11 where 0.5 mg/kg/day was determined to be a NOAEL in Dupont's 90-day mouse study, despite cytoplasmic alteration in 10/10 males). However, the dose-response data modeled in Appendix F suggest otherwise (e.g., 24/24 female mice in the high-dose group were selected for dose-response modeling, yet animal numbers 5027, 5033 and 5035 were diagnosed with mild cytoplasmic alteration and nothing more). Shouldn't the absence of hepatocellular necrosis or apoptosis disqualify an animal from inclusion in dose-response modeling? Admittedly, as the slide review worksheets do not indicate which animals were in a given dose group, this question may be for naught.

EPA Response: The reviewer is, in part, correct. Two (not three) out of 24 female mice in the high-dose group in Dupont-18405-1037 (2010) were diagnosed with only mild cytoplasmic alteration (5027 and 5035). The third animal, 5033, died before study termination and was not included in the high-dose group for the dose response modeling. EPA disagrees with the reviewer that the two mice (5027 and 5035) should be removed from the dose-response modeling. EPA interpreted the NTP PWG's description of a constellation of liver lesions as adverse endpoints to apply to the dose group instead of individual animals within a dose group since the histopathological evaluation represents a snapshot in time of a biological process and is conducted in one portion of the liver (see section 7.1). For example, some animals were diagnosed with liver necrosis without additional liver lesions (e.g., animals 4974 and 5072); yet, EPA would still consider the liver effects in these animals to be adverse. Therefore, when multiple liver lesions representing the progression of adverse liver changes (e.g., necrosis or apoptosis) were observed within a dose group, all animals in that dose group were considered for dose-response modeling. This was not the case for the males in the 0.5 mg/kg/day dose group in the 90-day mouse study as the NTP PWG reported that 10 out of 10 male mice in this dose group exhibited only cytoplasmic alteration. No other liver lesions (i.e., single-cell or focal necrosis or apoptosis) were observed at the 0.5 mg/kg/day dose level in males. Consistent with the Hall criteria, EPA did not consider the cytoplasmic alteration findings alone as an adverse effect in the 0.5 mg/kg/day dose group; instead EPA considered the constellation of liver lesions observed across the male mice in the

high-dose group as adverse. Compared to the males, 22 out of 24 pregnant female mice in the 5 mg/kg/day dose group in the reproductive/developmental study exhibited necrosis or apoptosis, demonstrating the progression of adverse liver effects among animals within this dose group. To address this comment, EPA revised section 7.1 clarifying our interpretation of the NTP PWG's definition that a constellation of liver lesions is adverse at the dose group level instead of at the individual animal level.

Charge Question 3

EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. **Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.**
b. **Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.**

Chou

The reviewer agrees to the decision of applying a databased uncertainty factor of 10 to derive the RfDs, based on a lack of epidemiological studies, limited information on development toxicity, and immunological responses.

The reviewer supports the use of UF of database deficiency for two additional reasons. First, there is a lack of data to explain the decreased maternal serum concentrations on Day 17.5, when compared with that on Day 11.5 (Blake et all, 2020). In addition, during the same period, from Day 11.5 to Day 17.5, the concentrations in the embryos increased 3.5 times in the 2 mg/kg treatment group and 2.4 times in the 10 mg/kg treatment group. The kinetics of the two incongruent observations, decreasing maternal serum concentration during repeated exposure, and the accompanied increasing accumulation of embryonic concentration, are yet to be explained by the mechanisms of toxicokinetics.

Second, the study by Coney et al. (2019) demonstrated that maternal exposure to GenX up-regulates genes in the pathways of fatty acid metabolism. Qualitatively, more genes are up-regulated in fetal liver than that in the maternal liver, and quantitatively, the magnitude of up-regulation of Cpt1b, Angptl4, and Acox1 are higher in the fetal liver than that in the maternal liver. Metabolic disturbance during fetal development is

likely to lead to long-term negative metabolic outcomes in the offspring. It is important to recognize the database deficiency for developmental metabolic effects in the offspring, and specifically, the need for a two-generation developmental toxicity study with an emphasis on the effects on lipid and carbohydrate metabolism in the offspring.

Based on the physical and chemical properties of GenX being a surfactant, there could be another rationale for applying a UF of database deficiency, which is provided in the answer to the next charge question.

EPA Response: Thank you for your response. The additional rationale for a two-generation developmental toxicity study that you provided has been added to the assessment. The point about the accumulation of HFPO dimer acid in the embryo was already included in the database uncertainty factor (UF_D) rationale.

Faustman

This reviewer agrees with the conclusion of the internal draft that in fact, the uncertainty has increased. This should not be surprising given the intensity of investigation of the perflorinated compounds and the expanded portfolio of endpoints that are being revealed. The internal report identifies additional uncertainties in observations in immune response, molecular responses that appear to be beyond PPAR alpha dependent responses and which identify further concerns regarding developmental sensitivity and kinetics. Since the uncertainties have now been expanded and cover both kinetic and dynamic considerations, the increase of the uncertainty factor from 3 to 10 is appropriate.

EPA Response: Thank you for your response.

Kamendulis

a. As noted in the document, important data gaps related to developmental toxicity exist for GenX chemicals. Since the 2018 draft document for GenX chemicals, 3 studies Conley 2019, 2021, and Blake 2020 have been published demonstrating that exposure to GenX chemicals are associated with reproductive and developmental toxicities (albeit at higher doses than the liver NOAEL). As a two-generation reproductive and developmental toxicity study is not available, it is unclear what effects will occur following exposure to GenX chemicals during development. Given that Blake et al. (2020) demonstrated that HFPO dimer acid accumulates in whole mouse embryos at early life stages, evaluation of developmental toxicities that occur during early organogenesis that have been observed following exposure to PFOA (delayed skeletal ossification and mammary gland development), appear as critical developmental endpoints to evaluate for GenX chemicals. Further, the available studies evaluating developmental and reproductive toxicities have not evaluated GenX chemicals at doses below the proposed NOAEL derived from the critical study. Other gaps in the database for GenX chemicals exist for immune, hematological and neurological toxicities. In addition, the available data indicate that the mouse is the more sensitive to the liver effects resulting from GenX chemicals compared to rats. The lack of a chronic bioassay for GenX chemicals evaluating cancer in mice is also considered a database deficiency (this also impacts my response to charge question 4). Collectively, these database limitations support applying a UF of 10.

b. Yes, the scientific rationale provided support the application of this uncertainty factor.

EPA Response: Thank you for your response.

Leung

a. The concern of adverse reproductive/developmental effects stems from 4 mice and rat dose-response studies (2 from DuPont/Chemours and 2 from the published scientific literature; all deemed high quality). Adverse effects from these studies include decreased pup weights, delays in the attainment of balanopreputial separation and vaginal patency, increased premature birth, decreased fetal weight, decreased gravid uterine weight, both increased/decreased gestational weight gain, decreased maternal serum total T3 and T4 levels, evidence of reduced body and tissue weights in F1 animals, increased abnormal placental lesions, decreased pup survival, and increased number of litters with a 14^{th} rudimentary rib. These statistically significant findings support the uncertainty of previously unrecognized potential reproductive/developmental effects due to GenX chemical exposure, although the clinical significance of these may be less clear. Nonetheless, I agree that this new information justifies the increase of the database uncertainty factor.

b. The selection of the revised uncertainty factor (10 for potential reproductive/developmental risks, increased from 3 previously) is not my area of expertise, and I defer to the other reviewers. However, this selection was reported to take into account variability in the human population, database uncertainties, and possible differences in the ways in which humans and rodents respond to HFPO dimer acid and/or its ammonium salt that reaches their tissues. Although selected RfDs were also based on adverse hepatic effects observed in parental females, thus expected to also account for adverse effects to their offspring population, developing offspring may be even more sensitive to the adverse effects of toxicant exposures.

EPA Response: Thank you for your response.

Salmon

The additional recently published information indicates that there is considerable uncertainty as to the developmental impact of the GenX chemicals, as described and explained in the document. These findings are of particular concern since the database does not include a full multigenerational study. The increased database uncertainty factor is therefore justified.

EPA Response: Thank you for your response.

Slitt

a. Yes, I do support this because additional evidence has been published since the 2018 review that indicate developmental and reproductive effects. The studies indicate that HFPO dimer acid can pass the placenta, accumulate in the fetus, and also cause histological changes to the placenta. The Blake et al., 2020 publication had similar findings to the DuPont-18405-1037, 2010 study. Blake et al., 2020 demonstrated maternal GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at gestational day 17.5. Since 2018, Blake et al., 2020 also demonstrated that Blake et

al. (2020) demonstrated that dosing or 2 or 10 mg/kg/day of HFPO dimer acid to pregnant dams resulted in measurable HFPO dimer acid in amniotic fluid and whole embryos at Embryonic day 11.5 (E11.5) and 17.5 (E17.5). Conley et al., 2019 also demonstrated transfer of HFPO dimer acid to the fetus in rats. Conley et al., 2021 demonstrated transfer of HFPO dimer acid from dam to pup in pregnant rats exposed from GD8 through PND2. Lastly, Blake et al, 2020, described increased placental lesions.

b. Yes, this provided rationale supports the application of an uncertainty factor of 10.

EPA Response: Thank you for your response.

Warren

Yes, I agree that additions to the GenX database following development of the original toxicity assessment, including the Conley et al. publication listed in my response to question no. 1, warrant an increase in the database uncertainty factor (UF_D). Clearly, there is a laundry list of emerging concerns for toxicities not fully characterized, reproductive/developmental and endocrine chief among them. In addition, the epidemiological data for GenX severely lag that of legacy PFAS, and the GenX toxicity profile to date, bears an eerie resemblance to that of PFOA, qualitatively and quantitatively. Pages 91-93 of the toxicity assessment are very effective at providing the scientific rationale for an increase in the UF_D. However, an increase from 3 to 10 in the UF_D becomes, in my opinion, problematic when coupled with an increase of the same magnitude in the subchronic-to-chronic uncertainty factor (UF_S). While the use of a UF_S in chronic RfD derivation is justified, a factor of 10 seems excessive given that uncertainty over exposure duration (including the absence of a chronic mouse study) is partially accounted for by maximizing the UF_D. That the UF_D and UF_S can overlap and address the same uncertainty over exposure duration is actually acknowledged in the toxicity assessment (see last paragraph of p. 94). In addition, the UF_D was increased to account for the lack of chronic studies in the recent derivation of toxicity values for the PFOA replacement, PFBS. Furthermore, maintaining both the UF_D and UF_S at 10 in the revamped toxicity assessment results in a chronic RfD of 0.003 µg/kg/day, a value nearly an order of magnitude below the RfD used to set lifetime drinking water health advisories for PFOA and PFOS (i.e., 0.02 µg/kg/day). Lastly, the changes in composite uncertainty factors (UF_C) from the original toxicity assessment are 3- (100 to 300) and 10-fold (300 to 3000) in the case of the subchronic and chronic RfDs, respectively. This, coupled with a slight decrease in POD_{HED}, translates into 7- and 27-fold decreases in the subchronic and chronic RfDs from those in the original toxicity assessment. While acknowledging the need for health conservative toxicity values in the face of uncertainty, a UF_C of 3000 is an extreme application of the precautionary principle. As it stands, I support the UF_C of 300 for subchronic RfD derivation, but suggest a reduction in the UF_S from 10 back to 3 (for a UF_C of 1000) before derivation of a chronic RfD. While slightly less health conservative, such a reduction in UF_S still results in a chronic RfD of 0.01 µg/kg/day, a toxicity value one-half that of PFOA and PFOS.

EPA Response: Thank you for your response on the UF_D rationale. Note that the Conley et al. (2021) study was included in the UF_D rationale.

With respect to the reviewer's comments on the subchronic-to-chronic-duration UF, EPA realizes that the two sentences in question—"EPA acknowledges that the lack of a chronic study in the mouse, which appears to be more sensitive to GenX chemical exposure than the rat, is a data gap. However, this uncertainty is also addressed in the subchronic-to-chronic UF."—can be

misinterpreted. To address the reviewer's comment, EPA removed mention of a lack of a chronic study in mice from the UF_D rationale because EPA considered the lack of a chronic study only in the subchronic-to-chronic UF rationale. The lack of a chronic study is appropriate for the subchronic-to-chronic UF rationale because the RfD for GenX chemicals is based on a subchronic study and a chronic study in the mouse does not exist. EPA guidance states that "a default value of 10 for [the subchronic-to-chronic-duration] UF is applied to the BMDL from the subchronic study on the assumption that effects from a given compound in a subchronic study occur at a 10-fold higher concentration than in a corresponding (but absent) chronic study" (EPA, 2002). The reviewer agrees with the selection of the critical study, the critical effect, and that the mouse appears to be more sensitive than the rat. Comparing the available studies of differing durations, the data demonstrate a progression of liver effects as duration of exposure increases, underscoring the need for a subchronic-to-chronic UF of 10.

The reviewer suggests maintaining consistency between the database UF rationales for perfluorobutanesulfonic acid (PFBS) and GenX chemicals. However, there are important differences between PFBS and GenX chemicals critical effects and available data. The lack of a chronic study was likely not cited in the UF_D write-up for the PFBS toxicity assessment (https://www.epa.gov/pfas/learn-about-human-health-toxicity-assessment-pfbs) because the critical effect for PFBS is a developmental effect and a two-generation reproductive toxicity study and multiple developmental exposure studies have been conducted. Therefore, given the developmental critical effect, the appropriate exposure durations are captured in the available database. As highlighted above, this is not the case for the GenX chemicals critical effect and available database.

Finally, altering the UFs to achieve an RfD for GenX chemicals that is closer to the RfDs derived for perfluorooctanoic acid (PFOA) and perfluorooctanesulfonic acid (PFOS) is not in line with EPA guidance. It is not surprising that more uncertainty is associated with GenX chemicals given their more recent (2015) detection in drinking water. PFOA and PFOS have been extensively studied in the peer reviewed literature for at least the past 30 years and the toxicological data for these chemicals is quantitatively large and comprehensive when compared to the GenX chemicals toxicological database.

Charge Question 4

EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. **Given the evidence provided above and EPA's guidance on selection of uncertainty factors (EPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?**
b. **Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.**

Chou

The reviewer agrees to the decision of applying a UF of 10 for the extrapolating toxicity values from sub-chronic exposure to chronic exposure. Existing toxicokinetic data do not support the notion that sub-chronic exposure of 90 days or shorter period has reached a steady state of toxicity or body burden. Continuous redistribution and additional exposure could lead to additional types of toxicity or additional target cellular compartments.

GenX is a biologically persistent surfactant. There is a lack of long-term studies that are designed to observe the potential long-term effects of small concentrations of surfactants in packets of cellular compartments. Interference of the dynamic surface tension of compartmentalized fluids at the cellular level predictably disrupts biochemical functions. The level and types of interference will depend on the normal function of a given compartmentalized surface tension. The characteristics of surface tension in various micro-compartments also depend on age, sex, other physiological states, and disease conditions. In addition, the dose-response relationship of surfactants is driven by the biphasic kinetics of the properties of a given surfactant. Genx's critical micelle concentration and its synergistic micelle formation with other surfactants in the cellular compartments will likely to result in a non-linear dose-response relationship. This rationale is offered to illustrate the data gap that contributes to the uncertainty of long-term of per- and polyfluoroalkyl surfactants, in general, and to support, specifically, the use of a UF of 10 to account for extrapolation from sub-chronic to chronic exposure duration to derive the chronic RfD in the current toxicity assessment.

EPA Response: Thank you for your response.

Faustman

This reviewer agrees with a selection of uncertainty factor of 10 to account for extrapolation from a subchronic to a chronic exposure duration. Detailed support for this number is provided by EPA and includes the following considerations: complexity of kinetics especially over time and lifestage, clarification of the adversity of the hepatic alterations observed in the Dupont study used for the critical effect (see the NTP re-assessment and use of the most current pathology classification guidance) that now highlight more concern over the long term manifestations of these adverse impacts in the hepatic system, further identification of cholesterol changes and concerns over adiposity and chronic health impacts and dose response for these complex endpoints across sex and time.

EPA Response: Thank you for your response.

Kamendulis

It is agreed that evidence supports that rats appear to be less sensitive than mice to the toxicities elicited following exposure to GenX chemicals, and that because a 2-year chronic mouse study is unavailable, the effect of a longer dosing duration on the incidence and severity of liver effects in mice is unknown. However, similar to the current draft assessment, the 2018 EPA draft assessment for GenX chemicals did not use the chronic bioassay in rats for the derivation of a chronic RfD and justified using a UF of 3 to account for extrapolation from a subchronic to a chronic exposure duration. In part, the justification for a UF was that the 2-year study identified a NOAEL based on liver effects (increased liver enzyme levels and centrilobular hepatocellular hypertrophy and cystic focal degeneration in males and centrilobular necrosis in both sexes), that were consistent with the liver effects observed in the oral reproductive/ developmental study in mice used to derive the RfDs (DuPont-18405-1037, 2010). Further, the lack of a chronic bioassay for GenX chemicals evaluating cancer in mice is also considered a database deficiency, and in part, is accounted for in the proposed UF of 10 for database uncertainty (see response to charge question 3). Application of a UF of 3 to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD appears appropriate.

EPA Response: Thank you for your response. There are two critical differences in the analysis that was completed in 2018 compared with the current analysis. First, the critical effect selected for RfD derivation changed from male mice to female mice based on the NTP PWG reanalysis of liver effects in DuPont-18405-1037 (2010). This is important because in DuPont-18405-1037 (2010), female mice were dosed well below the 90-day exposure window typically employed in a subchronic study. Specifically, parent generation (F_0) females that delivered were dosed daily starting 14 days prior to pairing and were dosed through lactation day (LD20) for a total of 53 to 64 days of exposure, depending on delivery date. By contrast, F_0 males in this study were dosed 70 days prior to mating and throughout mating through 1 day prior to scheduled termination, for a total of 84–85 days of exposure. The critical effect in female mice had a shorter exposure duration than the males, providing support for increasing the subchronic-to-chronic duration UF.

The second difference is that female rodents demonstrate progression of liver effects as duration of exposure increases. Specifically, necrosis in female rats was not reported in the 28- or 90-day

rat studies or the interim 1-year time point in the 2-year chronic rat study, which dosed the rats from 3 to 1,000 mg/kg/day. However, at the completion of the 2-year chronic rat study, centrilobular and single-cell necrosis are reported in the 500 mg/kg/day-dose group. Moreover, treatment-related liver tumors were observed in the 500 mg/kg/day rat dose group (0/70 in control versus 11/70 in the 500 mg/kg/day group). These data demonstrate progression of liver effects over the 2-year dosing period. Additionally, Blake et al. (2020) did not find clear evidence of changes in maternal liver serum enzymes (i.e., alkaline phosphatase (ALP), alanine aminotransferase (ALT) or aspartate transaminase (AST)) or increases in liver necrosis after 10–16 days of dosing at 2 mg/kg/day compared to controls. Similarly, DuPont-24459 (2008) did not report single cell necrosis in female mice treated with 0.1 or 3 mg/kg/day after 28 days of dosing, though 4/10 mice displayed single cell necrosis in the 30 mg/kg/day dose group. However, DuPont-18405-1037 (2010) found liver necrosis in mice after 53–85 days of dosing at 0.5 mg/kg/day, indicating a progression of liver effects with increasing duration of treatment.

Though the liver effects observed in the 2-year chronic rat study are consistent with the liver effects observed in the oral reproductive/developmental study in mice, the LOAEL for liver effects in the rats is 50 mg/kg/day while the LOAEL in the mice is 0.5 mg/kg/day, a difference of two orders of magnitude. EPA acknowledges that comparing study no-observed-adverse-effect levels (NOAELs) was not an appropriate justification for a subchronic-to-chronic duration UF of 3 in the draft assessment and has removed this point from the current assessment.

Finally, EPA considered the lack of a chronic study only in the subchronic-to-chronic UF rationale. This is appropriate because the RfD for GenX chemicals is based on a subchronic study, a chronic study in the mouse does not exist, and EPA guidance states that "a default value of 10 for [the subchronic-to-chronic-duration] UF is applied to the BMDL from the subchronic study on the assumption that effects from a given compound in a subchronic study occur at a 10-fold higher concentration than in a corresponding (but absent) chronic study" (EPA, 2002). The reviewer agrees with the selection of the critical study, the critical effect, and that the mouse appears to be more sensitive than the rat. For these reasons, EPA increased the UF from a 3 to a 10 to account for duration of exposure for the chronic RfD.

Leung

Selection of uncertainty factors is not my area of expertise; I defer to the other reviewers.

EPA Response: Thank you for your response.

Salmon

The increased subchronic to chronic uncertainty factor is consistent with risk assessment guidelines, which allow for a value of either 3 or 10 for this UF, depending on the uncertainty implied by this extrapolation. Selection of an appropriate value for UF_S is based on the nature of the critical effect and any toxicological, toxicokinetic or mechanistic evidence that has bearing on the likely timescale for appearance of that effect. Selection of a value of 10 for UF_S is justified by the considerations laid out in the document. There is a difference in timescale for appearance of necrosis in the liver between mice and rats, and there is no chronic study in mice. The new pathology analysis by NTP highlights the significance of this timescale difference.

EPA Response: Thank you for your response.

Slitt

a. Yes. The use of uncertainty factors is consistent with EPA guidance (USEPA, 2011b).

Given that there are no published chronic studies in mice, but mice are a more sensitive species, it is reasonable to assume that a longer duration would result in a magnitude of response or a lower LOAEL dose.

b. I agree with the rationale provided and the use of UF 10 for subchronic to chronic.

EPA Response: Thank you for your response.

Warren

See my response to question no. 3 above.

EPA Response: See response to question number 3 above and the response to Kamendulis under charge question number 4.

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SECTION III: REVIEWER ADDITIONAL AND EDITORIAL COMMENTS

Chou

1. **P. 4, 1.3 Occurrence**: The draft document has provided evidence for relatively short-distance (24 kilometers) downwind atmospheric transport. In case long-range transport of HFPO-DA to remote regions is also desired, see study by Joerss et al. (2020).

   EPA Response: Thank you for the reference. It has been added to section 1.3 of the assessment.

2. **P. 10, Bioaccumulation:** The publication by Hoke et al. (2016) is not a "replicate" of the DuPont study (DuPont-A080560, 2009). The same study by DuPont was published by Hoke et al in 2016.

   EPA Response: Thanks, "replicated" has been changed to "observed".

3. **P. 10, Bioaccumulation**: When the sample peak area is ND and the mean BCF is NA, there can be no claim of "steady state" or any BCFss values. The DuPont A080560 document did state, "the bioaccumulation potential of the test substance in fish tissues is judged to be low" and the publication by Hoki et al. (2016), "the substance is unlikely to bioconcentrate in aquatic organisms." Nonetheless, the reviewer believes that these statements are not substantiated by the data presented in DuPont A080560 or Hoki et al. (2016). This assertion "low potential to bioaccumulate in biota" (second line under Bioaccumulation) may or may not be true, the point is that the cited references do not support it. The reviewer understand that this statement dose not impact on the toxicity values derived in this Draft, but it may not be desirable.

   EPA Response: Thanks for this clarification. The statement has been removed.

Faustman

This reviewer would like to compliment EPA. The interim report was detailed, clear, and thorough. It included a conceptual diagram that helped provide context for this detailed discussion and was methodical in reviewing this extensive database. They identified critical data gaps and also were transparent in saying what was known versus unknown about specific impacts.

EPA Response: Thank you for your response.

Kamendulis

The table on page 8 (document page #34) is identified as "Table X" – numbering sequentially, this should be Table 5. If this change is made, all subsequent tables are misnumbered.

Page 80-81 (document pages 96-97) – mode of action discussion. The proposed PPARα MOA pertains to liver tumors, as an MOA for pancreatic acinar tumors has not been proposed. To clarify that the proposed PPARα MOA refers to liver tumors, paragraphs on pages 80 and 81 that pertain to PPARα could reference the tissue (presumably liver) that the data is describing.

EPA Response: Thanks, the suggested changes have been made.

Leung

> I would suggest amending to the following syntax (additions shown in **bold**), when referring to the data supporting the overall RfD, throughout the document for improved readability:

Example as shown in Section 7.4 (page 97): "The oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and **its pathologic demonstration of** liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the critical study and **organ** effects, **respectively**, for deriving the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt."

EPA Response: Thanks, the suggested changes have been made.

> In Table 11 regarding the Conley 2019 study, suggest inserting: "decreased maternal **serum** total T4 levels…" and clarifying "indications of reduced body (females) and **reproductive and non-reproductive** organ weights in F1 animals".

EPA Response: Thanks, the suggested changes have been made.

> In Table 11, recommend adding in the significant changes in serum total T3 levels observed from the 3 relevant studies, as well as the findings of decreased pup survival and increased number of litters with a 14^{th} rudimentary rib, for completeness.

EPA Response: Thanks, the effects listed in Table 11 (now numbered Table 12) are effects observed at the study LOAELs. Changes in serum total triiodothyronine (T3) levels were added to the Conley et al. (2019, 2021) list of effects since these effects were observed at the study LOAELs. However, serum total T3 levels were not significantly different between control and treated animals in Blake et al. (2020) so this effect was not added to Table 12. Decreased pup survival observed in Conley et al. (2021) occurred at doses higher than the study LOAEL so it was not added to Table 12. Finally, the number of litters with a 14^{th} rudimentary rib observed in DuPont-18405-841 (2010) was not significantly increased at the study LOAEL, so this effect was not added to Table 12.

Salmon

The only additional comments I have are of a minor editorial nature:

a. There are some broken links in the list of Figures (page v) and Tables (page vii).

b. In Table 12 (page 90) there are problems with the column widths causing badly placed line breaks in columns 5, 6 and 7. While the actual information is intact, this impairs the readability.

EPA Response: Thanks, the links and tables have been formatted.

Slitt

The document cites studies by Cannon et al. that investigate whether HFPO Dimer Acid is a substrate for BCRP. The study cited used a vesicle-based ATPase assay. These assays have a limitation that they can

produce false negatives, especially with drugs/chemicals that are permeable. So, it should be acknowledged that only in this assay it was not considered to be a substrate. The significance of this relates to the notion that GenX has reproductive effects and causes placental lesions. BCRP is highly enriched in placenta and is a potential transport mechanism that could explain GenX effects in placenta and the mechanism by which HFPO dimer acid accumulates in fetuses with exposure to the dams. For that reason, it is discouraged to make the assertion that BCRP does not transport HFPO dimer acid.

EPA Response: Thanks for this comment. The sentence concluding that HFPO dimer acid is not a substrate for breast cancer resistance protein (BCRP) has been updated to: "HFPO dimer acid ammonium salt did not alter ATPase activity associated with P-gp or BCRP transport either when the substrate was stimulated or when no substrate was added, indicating that HFPO dimer acid ammonium salt was not a substrate for either transporter using this particular in vitro reconstituted transport assay system."

Warren

a. Page E-56: I believe Project 18405-1307 Females should read Project 18405-1037 Females. The heading, Project 18405-1307 Females, is used previously on page E-34.

EPA Response: Thanks, page D-37 (previously page E-34) has been updated to Project 18405-1037.

b. Tables F-1 and F-3: The column headings should read Constellation of Lesions rather than Incidence of combined necrosis.

EPA Response: Thanks, Tables E-1 and E-3 column headings now read Constellation of Liver Lesions.

c. Last sentence, page 42: This information on NOAEL/LOAEL is confusing without knowledge as to what the NTP PWG considers adverse. As such, similar or identical language to that at the very bottom of page 85 should be included near the bottom of page 42, if not earlier in the text.

EPA Response: Thanks, text has been added to page 43 to describe what the NTP PWG considered adverse and how EPA selected the study NOAEL.

d. Last paragraph, page 58: Change deceased to decreased.

EPA Response: Thanks, the suggested change has been made.

e. Table of Contents, page vii: Tables F-5 and F-6 dealing with placental lesions are not included in the toxicity assessment.

EPA Response: Thanks, the suggested change has been made.

f. Table 3, footnotes, page. 15: Singularize measurements.

EPA Response: Thanks, the suggested change has been made.

g. Table 9, footnotes, page 43: Change misdoing to misdosing.

EPA Response: Thanks, the suggested change has been made.

h. Page 85, 6th line from the bottom: The sentence beginning with The NTP should be corrected.

EPA Response: Thanks, edits have been to the paragraph to improve clarity.

i. Page 85, 7th line from the bottom: Insert ",less cytoplasmic alteration in males," between the closed parentheses and were.

EPA Response: Thanks, edits have been to the paragraph to improve clarity.

j. Page 90, 2nd line of text: Unless I'm missing part of the toxicity assessment, candidate RfDs were not calculated based on Blake et al. (2020).

EPA Response: Thanks, the suggested change has been made.

k. Page 91, last line of text: Pluralize rat.

EPA Response: Thanks, the suggested change has been made.

l. Page 102, 4th line of text: Insert of after levels.

EPA Response: Thanks, the suggested change has been made.

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Joerss, H., Z.Y. Xie, C.C. Wagner, W.J. Von Appen, E.M. Sunderland, and R. Ebinghaus. 2020. Transport of legacy perfluoroalkyl substances and the replacement compound HFPO-DA through the Atlantic Gateway to the Arctic Ocean — is the Arctic a sink or a source? *Environmental Science & Technology* 54:9958–9967. doi:10.1021/acs.est.0c00228.

APPENDIX A: INDIVIDUAL REVIEWER COMMENTS

COMMENTS SUBMITTED BY

Karen Chou, Ph.D.

Associate Professor, Department of Animal Science
Michigan State University
East Lansing, Michigan

Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

The review does not know any recent pertinent literature for GenX chemicals that is not included in this document.

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**

      i. **If so, please explain your reasoning.**

      ii. **If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.**

      iii. **Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.**

The reviewer agrees to the constellation approach of applying hepatic lesions as one lesion and the consequent selection of female hepatic lesion as the critical effect.

The two recent studies by Conley et al. (2019) and Blake et al. (2020) have provided additional information to strengthen the hypothesis that the MOA of GenX is associated with the disruption of lipid and carbohydrate metabolism. Collectively, existing data of GenX toxicity indicate that liver lesion is the most sensitive and the earliest observable (i.e. with shortest latency) and measurable target after exposure. It also makes sense that pregnant animals are a sensitive model in the principal study because pregnancy is

associated with extra metabolic demands; its synthetic, metabolic, and excretory functions are physiologically tuned into elevated gears to meet the anabolic challenges during early gestation, followed by catabolic challenges in advanced pregnancy. It is therefore not surprising that liver damage caused by GenX appeared sooner and more apparent when compared with non-pregnant animals. The selection of the critical effect is also in agreement with the known mechanisms of toxicity, specifically, disturbance of lipid and carbohydrate metabolism. The draft document has already provided sufficient evidence to support the reviewer's comments. In case there is a need for a reference on the role of liver in lipid and carbohydrate metabolism during pregnancy, the reviewer provides a recent publication by Lu et al. (2021).

Lu, J. Y., Y. Gong, X. H. Wei, Z. Y. Yao, R. Yang, J. X. Xin, L. Gao & S. S. Shao (2021) Changes in hepatic triglyceride content with the activation of ER stress and increased FGF21 secretion during pregnancy. Nutrition & Metabolism, 18.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

The reviewer agrees to the decision of applying a databased uncertainty factor of 10 to derive the RfDs, based on a lack of epidemiological studies, limited information on development toxicity, and immunological responses.

The reviewer supports the use of UF of database deficiency for two additional reasons. First, there is a lack of data to explain the decreased maternal serum concentrations on Day 17.5, when compared with that on Day 11.5 (Blake et all, 2020). In addition, during the same period, from Day 11.5 to Day 17.5, the concentrations in the embryos increased 3.5 times in the 2 mg/kg treatment group and 2.4 times in the 10 mg/kg treatment group. The kinetics of the two incongruent observations, decreasing maternal serum concentration during repeated exposure, and the accompanied increasing accumulation of embryonic concentration, are yet to be explained by the mechanisms of toxicokinetics.

Second, the study by Coney et al. (2019) demonstrated that maternal exposure to GenX up-regulates genes in the pathways of fatty acid metabolism. Qualitatively, more genes are up-regulated in fetal liver than that in the maternal liver, and quantitatively, the magnitude of up-regulation of Cpt1b, Angptl4, and Acox1 are higher in the fetal liver than that in the maternal liver. Metabolic disturbance during fetal development is

likely to lead to long-term negative metabolic outcomes in the offspring. It is important to recognize the database deficiency for developmental metabolic effects in the offspring, and specifically, the need for a two-generation developmental toxicity study with an emphasis on the effects on lipid and carbohydrate metabolism in the offspring.

Based on the physical and chemical properties of GenX being a surfactant, there could be another rationale for applying a UF of database deficiency, which is provided in the answer to the next charge question.

4. **EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.**

   The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

   Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

   a. **Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?**

   b. **Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.**

The reviewer agrees to the decision of applying a UF of 10 for the extrapolating toxicity values from sub-chronic exposure to chronic exposure. Existing toxicokinetic data do not support the notion that sub-chronic exposure of 90 days or shorter period has reached a steady state of toxicity or body burden. Continuous redistribution and additional exposure could lead to additional types of toxicity or additional target cellular compartments.

GenX is a biologically persistent surfactant. There is a lack of long-term studies that are designed to observe the potential long-term effects of small concentrations of surfactants in packets of cellular compartments. Interference of the dynamic surface tension of compartmentalized fluids at the cellular level predictably disrupts biochemical functions. The level and types of interference will depend on the normal function of a given compartmentalized surface tension. The characteristics of surface tension in various micro-compartments also depend on age, sex, other physiological states, and disease conditions. In addition, the dose-response relationship of surfactants is driven by the biphasic kinetics of the properties of a given surfactant. Genx's critical micelle concentration and its synergistic micelle formation with other surfactants in the cellular compartments will likely to result in a non-linear dose-response relationship. This rationale is offered to illustrate the data gap that contributes to the uncertainty of long-term of per- and polyfluoroalkyl surfactants, in general, and to support, specifically, the use of a UF of 10 to account for extrapolation from sub-chronic to chronic exposure duration to derive the chronic RfD in the current toxicity assessment.

5. 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.

1) **P. 4, 1.3 Occurrence**: The draft document has provided evidence for relatively short-distance (24 kilometers) downwind atmospheric transport. In case long-range transport of HFPO-DA to remote regions is also desired, see study by Joerss et al. (2020).

   Joerss, H., Z. Y. Xie, C. C. Wagner, W. J. Von Appen, E. M. Sunderland & R. Ebinghaus (2020) Transport of Legacy Perfluoroalkyl Substances and the Replacement Compound HFPO-DA through the Atlantic Gateway to the Arctic Ocean-Is the Arctic a Sink or a Source? Environmental Science & Technology, 54, 9958-9967.

2) **P. 10, Bioaccumulation**: The publication by Hoke et al. (2016) is not a "replicate" of the DuPont study (DuPont-A080560, 2009). The same study by DuPont was published by Hoke et al in 2016.

3) **P. 10, Bioaccumulation**: When the sample peak area is ND and the mean BCF is NA, there can be no claim of "steady state" or any BCFss values. The DuPont A080560 document did state, "the bioaccumulation potential of the test substance in fish tissues is judged to be low" and the publication by Hoki et al. (2016), "the substance is unlikely to bioconcentrate in aquatic organisms." Nonetheless, the reviewer believes that these statements are not substantiated by the data presented in DuPont A080560 or Hoki et al. (2016). This assertion "low potential to bioaccumulate in biota" (second line under Bioaccumulation) may or may not be true, the point is that the cited references do not support it. The reviewer understand that this statement dose

COMMENTS SUBMITTED BY

Elaine M. Faustman, Ph.D., DABT

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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals")

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

This reviewer is not aware of additional relevant literature. Note that only Pub med via NLM was searched and although there is a tremendous increase in perflorinated compound associated literature this reviewer's search did not identify any highly relevant publications that would have modified their comments on the draft EPA document.

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**

      i. **If so, please explain your reasoning.**

This reviewer fully supports the use of the DuPont -18405-1037,2010 oral reproductive/developmental toxicity study in mice for the use of deriving the subchronic and chronic RfDs for Gen X chemicals. This reviewer found the detailed discussion of this study in the Draft GenX Chemicals Toxicity Assessment to be very well supported and that the discussion of other possible alternative assays for setting this RfD to be fully supported. The discussion of the study findings across specific organ effects included a thorough discussion of dose-response, histology and adversity. In fact, given the challenges that the assessment of the perflorinated compounds pose to regulators, this internal draft could be used as an excellent example of how toxicological signals across multiple organs, species, sex and dose is evaluated in a systems-based approach.

      iii. **Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.**

EPA provides a discussion within their review of the DuPont-18405-1037, 2010 study that demonstrates that they were very interested in determining the species differences, the dose response differences and the consistency or inconsistency of this study in relationship to the larger literature that discusses the types of endpoints occurring following PPAR activation. EPA provided evidence and re-assessment of the liver histology to ensure detailed histological analysis was conducted (see NTP reassessment and use of the INHAND criteria) that was focused on clarifying apoptotic and necrotic histological manifestation and further clarification of adverse histological and cytology impacts. This information is provided in the draft and a thorough discussion of mechanistic studies available is also currently included. Another area of study that can inform the interpretation of the oral reproductive/developmental toxicity study are the mechanistic studies that look in detail at molecular responses across doses. The draft report developed by EPA does an excellent job of reviewing these diverse array of studies and both highlighting their strengths, context for informing the overall assessment of in vivo studies and identifying critical data gaps and limitations in studies (see for example comments on use of DMSO as a confounder in many of the mechanistic studies). Although the MOA for the HFPO dimer acid and salt is plausible, there is also plausibility in a MOA that looks beyond just PPAR alpha. These studies have helped to support the choice of the critical endpoint of liver effects from the reproductive and developmental study by Dupont for the derivation of the RfD.

3. **EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.**

   EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

   a. **Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.**

   b. **Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.**

This reviewer agrees with the conclusion of the internal draft that in fact, the uncertainty has increased. This should not be surprising given the intensity of investigation of the perflorinated compounds and the expanded portfolio of endpoints that are being revealed. The internal report identifies additional uncertainties in observations in immune response, molecular responses that appear to be beyond PPAR alpha dependent responses and which identify further concerns regarding developmental sensitivity and kinetics. Since the uncertainties have now been expanded and cover both kinetic and dynamic considerations, the increase of the uncertainty factor from 3 to 10 is appropriate.

4. **EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a**

standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. **Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?**
b. **Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.**

This reviewer agrees with a selection of uncertainty factor of 10 to account for extrapolation from a subchronic to a chronic exposure duration. Detailed support for this number is provided by EPA and includes the following considerations: complexity of kinetics especially over time and lifestage, clarification of the adversity of the hepatic alterations observed in the Dupont study used for the critical effect (see the NTP re-assessment and use of the most current pathology classification guidance) that now highlight more concern over the long term manifestations of these adverse impacts in the hepatic system, further identification of cholesterol changes and concerns over adiposity and chronic health impacts and dose response for these complex endpoints across sex and time.

5. **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.**

This reviewer would like to compliment EPA. The interim report was detailed, clear, and thorough. It included a conceptual diagram that helped provide context for this detailed discussion and was methodical in reviewing this extensive database. They identified critical data gaps and also were transparent in saying what was known versus unknown about specific impacts.

COMMENTS SUBMITTED BY

Lisa M. Kamendulis, Ph.D.

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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

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

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**
      i. **If so, please explain your reasoning.**
      ii. **If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.**
      iii. **Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.**

I agree with the selection of the critical study selected for deriving RfD's for GenX chemicals (DuPont-18405-1037 2010), and that the derivation of RfDs for GenX chemicals is scientifically justified and clearly detailed in the document. The oral reproductive/developmental toxicity study in mice identified liver effects as the critical effect and was used to derive the subchronic and chronic RfDs for GenX chemicals. Further, an independent review (re-analysis) of the pathology slides from the 2010 DuPont study was performed by an NTP PWG (2019), who confirmed that the study NOAEL for DuPont-18405-1037, 2010 is 0.1 mg/kg/day and the LOAEL is 0.5 mg/kg/day based on liver effects classified under current INHAND diagnostic criteria (cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) in male and female mice.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

As noted in the document, important data gaps related to developmental toxicity exist for GenX chemicals. Since the 2018 draft document for GenX chemicals, 3 studies Conley 2019, 2021, and Blake 2020 have been published demonstrating that exposure to GenX chemicals are associated with reproductive and developmental toxicities (albeit at higher doses than the liver NOAEL). As a two-generation reproductive and developmental toxicity study is not available, it is unclear what effects will occur following exposure to GenX chemicals during development. Given that Blake et al. (2020) demonstrated that HFPO dimer acid accumulates in whole mouse embryos at early life stages, evaluation of developmental toxicities that occur during early organogenesis that have been observed following exposure to PFOA (delayed skeletal ossification and mammary gland development), appear as critical developmental endpoints to evaluate for GenX chemicals. Further, the available studies evaluating developmental and reproductive toxicities have not evaluated GenX chemicals at doses below the proposed NOAEL derived from the critical study. Other gaps in the database for GenX chemicals exist for immune, hematological and neurological toxicities. In addition, the available data indicate that the mouse is the more sensitive to the liver effects resulting from GenX chemicals compared to rats. The lack of a chronic bioassay for GenX chemicals evaluating cancer in mice is also considered a database deficiency (this also impacts my response to charge question 4). Collectively, these database limitations support applying a UF of 10.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

Yes, the scientific rationale provided support the application of this uncertainty factor.

4. EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. **Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?**

See response below

b. **Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.**

It is agreed that evidence supports that rats appear to be less sensitive than mice to the toxicities elicited following exposure to GenX chemicals, and that because a 2-year chronic mouse study is unavailable, the effect of a longer dosing duration on the incidence and severity of liver effects in mice is unknown. However, similar to the current draft assessment, the 2018 EPA draft assessment for GenX chemicals did not use the chronic bioassay in rats for the derivation of a chronic RfD and justified using a UF of 3 to account for extrapolation from a subchronic to a chronic exposure duration. In part, the justification for a UF was that the 2-year study identified a NOAEL based on liver effects (increased liver enzyme levels and centrilobular hepatocellular hypertrophy and cystic focal degeneration in males and centrilobular necrosis in both sexes), that were consistent with the liver effects observed in the oral reproductive/ developmental study in mice used to derive the RfDs (DuPont-18405-1037, 2010). Further, the lack of a chronic bioassay for GenX chemicals evaluating cancer in mice is also considered a database deficiency, and in part, is accounted for in the proposed UF of 10 for database uncertainty (see response to charge question 3). Application of a UF of 3 to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD appears appropriate.

5. **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 table on page 8 (document page #34) is identified as "Table X" – numbering sequentially, this should be Table 5. If this change is made, all subsequent tables are misnumbered.

Page 80-81 (document pages 96-97) – mode of action discussion. The proposed PPARα MOA pertains to liver tumors, as an MOA for pancreatic acinar tumors has not been proposed. To clarify that the proposed PPARα MOA refers to liver tumors, paragraphs on pages 80 and 81 that pertain to PPARα could reference the tissue (presumably liver) that the data is describing.

COMMENTS SUBMITTED BY

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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

Several literature searches and search updates were performed of submitted DuPont/Chemours studies and of publicly-available scientific published studies between July 2017-March 2020 on this topic. Inclusion criteria of retrieved studies were conducted in accordance with PECO criteria for systematic reviews. The references studies appear to be complete; I am not aware of any other available literature that may be pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals.

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**

      i. **If so, please explain your reasoning.**

Following the application of PECO inclusion criteria to the retrieved studies, there remain limited dose-response data for GenX chemicals (11 animal studies, all via the oral route; no human studies), from which mainly hepatic, hematologic, reproductive/developmental, renal, and immune effects were evaluated. These available data (all scored to be high quality) provide evidence that hepatic changes, as seen in both male and female mice and rats, at varying doses (0.5-1000 mg/kg/day), and of varying durations of exposure (15 days to 2 years), appear to be the most sensitive adverse health effects from GenX oral exposure. Hepatic damage included increased liver enzymes, increased liver weight, and the increase in a constellation of liver lesions by pathology (cytoplasmic alteration, single-cell necrosis, focal necrosis, and hepatocellular apoptosis) from several studies. From these, the DuPont-18405-1037, 2010 study and its pathologic demonstration of liver lesions (as observed among female mice) were selected for the derivation of the subchronic and chronic RfDs for GenX chemicals. I agree that this study appears to provide the best available

evidence of the most sensitive adverse effects, and its selection as the critical study for the purposes of RfD derivation is scientifically justified.

ii. If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.

Not applicable.

iii. Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.

There does not appear to be sufficient evidence from the available GenX studies to support other adverse health effects that would be more sensitive and/or provide more robust data supporting its RfD derivations.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

The concern of adverse reproductive/developmental effects stems from 4 mice and rat dose-response studies (2 from DuPont/Chemours and 2 from the published scientific literature; all deemed high quality). Adverse effects from these studies include decreased pup weights, delays in the attainment of balanopreputial separation and vaginal patency, increased premature birth, decreased fetal weight, decreased gravid uterine weight, both increased/decreased gestational weight gain, decreased maternal serum total T3 and T4 levels, evidence of reduced body and tissue weights in F1 animals, increased abnormal placental lesions, decreased pup survival, and increased number of litters with a 14^{th} rudimentary rib. These statistically significant findings support the uncertainty of previously unrecognized potential reproductive/developmental effects due to GenX chemical exposure, although the clinical significance of these may be less clear. Nonetheless, I agree that this new information justifies the increase of the database uncertainty factor.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

The selection of the revised uncertainty factor (10 for potential reproductive/developmental risks, increased from 3 previously) is not my area of expertise, and I defer to the other reviewers. However, this selection was reported to take into account variability in the human population, database uncertainties, and possible differences in the ways in which humans and rodents respond to HFPO dimer acid and/or its ammonium salt that reaches their tissues. Although selected RfDs were also based on adverse hepatic effects observed in parental females, thus expected to also account for adverse effects to their offspring population, developing offspring may be even more sensitive to the adverse effects of toxicant exposures.

4. **EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.**

   The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

   Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

   a. **Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?**

Selection of uncertainty factors is not my area of expertise; I defer to the other reviewers.

   b. **Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.**

Selection of uncertainty factors is not my area of expertise; I defer to the other reviewers.

5. 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.

> I would suggest amending to the following syntax (additions shown in **bold**), when referring to the data supporting the overall RfD, throughout the document for improved readability:

Example as shown in Section 7.4 (page 97): "The oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and **its pathologic demonstration of** liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the critical study and **organ** effects, **respectively**, for deriving the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt."

> In Table 11 regarding the Conley 2019 study, suggest inserting: "decreased maternal **serum** total T4 levels…" and clarifying "indications of reduced body (females) and **reproductive and non-reproductive** organ weights in F1 animals".

> In Table 11, recommend adding in the significant changes in serum total T3 levels observed from the 3 relevant studies, as well as the findings of decreased pup survival and increased number of litters with a 14^{th} rudimentary rib, for completeness.

COMMENTS SUBMITTED BY

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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

Response: I am not aware of any recent literature which was not identified in the Toxicity Assessment document.

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**

      i. **If so, please explain your reasoning.**

      ii. **If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.**

      iii. **Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.**

Response: The selection of DuPont-18405-1037 (2010), the oral reproductive/developmental toxicity study in mice, as the critical study for derivation of the RfDs is thoroughly described and justified in the document, along with detailed review of other candidate studies and various endpoints. The endpoint chosen in the critical study appears consistently in other similar studies, but the critical study selected is the most sensitive, following standard risk assessment guidelines. Other possible endpoints are either less sensitive, of uncertain biological significance, or do not show consistent dose response and time dependence. The duration of the critical study is directly appropriate for determination of the subchronic RfD, but for the

chronic RfD the only chronic study available is in the rat, which is shown in the subchronic studies to be less sensitive to the effects of GenX chemicals than the mouse. Use of DuPont-18405-1037 (2010) as the critical study for the chronic RfD, with the appropriate subchronic-to chronic uncertainty factor, is therefore justified.

3. **EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.**

   EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

   a. **Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.**
   b. **Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.**

Response: The additional recently published information indicates that there is considerable uncertainty as to the developmental impact of the GenX chemicals, as described and explained in the document. These findings are of particular concern since the database does not include a full multigenerational study. The increased database uncertainty factor is therefore justified.

4. **EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.**

   The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

   Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of

dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?

b. Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment?

Response: The increased subchronic to chronic uncertainty factor is consistent with risk assessment guidelines, which allow for a value of either 3 or 10 for this UF, depending on the uncertainty implied by this extrapolation. Selection of an appropriate value for UF_S is based on the nature of the critical effect and any toxicological, toxicokinetic or mechanistic evidence that has bearing on the likely timescale for appearance of that effect. Selection of a value of 10 for UF_S is justified by the considerations laid out in the document. There is a difference in timescale for appearance of necrosis in the liver between mice and rats, and there is no chronic study in mice. The new pathology analysis by NTP highlights the significance of this timescale difference.

5. 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.

Response: The only additional comments I have are of a minor editorial nature:

a. There are some broken links in the list of Figures (page v) and Tables (page vii).

b. In Table 12 (page 90) there are problems with the column widths causing badly placed line breaks in columns 5, 6 and 7. While the actual information is intact, this impairs the readability.

COMMENTS SUBMITTED BY

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University of Rhode Island
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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. **Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.**

Response:

I am not aware of any additional studies to include. The most recent PubMed search I performed a PubMed was on May 20, 2021 and did not retrieve any additional publications that would quantitatively impact the RfDs.

Overall, 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 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 importance used for weighting the criteria was appropriate. Overall, this semi-quantitative approach in evaluating the data/studies is considered appropriate and thorough.

2. **In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.**

   In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

   The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

   a. **Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?**

      i. **If so, please explain your reasoning.**

Response:

The scientific justification for the selection of DuPont-18405-1037, 2010 for the derivation of subchronic and chronic RfDs for GenX is based on a search that yielded 75 studies as of March 2020. Of those 75 studies, rigorous criteria and metrics were applied to weight each study for quality of the study and ultimately whether the study was able to demonstrate a change in health outcome, if the outcome was more likely than not attributable to test article exposure, and the dose at which the change was observed. From these, ten studies in rats or mice were identified to determine NOAEL and LOAELs, with mice being a more sensitive species. Of these studies both sub-chronic and chronic studies, four describe liver effects, that include increased liver weight, single-cell necrosis, and cytoplasmic alterations. The DuPont-18405-1037, 2010 study meets the criteria listed in almost all elements for being considered of high quality. In addition, livers from this study were re-analyzed by a panel of eight NTP pathologists (NTP PWG, 2019) and concluded that the NOAEL in the F0 generation was 0.1 mg/kg and 0.5 mg/kg was the LOEAL. The study meets every metric as high or medium, such as test substance, test setup, exposure characterization, etc. The critical effect of single cell necrosis is based on a large n=24-25. The selection of this study is scientifically justifiable based on it sufficiently meeting the review criteria. The selection of liver weight and cytotoxicity is a reasonable measure to use as a critical effect and meets the Hall criteria. This measure has been used previously for other perfluoroalkyl substances, such as PFOA and PFOS, in which rodent studies that have demonstrated hepatotoxicity in rodents is concordant with studies in human populations that describe adverse liver effects, such as increased serum ALT and AST enzyme activity. In addition, the DuPont-18405-1037, 2010 describes developmental effects to the F1 generation (i.e. decreased birth weight) at a NOAEL of 0.5 mg/kg and LOAEL of 5 mg/kg.

ii. If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.

Response:

I do not disagree with the selected critical study. Other studies included have similar limitations, like test article purity – which is either low (84%) or not described.

iii. Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.

Response:

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 higher, this study should could be considered an alternative to DuPont 18405-1037, 2010 for considering 0.1 mg/kg in male mice for the RfD.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of

developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

Response:

Yes, I do support this because additional evidence has been published since the 2018 review that indicate developmental and reproductive effects. The studies indicate that HFPO dimer acid can pass the placenta, accumulate in the fetus, and also cause histological changes to the placenta. The Blake et al., 2020 publication had similar findings to the DuPont-18405-1037, 2010 study. Blake et al., 2020 demonstrated maternal GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at gestational day 17.5. Since 2018, Blake et al., 2020 also demonstrated that Blake et al. (2020) demonstrated that dosing or 2 or 10 mg/kg/day of HFPO dimer acid to pregnant dams resulted in measurable HFPO dimer acid in amniotic fluid and whole embryos at Embryonic day 11.5 (E11.5) and 17.5 (E17.5). Conley et al., 2019 also demonstrated transfer of HFPO dimer acid to the fetus in rats. Conley et al., 2021 demonstrated transfer of HFPO dimer acid from dam to pup in pregnant rats exposed from GD8 through PND2. Lastly, Blake et al, 2020, described increased placental lesions.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

Response:

Yes, this provided rationale supports the application of an uncertainty factor of 10.

4. EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?

Response:

Yes. The use of uncertainty factors is consistent with EPA guidance (USEPA, 2011b).

Given that there are no published chronic studies in mice, but mice are a more sensitive species, it is reasonable to assume that a longer duration would result in a magnitude of response or a lower LOAEL dose.

b. Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.

Response:

I agree with the rationale provided and the use of UF 10 for subchronic to chronic.

5. 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.

Response:

The document cites studies by Cannon et al. that investigate whether HFPO Dimer Acid is a substrate for BCRP. The study cited used a vesicle-based ATPase assay. These assays have a limitation that they can produce false negatives, especially with drugs/chemicals that are permeable. So, it should be acknowledged that only in this assay it was not considered to be a substrate. The significance of this relates to the notion that GenX has reproductive effects and causes placental lesions. BCRP is highly enriched in placenta and is a potential transport mechanism that could explain GenX effects in placenta and the mechanism by which HFPO dimer acid accumulates in fetuses with exposure to the dams. For that reason, it is discouraged to make the assertion that BCRP does not transport HFPO dimer acid.

COMMENTS SUBMITTED BY

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Additional Focused External Peer Review of EPA's Draft Human Health Toxicity Assessment for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3) Also Known as "GenX Chemicals"

1. Are you aware of any recent literature pertinent to the derivation of subchronic and chronic RfDs for GenX chemicals that is not identified in this document? If so, please provide citations along with a justification for why the studies might quantitatively impact the calculation of the RfDs.

It appears that the six publications listed below only became available, even electronically, after the last literature search update on March 3, 2020. Even so, the authors of the GenX toxicity assessment were likely aware of their existence before finalization and distribution of the draft for external peer review (e.g., Conley et al. (2021) is referred to on p. 94, yet does not appear in the reference list). As such, I hesitate to mention them, though all are informative in one way or another. Of the six, there is one *in vivo* (a) and two *in vitro* (b, c) toxicity studies, two studies that address the occurrence of PFAS in North Carolina (d, e) and an informative review (f). Conley et al. is not a viable candidate for principal study with its relatively high doses and short exposure durations. However, like the two *in vitro* studies and Blake and Fenton review, it increases concern for developmental effects yet to be fully characterized, and in so doing, lends support for a full database uncertainty factor (UF_D) of 10. Interestingly, despite detecting elevated levels of several legacy PFAS in the blood of Wilmington, NC residents, Kotlarz et al. failed to detect GenX above analytical reporting limits.

a. Conley JM, Lambright CS, Evans N, et al. Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. https://doi.org/10.1016/j.envint.2020.106204.

b. Coperchini F, Croce L, Denegri M, et al. Adverse effects of in vitro GenX exposure on rat thyroid cell viability, DNA integrity and thyroid-related gene expression. https://doi.org/10.1016/j.envpol.2020.114778.

c. Bangma J, Szilagyi J, Blake, BE, et al. An assessment of serum-dependent impacts on intracellular accumulation and genomic response of per- and polyfluoroalkyl substances in a placental trophoblast model. https://doi.org/10.1002/tox.23004.

d. Petre M-A, Genereux DP, Koropeckyi-Cox L, et al. Per- and Polyfluoroalkyl Substance (PFAS) Transport from Groundwater to Streams near a PFAS Manufacturing Facility in North Carolina, USA. https://doi.org/10.1021/acs.est.0c07978.

e. Kotlarz N, McCord J, Collier D, et al. Measurement of Novel, Drinking Water-Associated PFAS in Blood from Adults and Children in Wilmington, North Carolina. https://doi.org/10.1289/EHP6837.

f. Blake, RE and Fenton SE. Early life exposure to per- and polyfluoroalkyl substances (PFAS) and latent health outcomes: A review including the placenta as a target tissue and possible driver of peri- and postnatal effects. https://doi.org/10.1016/j.tox.2020.152565.

2. In the draft toxicity assessment that was peer reviewed in 2018, EPA derived subchronic and chronic RfDs. The critical study chosen for determining these values was the oral reproductive/developmental toxicity screening study in adult mice (DuPont-18405-1037, 2010) and the critical effect was liver

effects (single cell necrosis) in adult males. Overall, the peer review affirmed selection of this study and effect as the basis for the derivation of the RfDs.

In this updated assessment, candidate subchronic and chronic RfDs were calculated for GenX chemicals based on the NTP PWG review of the same liver pathology slides from the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Candidate RfDs were developed based on liver effects identified by the NTP PWG as a constellation of lesions (cytoplasmic alteration, hepatocellular single cell and focal necrosis, and hepatocellular apoptosis) in parental males and females.

The candidate RfDs derived from the oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the subchronic and chronic RfDs for GenX chemicals. The RfDs based on this grouping of effects are the most health-protective of the modeled endpoints.

a. Is the selection of the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010) for the derivation of the subchronic and chronic RfDs for GenX chemicals scientifically justified and clearly described?

I. If so, please explain your reasoning.

II. If you disagree with the selected critical study and effect, please provide your rationale and identify an alternative key study to support the derivation of the subchronic and chronic RfDs and provide the scientific support for the alternative choice.

III. Should any other studies or effects be considered for the derivation of subchronic and chronic RfDs for GenX chemicals? Please provide the scientific support for any other choices.

Selection of Dupont-18405-1037, 2010 for RfD derivation is scientifically justified and clearly described in the current toxicity assessment, as it was in 2018. Confidence in the study is only increased by the NTP PWG's review of liver pathology, as it is essentially confirmatory of the original findings. Also, despite an adverse response (i.e., critical effect) being defined by a constellation of lesions rather than a single one, the resulting POD_{HED} changed very little from 2018 (i.e., it was reduced from 0.023 to 0.01 mg/kg/day). It is also noteworthy that the reduction in POD_{HED} remained minor despite the change from male to female mice as its basis. Though I support the use of Dupont's reproductive/developmental study for RfD derivation, the new definition of adversity raises an issue for dose-response modeling. Based on the slide review worksheets in Appendix E, several mice were diagnosed by the NTP PWG with varying degrees of cytoplasmic alteration only, or in some cases, cytoplasmic alteration accompanied by mixed cell infiltration. In the absence of necrosis or apoptosis, it would seem appropriate to consider these diagnoses as adaptive and non-adverse (as was done in Table 11 where 0.5 mg/kg/day was determined to be a NOAEL in Dupont's 90-day mouse study, despite cytoplasmic alteration in 10/10 males). However, the dose-response data modeled in Appendix F suggest otherwise (e.g., 24/24 female mice in the high-dose group were selected for dose-response modeling, yet animal numbers 5027, 5033 and 5035 were diagnosed with mild cytoplasmic alteration and nothing more). Shouldn't the absence of hepatocellular necrosis or apoptosis disqualify an animal from inclusion in dose-response modeling? Admittedly, as the slide review worksheets do not indicate which animals were in a given dose group, this question may be for naught.

3. EPA applied a database uncertainty factor of 3 to derive the RfDs in the toxicity assessment that was peer reviewed in 2018 based on uncertainty due to a lack of epidemiological studies, limited testing of developmental toxicity and immunological responses, and inconsistent hematological effects observed in many of the studies. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

EPA has identified new toxicological and toxicokinetic information published since the last peer review of this document that demonstrate accumulation of GenX chemicals in the whole embryo and identified additional adverse effects that EPA had not considered in applying a database uncertainty factor of 3. Based on this new information, EPA has increased the uncertainty factor to 10 to address database limitations on the impact of GenX chemicals exposure specifically on reproduction and development.

a. Do you agree that this new information increases uncertainty regarding GenX reproductive and developmental effects and that this justifies an increase in the database uncertainty factor? If not, how should EPA account for this new information in the assessment.

b. Does the provided scientific rationale support the application of the selected uncertainty factors? If not, please explain.

Yes, I agree that additions to the GenX database following development of the original toxicity assessment, including the Conley et al. publication listed in my response to question no. 1, warrant an increase in the database uncertainty factor (UF_D). Clearly, there is a laundry list of emerging concerns for toxicities not fully characterized, reproductive/developmental and endocrine chief among them. In addition, the epidemiological data for GenX severely lag that of legacy PFAS, and the GenX toxicity profile to date, bears an eerie resemblance to that of PFOA, qualitatively and quantitatively. Pages 91-93 of the toxicity assessment are very effective at providing the scientific rationale for an increase in the UF_D. However, an increase from 3 to 10 in the UF_D becomes, in my opinion, problematic when coupled with an increase of the same magnitude in the subchronic-to-chronic uncertainty factor (UF_S). While the use of a UF_S in chronic RfD derivation is justified, a factor of 10 seems excessive given that uncertainty over exposure duration (including the absence of a chronic mouse study) is partially accounted for by maximizing the UF_D. That the UF_D and UF_S can overlap and address the same uncertainty over exposure duration is actually acknowledged in the toxicity assessment (see last paragraph of p. 94). In addition, the UF_D was increased to account for the lack of chronic studies in the recent derivation of toxicity values for the PFOA replacement, PFBS. Furthermore, maintaining both the UF_D and UF_S at 10 in the revamped toxicity assessment results in a chronic RfD of 0.003 µg/kg/day, a value nearly an order of magnitude below the RfD used to set lifetime drinking water health advisories for PFOA and PFOS (i.e., 0.02 µg/kg/day). Lastly, the changes in composite uncertainty factors (UF_C) from the original toxicity assessment are 3- (100 to 300) and 10-fold (300 to 3000) in the case of the subchronic and chronic RfDs, respectively. This, coupled with a slight decrease in POD_{HED}, translates into 7- and 27-fold decreases in the subchronic and chronic RfDs from those in the original toxicity assessment. While acknowledging the need for health conservative toxicity values in the face of uncertainty, a UF_C of 3000 is an extreme application of the precautionary principle. As it stands, I support the UF_C of 300 for subchronic RfD derivation, but suggest a reduction in the UF_S from 10 back to 3 (for a UF_C of 1000) before derivation of a chronic RfD. While slightly less health conservative, such a reduction in UF_S still results in a chronic RfD of 0.01 µg/kg/day, a toxicity value one-half that of PFOA and PFOS.

4. EPA applied an uncertainty factor of 3 to account for extrapolating from a subchronic to chronic exposure duration to derive the chronic RfD in the toxicity assessment that was peer reviewed in 2018. This uncertainty factor accounted for the dosing of parental males (84–85 days) falling short of a standard subchronic study and below the duration of a chronic study. It was concluded that because the NOAELs for the oral reproductive/developmental toxicity study in mice and the chronic rat study were within one order of magnitude of each other, that there was consistency in dose-response relationships between these studies. This rationale for designating a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. The peer review affirmed application of this uncertainty factor for the derivation of the RfDs.

The reanalysis of pathology slides by the NTP identified a constellation of liver effects in both parental males and females. The dose response in the females provided the most health protective point of departure between the two sexes and was selected for derivation of the RfDs. Females were dosed for a shorter duration (a total of 53 to 64 days) in the critical study as compared to the male mice (84-85 days). 53-64 days falls well below the standard subchronic or chronic study.

Because a 2-year chronic mouse study is unavailable, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because the new analysis by NTP indicates that the duration of exposure appears to play a larger role than previously understood in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, female rats do not exhibit liver lesions until after two years of dosing and these liver lesions progressed into liver tumors. The mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat, thus a 2-year chronic study in the mouse would provide information critical to understand the progression of these liver effects. Specifically, it is possible that a longer duration study would result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies.

a. Given the evidence provided above and EPA's guidance on selection of uncertainty factors (USEPA, 2002), is the subchronic to chronic uncertainty factor of 10 appropriate to account for extrapolation from a subchronic to a chronic exposure duration?

b. Do you agree that the rationale provided here and in the assessment for a subchronic to chronic uncertainty factor of 10 is justified? If not, how should EPA account for this new analysis in the assessment.

See my response to question no. 3 above.

5. 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.

a. Page E-56: I believe Project 18405-1307 Females should read Project 18405-1037 Females. The heading, Project 18405-1307 Females, is used previously on page E-34.

b. Tables F-1 and F-3: The column headings should read Constellation of Lesions rather than Incidence of combined necrosis.

c. Last sentence, page 42: This information on NOAEL/LOAEL is confusing without knowledge as to what the NTP PWG considers adverse. As such, similar or identical language to that at the very bottom of page 85 should be included near the bottom of page 42, if not earlier in the text.

d. Last paragraph, page 58: Change deceased to decreased.

e. Table of Contents, page vii: Tables F-5 and F-6 dealing with placental lesions are not included in the toxicity assessment.

f. Table 3, footnotes, page. 15: Singularize measurements.

g. Table 9, footnotes, page 43: Change misdoing to misdosing.

h. Page 85, 6th line from the bottom: The sentence beginning with The NTP should be corrected.

i. Page 85, 7th line from the bottom: Insert ",less cytoplasmic alteration in males," between the closed parentheses and were.

j. Page 90, 2nd line of text: Unless I'm missing part of the toxicity assessment, candidate RfDs were not calculated based on Blake et al. (2020).

k. Page 91, last line of text: Pluralize rat.

l. Page 102, 4th line of text: Insert of after levels.