

EPA Response to Public Comments

on

Draft Human Health Toxicity Values for

**Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its
Ammonium Salt (CASRN 13252-13-6 and
CASRN 62037-80-3)**

Also Known as “GenX Chemicals”

(Docket ID No. EPA-HQ-OW-2018-0614)

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CONTENTS

ACRONYMS AND ABBREVIATIONS	iii
INTRODUCTION	1
LIST OF COMMENTERS	2
1 TECHNICAL COMMENTS	4
1.1 CHOICE OF CRITICAL STUDY AND EFFECT	4
1.2 ALLOMETRIC SCALING OF DOSES BETWEEN TEST SPECIES	18
1.3 UNCERTAINTY AND DATA QUALITY	21
1.3.1 Uncertainty Factors—Database Uncertainty	21
1.3.2 Uncertainty Factors—Subchronic-to-Chronic Extrapolation	24
1.3.3 Uncertainty Factors—Interspecies Uncertainty	27
1.3.4 Uncertainty Factors—Total Uncertainty	28
1.3.5 Uncertainty Factors—Consideration of Other PFAS Data in Assigning Uncertainty	29
1.3.6 Quality of the Data Used in the Assessment	31
1.4 MODE OF ACTION (MOA)—PPAR α	33
1.5 LITERATURE SEARCH AND SCREENING	37
1.5.1 Identification of New Literature	37
1.5.2 Systematic Literature Review	37
1.6 PFAS USES AND TSCA	41
2 POLICY QUESTIONS	43
2.1 POTENTIAL RISK MANAGEMENT AND REGULATORY APPROACHES	43
2.2 ASSESSMENT/REGULATION OF PFAS AS A GROUP OR AS A MIXTURE	46
2.3 RISK COMMUNICATION	49
2.4 IMPLEMENTATION TOOLS	53
3 GENERAL	54
3.1 GENERAL SUPPORT OF THE TOXICITY ASSESSMENT	54
4 PUBLIC COMMENT PERIOD	54
4.1 REQUEST FOR EXTENSION	54
5 REFERENCES	55

ACRONYMS AND ABBREVIATIONS

A/G	albumin/globulin
ACC	American Chemistry Council
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ARAR	Applicable or Relevant Requirement
ASDWA	Association of State Drinking Water Administrators
AST	aspartate aminotransferase
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
AWWA	American Water Works Association
BMD	benchmark dose
BMDL	benchmark dose lower limit, which is the 95% lower bound of the BMD
BMDL ₁₀	lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level
BW	body weight
CASRN	Chemical Abstracts Service Registry Number
CDC	Centers for Disease Control and Prevention
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
DAF	dosimetric adjustment factor
DoD	U.S. Department of Defense
DOE	U.S. Department of Energy
E	embryonic day
ECOS	Environmental Council of the States
EPA	U.S. Environmental Protection Agency
EPN	Environmental Protection Network
EWG	Environmental Working Group
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
GWG	gestational weight gain
H&E	hematoxylin and eosin
HA	Health Advisory
HAL	Health Advisory Level
HAWC	Health Assessment Workspace Collaborative
HED	human equivalent dose
HERO	Health & Environmental Research Online
HFPO	hexafluoropropylene oxide

HFPO dimer acid	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy) propanoate
HFPO-TeA	HFPO tetramer acid
HFPO-TrA	HFPO trimer acid
HHS	U.S. Department of Health and Human Services
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
IRIS	Integrated Risk Information System
K ⁺ PFBS	potassium perfluorobutane sulfonate
LOAEL	lowest observed adverse effect level
MCL	Maximum Contaminant Level
MCLG	Maximum Contaminant Level Goal
MDEQ	Michigan Department of Environmental Quality
MDHHS	Michigan Department of Health and Human Services
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
MOA	mode of action
MRL	Minimum Risk Level
NASA	National Aeronautics and Space Administration
NAWC	National Association of Water Companies
ng/mL	nanograms per milliliter
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NJDEP	New Jersey Department of Environmental Protection
NOAEL	no observed adverse effect level
NTP	National Toxicology Program
NTTC	National Toxics Tribal Council
NYSDEC	New York State Department of Environmental Conservation
NYSDOH	New York State Department of Health
OECD	Organization for Economic Cooperation and Development
OMB	Office of Management and Budget
OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
PADEP	Pennsylvania Department of Environmental Protection
PADOH	Pennsylvania Department of Health
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PECO	population, exposure, comparator, and outcome
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonic acid

PFDA	perfluorodecanoic acid
PFH _x A	perfluorohexanoic acid
PFH _x S	perfluorohexane sulfonic acid
PFMOAA	2,2-difluoro-2-(trifluoromethoxy)-acetic acid
PFNA	perfluorononanoic acid
PFO2H _x A	2-[difluoro(trifluoromethoxy)methoxy]-2,2-difluoroacetic acid
PFO3OA	2-[[difluoro(trifluoromethoxy)methoxy]difluoromethoxy]-2,2-difluoro-acetic acid
PFO4DA	3,5,7,9-tetraoxadecanoic perfluoro acid
PFO5DoDA	perfluoro-3,5,7,9,11-pentaoxadodecanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PMN	premanufacture notice
PND	postnatal day
POD	point of departure
POD _{HED}	point of departure human equivalent dose
PPAR	peroxisome proliferator-activated receptor
PPAR α	peroxisome proliferator-activated receptor alpha
PPAR γ	peroxisome proliferator-activated receptor gamma
PWG	Pathology Working Group
RfD	reference dose
RIVM	National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
SAB	Science Advisory Board
SDWA	Safe Drinking Water Act
SOT	Society of Toxicology
T4	thyroxine
TDAR	T cell-dependent antibody response
TEDX	The Endocrine Disruption Exchange
TG	Test Guideline
TSCA	Toxic Substances Control Act
TURI	Toxics Use Reduction Institute
UF	uncertainty factor
UF _A	interspecies uncertainty factor
UF _D	database uncertainty factor
UF _H	intraspecies uncertainty factor
UF _S	extrapolation from subchronic to a chronic exposure duration uncertainty factor
UF _{TOT}	total uncertainty factor
USGS	U.S. Geological Survey
VA	U.S. Department of Veterans Affairs

INTRODUCTION

On November 21, 2018, the U.S. Environmental Protection Agency (EPA) issued draft subchronic and chronic oral toxicity values (i.e., reference doses, or RfDs) as part of its toxicity assessment document for 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6)—or hexafluoropropylene oxide (HFPO) dimer acid—and ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt—also known as “GenX chemicals,” for public comment (83 FR 58768). The assessment draft document provided the available health effects information that forms the basis for deriving oral RfDs for subchronic and chronic durations for GenX chemicals. When finalized, the toxicity values, along with specific exposure and other relevant information, can be used, under the appropriate regulations and statutes, by EPA, states, tribes, and local communities to determine and address potential risk associated with human exposures to these chemicals.

EPA accepted public comments on the draft assessment for 60 days, from November 21, 2018 to January 22, 2019. EPA considered the public comments that were received during the finalization of the GenX chemicals toxicity assessment document and prepared the responses to those public comments (provided in this document). The sections that follow provide summaries of the comments received in the Public Docket EPA-HQ-OW-2018-0614 and EPA’s responses, which are organized into categories based on scientific points. Some of the comments received suggested changes that would require expanding the scope of the assessment (e.g., assessment of cumulative per- and polyfluoroalkyl substances (PFAS) risk, evaluation of inhalation or dermal routes of exposure, and development of drinking water regulations), which was not feasible and therefore, these comments were not addressed.

LIST OF COMMENTERS

Comment number	Author / Organization	Docket identifier
Not applicable; comments submitted by this author were relevant to the EPA public comment review document for human health toxicity values for perfluorobutane sulfonic acid (PFBS) and related compound potassium perfluorobutane sulfonate (K+PFBS)	Bruce Allen, Independent Consultant to 3M Company	EPA-HQ-OW-2018-0614-0021
1.1.g, 1.1.j, 1.1.k, 1.2.a, 1.3.1.a, 1.3.1.c, 1.5.2.c, 1.5.2.d, 4.1	American Chemistry Council, Chemical Products and Technology Division	EPA-HQ-OW-2018-0614-0011, EPA-HQ-OW-2018-0614-0032
1.4.a, 2.3.e, 2.3.f, 2.3.g, 2.3.h, 3.1	American Water Works Association; National Association of Water Companies	EPA-HQ-OW-2018-0614-0026
1.1.b, 2.1.a, 2.1.c, 2.1.e, 2.2.b, 2.3.b	Anonymous Citizens	EPA-HQ-OW-2018-0614-0006, -0007, -0008, -0012, -0013, -0014, -0015, -0016, -0027, -0029
4.1	Arnold & Porter, Legal Counsel to Chemours Company	EPA-HQ-OW-2018-0614-0030
2.1.d, 2.2.g, 2.4.c	Association of State Drinking Water Administrators	EPA-HQ-OW-2018-0614-0022
2.1.e, 2.2.e, 2.4.a, 3.1	Cape Fear Public Utility Authority	EPA-HQ-OW-2018-0614-0009
1.1.c, 1.1.d, 1.1.e, 1.2.a, 1.3.1.a, 1.3.1.b, 1.3.4.a, 1.3.5.b, 1.3.5.c, 1.3.6.a, 1.5.1.a, 1.5.2.a, 1.5.2.e, 1.5.2.f, 1.5.2.g, 2.1.c, 2.2.a, 2.2.c, 2.2.e, 2.3.a	The Endocrine Disruption Exchange; Natural Resources Defense Council; Sierra Club; Environmental Working Group; Center for Environmental Health	EPA-HQ-OW-2018-0614-0038
1.5.2.a, 2.1.a, 2.1.b	Environmental Protection Network	EPA-HQ-OW-2018-0614-0018, EPA-HQ-OW-2018-0614-0041
1.1.a, 1.3.1.a, 1.3.5.a, 1.6.b, 2.2.a, 2.2.c, 2.2.d, 2.3.b, 2.3.c	Environmental Working Group	EPA-HQ-OW-2018-0614-0024
1.1.h, 1.1.j, 1.1.k, 1.1.l, 1.1.m, 1.2.b, 1.3.2.a, 1.4.d	Green Toxicology LLC	EPA-HQ-OW-2018-0614-0037
Not applicable; comments submitted by this author were relevant to the EPA public comment review document for human health toxicity values for PFBS and related compound K+PFBS	Dr. Wendy Heiger-Bernays, Boston University School of Public Health	EPA-HQ-OW-2018-0614-0039
1.1.j, 1.1.k, 1.1.m, 1.4.b, 1.4.c, 1.4.h, 4.1	Dr. James Klaunig, Indiana University, Consultant to Chemours Company	EPA-HQ-OW-2018-0614-0034; follow-up comment submitted after January 22, 2019 (no identifier)
Not applicable; no specific comments on GenX provided	Paul Lutton	EPA-HQ-OW-2018-0614-0028

Comment number	Author / Organization	Docket identifier
Not applicable; comments submitted by this author were relevant to the EPA public comment review document for human health toxicity values for PFBS and related compound K ⁺ PFBS	Massachusetts Department of Environmental Protection	EPA-HQ-OW-2018-0614-0033
1.1.n, 1.2.a, 1.3.1.a, 1.3.2.a, 1.3.3.a, 1.3.6.a, 2.1.a, 3.1	Michigan Department of Environmental Quality; Michigan Department of Health and Human Services	EPA-HQ-OW-2018-0614-0025
Not applicable; comments submitted by this author were relevant to the EPA public comment review document for human health toxicity values for PFBS and related compound K ⁺ PFBS	Minnesota Department of Health	EPA-HQ-OW-2018-0614-0019
4.1	Natural Resources Defense Council; The Endocrine Disruption Exchange; Sierra Club; Environmental Working Group; Clean Water Action/ Clean Water Fund; Alaska Community Action on Toxics; Safer States	EPA-HQ-OW-2018-0614-0010
1.1.c, 1.1.e, 1.1.g, 1.1.i, 1.1.n, 1.2.a, 1.3.2.a, 1.3.3.a, 1.3.6.b, 1.4.d, 1.4.e, 1.4.f, 1.5.2.a, 1.6.d, 2.3.d	New Jersey Department of Environmental Protection	EPA-HQ-OW-2018-0614-0020
2.2.f	New York State Department of Environmental Conservation	EPA-HQ-OW-2018-0614-0017
1.1.e, 1.2.a	New York State Department of Health	EPA-HQ-OW-2018-0614-0031
1.1.e, 1.3.1.a, 2.2.c, 2.2.h, 2.3.f, 2.4.b, 3.1	Pennsylvania Department of Environmental Protection; Pennsylvania Department of Health	EPA-HQ-OW-2018-0614-0023
1.1.f, 1.1.h, 1.1.k, 1.4.d	Dr. Damian Shea, North Carolina State University, Researcher Funded by Chemours Company	EPA-HQ-OW-2018-0614-0035
1.2.c, 1.3.3.a, 1.6.a, 2.1.c, 2.1.e, 2.2.c	Silent Spring Institute	EPA-HQ-OW-2018-0614-0040
1.5.2.b, 1.6.c, 2.1.c, 2.1.e, 2.3.a	Toxics Use Reduction Institute, University of Massachusetts-Lowell	EPA-HQ-OW-2018-0614-0042
1.1.j, 1.1.k, 1.4.g, 1.4.h	ToxStrategies, Inc., Consultant to Chemours Company	EPA-HQ-OW-2018-0614-0036; follow-up comment submitted after January 22, 2019 (no identifier)

1 TECHNICAL COMMENTS

1.1 CHOICE OF CRITICAL STUDY AND EFFECT

1.1.a Comment: The Environmental Working Group (EWG) asked EPA to use human exposure and epidemiological evidence to develop the toxicity assessment for GenX. EWG mentioned that EPA noted in the draft assessment that human epidemiological studies were inadequate for use due to likelihood that humans are exposed to multiple PFAS. They noted, however, that numerous studies have found health impacts at levels of GenX detected both in contaminated communities and in the general population and that the reliance on animal testing for setting a safe exposure level for PFAS compounds is not adequately protective of health.

EPA Response: The GenX toxicity assessment document incorporates the available studies for GenX chemicals. There are no epidemiological studies available that evaluate health effects following exposure to GenX chemicals. The available biomonitoring studies, which were published after the draft was released for public comment, have been added to section 1.3, and the one available human half-life study is summarized in section 8.4 of the assessment (EPA, 2021a).

1.1.b Comment: An anonymous citizen noted that the potential for GenX to be immunotoxic should not be downplayed by a database that is characterized in the draft as weak according to criteria (i.e., not including sufficient measures of immunopathology, humoral immunity, cell-mediated immunity, nonspecific immunity, or host resistance) developed by the assessment authors because the database is not considered weak by agency (National Toxicology Program [NTP] and EPA) standards/guidelines. The citizen indicates that evidence for the biological plausibility of GenX to induce immunotoxicity is available in some of the toxicity studies from DuPont and from Rushing et al. (2017). The citizen further notes that the harmonized Health Effects Test Guidelines (TGs) for Immunotoxicity (OPPTS 870.7800) recommends a functional test to evaluate the response to an antigen and enumeration of splenic or peripheral blood T cells, B cells, or NK cells. Although immunotoxicity is a common health effect after PFAS exposure, adverse effects on immune system function and changes in early markers of immunotoxic effects have been associated with more well-studied PFAS; thus, EPA should not assume that there is no harm or effects when there are data gaps.

EPA Response: EPA agrees that immunotoxicity is an identified hazard for GenX chemicals. In section 5.5 (EPA, 2021a), EPA describes the data supporting immune system hazards. There is a single study in the published literature evaluating immune endpoints (Rushing et al., 2017). Additionally, DuPont conducted lymph node assays, but as pointed out in section 5.5, the results were equivocal. EPA considered immune endpoints such as T cell-dependent antibody response (TDAR) suppression as indicative of a potential hazard; however, these effects were observed at doses higher than the liver effects. Section 7.3 (EPA, 2021a) describes the database uncertainties, including immunotoxicity. In the final document, EPA has clarified that, while the database for immunotoxicity is incomplete, the available studies indicate potential immunotoxicity as a hazard.

1.1.c Comment: The Endocrine Disruption Exchange (TEDX), Natural Resources Defense Council, Sierra Club, EWG, Center for Environmental Health, and New Jersey Department of Environmental Protection (NJDEP) commented that exposure to GenX chemicals is linked to

adverse effects on the liver, kidney, immune system, and development, as well as cancer. Because these health effects have been associated with exposure to other PFAS (e.g., perfluorooctanoic acid (PFOA)), there is a need to account for simultaneous, cumulative exposure to multiple PFAS chemicals that impact the same target organs. The group notes that GenX chemicals and PFOA are associated with similar health effects at roughly comparable external dose levels. Given that a similar external dose would result in lower internal concentrations of GenX chemicals, it is possible that the toxicity of GenX on certain targets would be greater than PFOA.

EPA Response: EPA agrees that GenX chemicals are linked to adverse effects of the liver, kidney, and immune system; adverse development effects; and cancer, and that these health effects have been associated with other PFAS (e.g., PFOA). In this assessment, EPA assessed the human health hazards associated with exposure to only GenX chemicals and not with exposure to multiple PFAS.

EPA researchers are also applying computational and high-throughput toxicology tools for PFAS toxicity screening and testing on a larger scale to accelerate our understanding of potential toxicity for the large universe of PFAS, most of which have little or no published toxicity data. Assessments of human health hazards after exposure to other individual PFAS chemicals are also underway at EPA (EPA, 2020a). Thus, considerations of exposure to multiple PFAS as a class were not addressed in this assessment. However, future EPA actions on PFAS chemicals may include this cumulative assessment approach.

The commenter notes that exposure to GenX chemicals or PFOA are associated with similar health effects at roughly comparable external dose levels. Further, they assert that a similar external dose would result in lower internal concentrations of GenX chemicals, and it is possible that the toxicity of GenX on certain targets would be greater than PFOA. EPA recognizes that exposure to GenX chemicals can lead to adverse effects on the liver, kidney, and immune system; adverse developmental effects; and cancer, and that these health effects have also been associated with PFOA exposure. There are data available that demonstrate the toxicokinetic profiles differ between GenX chemicals and PFOA; GenX chemicals are more rapidly excreted than PFOA and appear not to bioaccumulate like PFOA. EPA agrees that these toxicokinetic findings would predict differences in internal dose after a similar administered dose of each of the chemicals (i.e., a similar dose could result in a lower internal dose for GenX chemicals compared with PFOA).

Since the release of the public comment draft (EPA, 2018a), two studies were published that compare the internal dose of HFPO dimer acid to the internal dose of PFOA (Blake et al., 2020) or PFOS (Conley et al., 2021). The results of these comparisons have been included in the final assessment in section 8.6 (EPA, 2021a).

1.1.d Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health supported EPA's overall critical review and analysis of the available studies but noted that delays in genital development after GenX chemical exposure should not be discounted. The group asked that EPA further clarify why it was reported that that no reproductive effects were associated with exposure to GenX chemicals, although there was a mention of 11 mating pairs not able to successfully produce litters in the DuPont 18405-1037 study. The Silent Spring Institute noted that the critical study used for developing the GenX RfD

was underpowered to assess reproductive toxicity because a third of the animals were missing uteri and not able to reproduce. They noted that while the study did not find an effect on pup weight overall, 9 of the 11 pups in one litter administered 5 milligrams per kilogram per day (mg/kg/day; highest administered dose) were dead by postnatal day (PND) 4.

EPA Response: EPA does not discount the reproductive and developmental effects observed following exposure to GenX chemicals. While 11 mating pairs failed to reproduce in DuPont 18405-1037 (2010), the biological significance of this finding is unclear because three mating pairs in the control group failed to reproduce. Specifically, 3, 4, 1, and 3 mating pairs failed to reproduce in the 0 (control), 0.1, 0.5, and 5 mg/kg/day groups, respectively, which suggests that the failure to reproduce was not test substance related (i.e., no dose response was observed). The study reports (page 52) that the uteri from the female mice that failed to produce a litter were placed in an ammonium sulfide solution to detect early implantation loss but were then discarded without microscopic examination. Therefore, according to the study authors, the cause of reproductive failure could not be determined. Although the study describes the uterus (and sometimes the uterus and cervix) as missing, EPA interpreted this to mean that these organs were missing from the microscopic examination because they were discarded after the ammonium sulfide treatment to detect early implantation loss, as outlined in the protocol.

EPA and the study authors concluded that a statistically significant decrease in mean pup body weights (BW) was observed in male and female pups in the 5 mg/kg/day group over the period of PND4–21 in DuPont 18405-1037 (2010). For example, on PND21 mean pup BW for male and female pups were 22% and 18% lower than control, respectively. This decrease in mean pup BW was considered in the selection of the critical effect (see Table 12 in section 5.0 in EPA, 2021a); however, the no observed adverse effect level (NOAEL) for this effect (0.5 mg/kg/day) was higher than the NOAEL for liver effects (0.1 mg/kg/day). Additionally, the number of litters per dose group was not significantly different (21, 18, 23, and 18 litters for 0, 0.1, 0.5, and 5 mg/kg/day, respectively), and the litter, not the pup, was considered the statistical unit.

EPA did consider the developmental endpoints of delayed attainment of balanopreputial separation (in males) and vaginal patency (in females) in the selection of the critical effect (see Table 12 in section 5.0 of EPA, 2021a). The NOAEL for these developmental effects (0.5 mg/kg/day) was higher than the NOAEL for liver effects (0.1 mg/kg/day), and the delays in vaginal patency (but not for balanopreputial separation) did not exhibit a dose response. Effects in rats (increased early deliveries, decreased fetal weights, and decreased gravid uterine weight) reported in DuPont-18405-841 (2010) were also considered in the selection of the critical effect (see Table 12 in section 5.0 of EPA, 2021a); however, the NOAEL for these effects (10 mg/kg/day) was higher than for the reported liver effects (0.1 mg/kg/day).

Finally, EPA considered other reproductive and developmental effects in mice and rats from studies published after the public comment draft was released (EPA, 2018a). Specifically, increases in gestational weight gain (GWG) and the incidence of placental lesions in mice reported by Blake et al. (2020) and decreases in GWG and maternal total thyroxine (T4) levels were considered adverse effects (see Table 12 in section 5.0 of EPA, 2021a). Changes in GWG in mice occurred at the same dose level as the liver effects in mice (0.5 mg/kg/day) but were not considered as a candidate RfD because of inconsistency in the direction of effect across species (i.e., mice gained weight during gestation while rats lost weight during gestation as compared to control). The placental lesions observed in Blake et al. (2020) exhibited a dose response;

however, only two dose groups were used in this study, and the study LOAEL (2 mg/kg/day) is much higher than the LOAEL observed in studies that observed liver effects (0.5 mg/kg/day). While the placental lesions observed are considered adverse, additional research is needed to determine whether these effects are seen at lower doses. Additionally, further research is needed to understand the consequence of the placental lesions on pregnancy and offspring. Towards this end, studies could evaluate the impact of GenX chemicals-induced placental lesions on the offspring's postnatal development, including latent health outcomes in the adult. For these reasons, placental lesions were not considered as a candidate RfD. All other reproductive and developmental effects reported as a result of gestational exposure to GenX chemicals (see Table 12 for a summary) were observed at higher doses than the placental lesions and changes in GWG and were, therefore, not considered for determination of the RfD derivation (see section 7.1 in EPA, 2021a for more detail).

Additionally, recent publications on the reproductive and developmental toxicity after GenX chemical exposure raise additional concern related to impacts on pregnancy that may lead to additional health effects later in life (Blake et al., 2020; Conley et al., 2019, 2021). Summaries of the studies published since the draft assessment was released have been added to the final document in section 4.5. This new toxicological information created additional uncertainty about the impact of exposure to GenX chemicals specifically on reproduction, development, and neurotoxicity, which justified an increase in the database uncertainty factor (UF_D) from 3 in the public comment draft (EPA, 2018a) to 10 in the final assessment (EPA, 2021a). See section 4.5 of the assessment (EPA, 2021a) for further details on these reproductive and developmental studies.

1.1.e Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, the Center for Environmental Health, NJDEP, the New York State Department of Health (NYSDOH), and the Pennsylvania Departments of Environmental Protection (PADEP) and Health (PADOH) supported EPA's selection of the subchronic reproductive/developmental toxicity study in mice (DuPont-18405-1037) over the chronic (2-year) toxicity study in rats (DuPont-18405-1238) because rats are less sensitive than mice to the effects of GenX chemicals.

EPA Response: Thank you for your comment. No response is needed.

1.1.f Comment: Dr. Damian Shea, in comments submitted on behalf of Chemours, stated that EPA made two critical errors in deriving the chronic RfD for GenX: (1) using the subchronic oral mouse reproductive/developmental toxicity study when a 2-year lifetime chronic rat study is available; and (2) use of a safety factor to account for duration of exposure. Shea suggested that EPA should use the 2-year chronic study with rats to derive a lifetime exposure health advisory and that the NOAEL for this study of 1.0 mg/kg/day should be used as the point of departure (POD), with protective uncertainty factors (UFs) of 10 each for interspecies and intraspecies, and a conservative relative source contribution from water of 20%. Shea further noted that data on the elimination of GenX from mammals suggest that its half-life in humans is in the range of 4 hours to 6 days, indicating no potential for significant accumulation in humans. Shea noted that the resulting lifetime health advisory value would be 70,000 ng/L for GenX.

EPA Response: EPA disagrees with the commenter. EPA followed existing risk assessment guidance and came to a different conclusion than the commenter. A review of the available data is consistent with the mouse being more sensitive than the rat to adverse effects after exposure to

GenX chemicals. There are no available chronic studies in mice. While typically a chronic study is used for developing a lifetime RfD, prenatal developmental toxicity studies, developmental neurotoxicity studies, and reproduction and fertility effects studies are also suitable for consideration in setting chronic RfDs or reference concentrations (EPA, 2002). In this case, the oral reproductive/developmental toxicity screening study in mice indicated adverse effects are observed at lower doses than those reported in the chronic study in rats. Additionally, in the 2-year chronic study in rats, just 25.4% of the test animals survived to planned terminal necropsy while 74.6% of the animals experienced unscheduled death/moribundity prior to the scheduled study termination at 104 weeks. While the authors stated that mean survival in males and females was unaffected by treatment, all females were sacrificed before study termination at 101 weeks because of decreased survival across all groups, including the control.

Additionally, it is EPA human health risk assessment practice to apply a UF to account for less-than-chronic data when they are used to derive the chronic RfD, as outlined in the most recent description of UF methodology in *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002). For GenX chemicals, a subchronic-to-chronic exposure uncertainty factor (UFs) was applied. This factor accounts for the likelihood that a lower concentration over a longer duration might induce a similar toxic effect to that observed in the subchronic study. Importantly, for GenX chemicals there is evidence of disease progression in the available rat studies. See section 7.3 of the assessment (EPA, 2021a) and responses to comments in section 1.3.2 of this document for further discussion of application of the UFs to account for exposure duration.

A relative source contribution term is not relevant to the development of toxicity values (RfDs), which is the purpose of this assessment. EPA is not deriving a health advisory value for GenX chemicals at this time.

1.1.g Comment: The American Chemistry Council (ACC) commented that EPA bases its toxicity value for the GenX chemicals on liver effects reported in a mouse reproductive/developmental toxicity screening study, despite the availability of a 90-day subchronic study (DuPont-18405-1307) which provides additional relevant hepatic measurements (e.g., alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST)). The ACC notes that EPA dismisses the results from the 90-day study because of the smaller sample size without addressing other significant aspects of the study. They mentioned that the longer exposure time in the 90-day study should improve chances to observe necrosis despite the lower statistical power. In addition, the commenters consider the consistency of the necrosis data with the liver enzyme results to provide a more complete picture of the liver effects than the data available from the reproductive/developmental study used as the critical study in the assessment. The NJDEP also commented on this issue; however, they agreed with EPA's selected study instead of the longer 90-day subchronic study for benchmark dose (BMD) modeling because of concerns that the higher LOAEL in the 90-day study may result from the smaller number of animals per dose group. Based on the liver effects reported in the 90-day study, the lowest observed adverse effect level (LOAEL) is 5.0 mg/kg/day and the NOAEL is 0.5 mg/kg/day.

EPA Response: The reproductive developmental study (DuPont-18405-1037, 2010) has greater statistical power than that of the 90-day subchronic study (DuPont-18405-1307, 2010) because of its greater sample size/group. In the reproductive/developmental study, DuPont evaluated

22–25 mice/dose group while the 90-day study in mice used 10 mice/dose group for liver endpoints. Additionally, one female mouse per HFPO dimer acid ammonium salt dose group died before study completion, bringing the sample size to nine mice/dose group in the 90-day toxicity study in mice (DuPont-18405-1307, 2010). The difference in the number of mice per dosing group between the 90-day study and the reproductive/developmental study might have an impact on statistical power (i.e., ability to observe liver effect levels in these studies). For example, in the 90-day study, adverse effects in the liver were observed in the high-dose 5 mg/kg/day group, yet there are indications of liver damage in the 0.5 mg/kg/day group. Specifically, absolute and relative liver weight increased relative to control mice in males by 12% and 11%, respectively, at 0.5 mg/kg/day. In males dosed with 0.5 mg/kg/day, 4/10 (40%) livers were observed to be discolored, compared to 0/10 (0%) for control mice. There were also increases in serum liver proteins at 0.5 mg/kg/day in males, although they did not differ significantly from control. AST, ALP, and ALT increased 35%, 40%, and 35%, respectively, compared to control (DuPont-18405-1307, 2010). Finally, histopathological liver effects were observed at 0.5 mg/kg/day in both sexes. Specifically, the NTP Pathology Working Group (PWG; see section 4.3 or appendix D of EPA, 2021a) noted that 10/10 (100%) male mice at the 0.5 mg/kg/day dose exhibited cytoplasmic alteration, compared to 0/10 (0%) in control.

ACC also claimed that the longer exposure time in the 90-day study should have improved chances to observe necrosis despite the lower statistical power. However, this was not the case as evidenced by the NOAEL (0.1 mg/kg/day) from the reproductive/developmental study, which was lower than the NOAEL from the 90-day study (0.5 mg/kg/day) (see Table 12 in the assessment). The fact that liver enzyme data were not collected in the reproductive/developmental study does not negate the liver findings of cell death that were observed and recorded by the NTP PWG (appendix D in EPA, 2021a) as part of the adverse constellation of liver lesions.

Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions that are considered adverse, 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 (Hall et al., 2012), EPA did not consider the cytoplasmic alteration alone as an adverse effect in this dose group. Additionally, 2/9 (22%) of the female mice in the 0.5 mg/kg/day dose group exhibited focal necrosis. Because 1/10 (10%) female mice in the control group also exhibited focal necrosis, a dose response was not observed for the constellation of liver lesions in the female mice in this study. Because of the significant uncertainties in the results of the 90-day study, EPA determined that the reproductive/developmental study was not only more sensitive for liver effects but also more completely represents the constellation of hepatic effects than the chronic study.

With regard to the issue of adversity of the critical effect selected, please see the response to comment 1.1.j.

1.1.h Comment: Green Toxicology LLC noted that the hepatocellular single cell necrosis dose-response data for male mice for 84 or 85 days and the 90-day male mouse study were carried out at the same dose levels and used the same species, strain and mouse supplier company. Therefore, these two bioassays should be considered of equivalent status, and treated as if they are a single experiment. Combination of the two studies was also supported in comments submitted by Dr. Damian Shea.

EPA Response: Liver effects observed in the 90-day study in mice (DuPont-18405-1307, 2010) were observed at higher doses (greater than or equal to 5 mg/kg/day) than in the oral reproductive/developmental toxicity study (DuPont-18405-1037, 2010) in mice (0.5 mg/kg/day). According to EPA's *Benchmark Dose Technical Guidance* (EPA, 2012), the 90-day dataset does not satisfy the minimum dataset criterion for modeling because only the highest dose shows a response and:

“...[t]he dataset should contain information on the dose-response relationship between the extremes of the control level and the maximal response observed...A dataset with only the highest dose showing a response would bracket the BMD at the low end but may provide limited information about the shape of the dose-response relationship.” (EPA, 2012)

Although these studies used the same strain of mice (CrI:CD1(ICR)), the reproductive/developmental study (DuPont-18405-1037, 2010) and 90-day study in mice (DuPont-18405-1307, 2010) were not conducted by the same laboratory, which increases the variability among the two datasets. The reproductive/developmental study (DuPont-18405-1037, 2010) was conducted by WIL Research Laboratories, LLC while the 90-day study in mice (DuPont-18405-1307, 2010) was conducted by E.I. du Pont de Nemours and Company DuPont Haskell Global Centers for Health & Environmental Sciences. EPA determined that it is inappropriate to combine the data from these separate experiments performed in two separate laboratories.

1.1.i Comment: NJDEP stated that when considering increased liver weight and hypertrophy in the development of a chronic RfD, duration-of-exposure issues must be considered (Health Canada, 2016; NJDEP, 2018; Michigan PFAS Science Advisory Panel, 2018). They also requested EPA to consider the duration of exposure (on page 51 of USEPA, 2018a) when applying the Hall criteria to studies of less-than-chronic duration for the purpose of chronic RfD development. NJDEP further noted that the primary focus of the Hall et al. (2012) study was hepatic effects observed in pre-clinical toxicity studies for pharmaceutical development. Because pharmaceuticals are normally administered for less than chronic exposure durations, hepatic effects from exposure to the drug might be adaptive. However, potential for reversibility when shorter-than-chronic exposure ends is not a reason to discount adversity of increased liver weight and hepatocellular hypertrophy in chronic RfD development because these lesions might progress with longer exposure.

EPA Response: The Hall et al. (2012) criteria aim to identify precursors to cancer. The commenter asserts that pharmaceuticals are normally administered for less-than-chronic exposure durations. EPA is unaware of published literature to support this assertion. GenX chemicals are not pharmaceuticals, but rather environmental contaminants that might have been released over a considerable time period in some locations. Hall et al. (2012) recognizes that:

“...prolonged exposure to a xenobiotic at levels that have previously been shown to be adaptive may eventually result in liver cell injury due to a failure of adaptive mechanisms.” (Hall et al., 2012)

EPA's approach for evaluating the toxicity of GenX chemicals does not discount increased liver weight and hepatocellular hypertrophy; in fact, EPA lists them as adverse effects associated with the study LOAEL when, in accordance with the Hall criteria, they are accompanied by liver cell

injury (e.g., necrosis or increases in serum enzymes indicative of cellular liver tissue damage) (Table 12 in EPA, 2021a).

1.1.j Comment: Dr. James Klaunig, in comments submitted on behalf of Chemours, requested that the hepatic effects discussion in sections 5.0 and 5.1 of the draft GenX document clarify the specific studies where adverse effects, adaptive effects, and no effect of the GenX compounds on the liver were reported, with special attention to the dose response characteristics of the reported effects. Klaunig indicated that the draft GenX document incorrectly combines all forms of necrosis together as indicated by the statement “Hepatocellular necrosis was detected in nearly all the available studies and at the lowest doses tested.” Klaunig further notes that liver necrosis is accompanied by inflammation and is not reversible. Single cell necrosis is different from liver necrosis and has been addressed and clarified since the time of the DuPont studies on the GenX compounds. Klaunig further notes that in the case of exposure to GenX chemicals, where single cell necrosis is observed at a dose where concomitant multifocal or focal necrosis is not evident, there is no apparent adverse effect on the liver and the designation of single cell necrosis/apoptosis should not be considered an adverse change based on the criteria of Hall et al. (2012). Similarly, the ACC and Green Toxicology LLC noted that the minimal liver necrosis observed in the reproductive/developmental study used in the assessment may suggest an adaptive, non-adverse reaction in mice or a response to other stressors for which no acknowledgement has been made.

Similarly, ToxStrategies, Inc. noted that only “single cell necrosis” was observed in the livers of male mice that were used as the basis of EPA’s RfD, and further, that if this was not necrosis and was actually apoptosis there would be no histological support for hepatotoxicity and the clear evidence of peroxisome proliferator-activated receptor alpha (PPAR α) involvement would result in a determination that the liver lesions in mice were non-adverse in terms of human health risk assessment. They cited experts from the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) Organ Working Groups (Elmore et al., 2016) as providing more recent, at the time, diagnostic criteria to allow pathologists to distinguish between apoptosis and single cell necrosis in standard hematoxylin and eosin (H&E) stained tissue sections. The commenter also suggests that based on the description in DuPont-18405-1307 (2010) of single cell necrosis (“...isolated eosinophilic bodies with occasional pyknotic nuclear fragments...thus was consistent with apoptosis”) and the Elmore et al. (2016) diagnostic criteria, it is conceivable that GenX exposure induced apoptosis rather than single cell necrosis in the critical study. Klaunig and Green Toxicology LLC agreed with ToxStrategies, Inc that the pathologic description of single cell necrosis after exposure to GenX compounds is consistent with the criteria of apoptosis in contrast to necrosis based on the Elmore et al., (2016) diagnostic criteria.

To evaluate their proposed hypothesis, ToxStrategies, Inc. presented a reanalysis of liver pathology slides from the 90-day mouse (male and female; DuPont-18405-1307, 2010) and reproductive and developmental mouse studies (males only; DuPont-18405-1037, 2010) using the diagnostic criteria described by Elmore et al. (2016) to score apoptosis and necrosis. Dr. John Cullen, a North Carolina State University board-certified veterinary pathologist, retained by ToxStrategies, Inc., concluded that for both studies, apoptosis was the primary adverse effect of note in the liver with sporadic occurrence of necrosis at lower doses in the parental males in the reproductive and developmental study, but not in the 90 day study (both males and females). (DuPont-18405-1307, 2010; DuPont-18405-1037, 2010). In summary, the authors concluded that

the reevaluation of liver slides from these two studies demonstrated that the previous diagnosis of ‘single cell necrosis’ is more accurately diagnosed as apoptosis using what they consider to be the most current diagnostic criteria.

Dr. John Cullen also observed increased mitosis at GenX doses with apparent increased apoptosis; the commenters asserted that it is well-established that peroxisome proliferator-activated receptor (PPAR) activators can increase mitosis and apoptosis *in vivo*. Therefore, they concluded that this effect is likely a part of PPAR α signaling pathways specific to rodents. ToxStrategies, Inc. noted that based on the evidence for peroxisomal proliferation and PPAR α involvement, liver hypertrophy would be considered non-adverse and should not be considered as the basis for risk assessment.

EPA Response: In response to this comment and similar claims in a 2019 publication describing a reanalysis of the slides from the critical study (Thompson et al., 2019), EPA requested that the National Institute of Environmental Health Sciences’ (NIEHS’s) NTP in Research Triangle Park, NC, convene a PWG to provide independent, expert review of selected tissues from the reproductive/developmental study (DuPont-18405-1037, 2010) and the 90-day mouse study (DuPont-18405-1307, 2010).

As part of this PWG, one pathologist reviewed all the slides from the two studies that DuPont submitted to EPA and classified liver cell death according to the INHAND Organ Working Group’s diagnostic criteria, which describe how pathologists can distinguish between apoptosis and single-cell necrosis in standard H&E-stained 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 PWG coordinator then confirmed the classifications and selected example slides representative of the observed liver effects for review by the other six members of the group. The selected slides included three examples each of normal liver, hepatocellular apoptosis, hepatocellular single-cell necrosis, and hepatocellular cytoplasmic alteration; two examples each of focal necrosis, pigment, increased mitoses, mixed-cell infiltrates, and cytoplasmic vacuolation; and one example of oval cell hyperplasia. There was a majority agreement on all reviewed lesions. The PWG consensus opinion for each slide, including any additional diagnoses made by the PWG panel, was recorded and presented in the final PWG report (appendix D in EPA, 2021a).

The PWG’s classification of liver lesions included, but was not limited to, the following: apoptosis, single-cell necrosis, cytoplasmic alteration, and focal necrosis. The PWG confirmed single-cell necrosis and focal necrosis in the mid- and high-dose groups of both studies. Both single-cell necrosis and focal necrosis exhibited a dose-response in male and female mice in the reproductive/developmental study (DuPont-18405-1037, 2010). Focal necrosis and single-cell necrosis were observed in the high-dose group for male and the mid- and high-dose groups for female mice in the 90-day toxicity study in mice (DuPont-18405-1307, 2010). The PWG agreed that the observed single-cell necrosis was accompanied with inflammation. Using the INHAND criteria outlined in Elmore et al. (2016), the pathologists separated single-cell necrosis from apoptosis. Findings of apoptosis were observed but limited to the highest dose groups in both sexes in both studies.

The PWG results confirm the conclusions presented in the studies submitted to EPA’s Office of Pollution Prevention and Toxics (OPPT) by DuPont and in the draft GenX chemicals toxicity

assessment (EPA, 2018a) that the observed liver lesions, which include single-cell necrosis, are treatment-related adverse effects. EPA updated the final assessment to include a description of the NTP PWG analysis and BMD modeling of these new dose response data. The reproductive/developmental study (DuPont-18405-1037, 2010), which was identified as the critical study, identified liver effects in females (i.e., the constellation of lesions as defined by the NTP PWG to include cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) as the critical effect and used it as the basis for the calculation of the subchronic and chronic RfDs.

1.1.k Comment: Dr. James Klaunig, in comments submitted on behalf of Chemours, stated that it is misleading that the term “hypertrophy” was equated with liver damage and was interpreted as an adverse effect. Klaunig mentioned that hypertrophy of the liver should be considered an adaptive change unless it is accompanied by focal or multifocal necrosis, inflammation, and/or fibrosis. Likewise, the ACC notes that additional consideration of the relevance of liver hypertrophy to humans should be considered (Hall et al. 2012). ToxStrategies, Inc. also noted that Hall et al. (2012) indicates that increased liver weight is non-adverse if there is evidence for PPAR α involvement without other evidence of hepatotoxicity (e.g., fibrosis, inflammation, steatosis, necrosis, etc.). Further, Klaunig mentioned that an increase in liver hypertrophy and liver weight can occur at doses that fail to induce adverse effects. Klaunig further noted that the document failed to address differences in adverse and adaptive effects as they relate to dose and emphasizes that an effect only seen at a high dose does not necessarily translate to lower doses. Green Toxicology LLC noted that hypertrophy would not be expected in human livers at potential human exposure levels because this liver cell hypertrophy is a consequence of PPAR α activation to which humans are much less sensitive than are rats and mice; they conclude that the rat- and mouse-based response is thus irrelevant for purposes of assessing risks to human health after exposures to GenX. Dr. Damian Shea also submitted comments supporting this point made by Green Toxicology LLC.

EPA Response: EPA disagrees with the comment. The observation of liver hypertrophy alone was not characterized as adverse in the document. Specifically, EPA evaluated the liver effects in studies after exposure to GenX chemicals in the context of the Hall criteria (Hall et al., 2012), that characterizes liver effects such as changes in liver weight or hepatocellular hypertrophy as adverse when they are accompanied with microscopic evidence of liver damage such as necrosis, inflammation, and/or fibrosis. Hall et al. (2012) also recognized that “prolonged exposure to a xenobiotic at levels that have previously been shown to be adaptive might eventually result in liver cell injury due to a failure of adaptive mechanisms.” In the public comment draft (Table 7, EPA, 2018a), EPA listed increased liver weight and hepatocellular hypertrophy as adverse effects associated with the study LOAEL only when they were accompanied by necrosis or other markers of liver damage (e.g., increases in liver serum enzymes) in the public comment draft. The NTP PWG review (appendix D in EPA, 2021a) included hepatocellular hypertrophy, along with eosinophilic change to the hepatocytes, under the category of “cytoplasmic alteration” and deemed this effect to be among the constellation of lesions that represent adversity among the findings from the DuPont 18405-1307 (2010) and DuPont 18405-1037 (2010) studies. Following the direction provided by the expert NTP PWG, EPA will continue to reference cytoplasmic alterations as part of the constellation of lesions that represents adversity based on the NTP’s evaluation of the DuPont 18405-1307 (2010) and DuPont 18405-1037 (2010) studies.

With respect to PPAR α , EPA agrees that some data are consistent with a PPAR α mode of action (MOA); however, the available data are not adequate to definitively conclude that a PPAR α MOA is the sole toxicologic MOA for HFPO dimer acid and/or ammonium salt in the liver or in other organ systems. EPA has clarified that the PPAR α MOA is plausible in the liver after GenX chemicals exposure in section 6.0 of the assessment (EPA, 2021a). However, the available data are also consistent with additional MOAs (e.g., peroxisome proliferator-activated receptor gamma (PPAR γ), mitochondrial dysfunction, and cytotoxicity) and the weight of the evidence indicates multiple MOAs. The synthesis of the MOA data is included in section 6.0.

1.1.i Comment: Green Toxicology LLC noted that the liver cell changes were not observed in either the initial rat 14-day developmental study nor in the rat 90-day study.

EPA Response: EPA disagrees with this comment. While single-cell necrosis was not noted in the rat 90-day study (DuPont-17751-1026, 2009), focal necrosis was observed in the rat 14-day developmental study (DuPont-18405-841, 2010; 9% and 23% of the dams dosed with 100 mg/kg/day and 1,000 mg/kg/day, respectively).

1.1.m Comment: Green Toxicology LLC and Dr. James Klaunig, in comments submitted on behalf of Chemours, indicated that there were concerns about the discussion and interpretation of the reported liver specific serum enzymes following GenX treatment. In the draft GenX document, the increase in liver serum enzymes is considered an adverse response; however, an increase in liver serum enzymes without hepatocyte membrane damage have been reported as a consequence of liver enzyme induction as well as cell proliferation, representing an adaptive response rather than an adverse effect. They noted that it is generally accepted that liver serum enzyme increases of two-fold or less from untreated control do not reflect a significant change in liver function. Green Toxicology LLC also noted that increases in liver serum enzymes should be considered in a dose response manner coupled with other liver endpoints (specifically necrosis). They asked that EPA address these points in the discussion and conclusions reached on GenX toxicity.

EPA Response: EPA agrees that increases in liver serum enzymes without hepatocyte membrane damage could be considered an adaptive response. However, Hall et al. (2012) also recognized that “prolonged exposure to a xenobiotic at levels that have previously been shown to be adaptive may eventually result in liver cell injury due to a failure of adaptive mechanisms.” In the draft and final GenX assessments (EPA, 2018a, 2021a), liver toxicity was evaluated against the Hall criteria (Hall et al., 2012) that consider increased liver weight and hepatocellular hypertrophy that is accompanied by histologic or clinical pathology indicative of liver toxicity to be adverse. Histologic or clinical pathology indicative of liver toxicity can include changes in liver enzyme concentrations in the serum, necrosis, inflammation, and degeneration. Only the doses associated with the effects classified as adverse were used for determining the NOAELs/LOAELs provided in Table 12 of the assessment (EPA, 2021a). Upon consideration of the Hall criteria, it can be concluded that the liver effects identified in Table 12 of the assessment indicate toxicity that is relevant to humans. EPA interpreted single-cell or focal necrosis to be the equivalent of the hepatocyte membrane damage cited by the commenter. Finally, in determining the POD for each study listed in Table 13, serum liver enzyme levels were not available for the studies selected for POD derivation. Rather, EPA used the constellation of liver lesions identified by the NTP PWG as adverse (i.e., apoptosis, single-cell necrosis, cytoplasmic alteration, and

focal necrosis) for POD derivation. To address these comments, EPA has clarified how liver serum enzymes were considered in the assessment (section 3.2 in EPA, 2021a).

1.1.n Comments: The Michigan Department of Environmental Quality (MDEQ) in collaboration with the Michigan Department of Health and Human Services (MDHHS), and NJDEP provided the following specific comments:

- MDEQ stated that Table 2 contains three entries that are inconsistent with the data provided in the cited reference.

EPA Response: Thank you for this comment. EPA revised Table 2 to be consistent with the data provided in DuPont-18405-1307 (2010).

- MDEQ also stated that there is a lack of evidence that steady state tissue concentrations had been reached in either of the two mouse studies considered for the final GenX toxicity value development. In the DuPont-18405-1307 (2010) study, steady state might not have been reached in mice out to 90 days of oral exposure (in two of the three dose groups). In Rushing et al. (2017), steady state had not been reached in mice out to 28 days of oral exposure. It is unclear whether the possible lack of steady state conditions was evaluated by EPA in the lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level (BMDL₁₀) or point of departure human equivalent dose (POD_{HED}) development, UF selection, or final oral dose determination.

EPA Response: There is limited evidence from a single mouse study (DuPont 18405-1307, 2010) indicating that the serum levels at either 28 days or 95 days are comparable in male mice (1,124 nanograms per milliliter (ng/mL) versus 1,276 ng/mL at day 28 and day 95, respectively) only in the 0.1 mg/kg/day (the NOAEL from the critical study selected in the assessment) dose group. This suggests that steady state is reached between 28 days and 95 days when mice receive 0.1 mg/kg/day. EPA agrees that based on the available data, it does not appear that steady state is reached at the higher doses. To respond to this comment, EPA updated section 2.3.6 (EPA, 2021a) to reflect this information.

- NJ DEP stated that on p. 13, first full paragraph: It is unclear why the increases in serum albumin and albumin/globulin (A/G) ratio observed in rats and mice support the hypothesis that GenX binds to albumin, as stated in the draft. To our knowledge, serum albumin levels are not affected by binding of xenobiotics to albumin. Additionally, it is stated later in the document (p. 29) that the increased A/G ratio is most likely due to decreased production of globulin, and that “the observed changes in albumin and A/G ratio...are considered early markers of potential immunotoxic effects” (p. 51).

EPA Response: To address this comment, EPA has revised the assessment to describe the studies that demonstrate the major serum protein interaction site for some PFAS, including PFOA and perfluorohexanoic acid (PFHxA), is albumin (D’eon et al., 2010; Han et al., 2003). Considering these points, and that albumin is the major transport protein in the blood, it is likely that GenX chemicals are also distributed via serum albumin (Peters, 1995). Indeed, Allendorf et al. (2019) demonstrated that bovine serum albumin binds HFPO dimer acid and that its albumin/water partition coefficient is in the same range as other PFAS (e.g., perfluorobutanoic acid (PFBA) and perfluorohexane sulfonic acid (PFHxS)). However, the evidence is not definitive, and EPA has revised the document to reflect this information (section 2.3.2 of EPA, 2021a).

- NJDEP stated that on p. 14. Section 2.3.4 – Metabolism, 2nd line: Hepatocytes were incubated with 5 micromolar (not 5 “micrometers”) of HFPO dimer acid ammonium salt.

EPA Response: Thank you for this comment. The text has been revised.

- NJDEP stated that on p. 14. Section 2.3.5. – Metabolism-Urine, 3rd line: The dose in the cited study (DuPont-18405-1017 RV1, 2011) was 30 milligrams per kilogram (mg/kg), not 10 mg/kg.

EPA Response: Thank you for this comment. The text has been revised.

- NJDEP stated that on p. 22-23. Section 3.2 – Overall Scientific Objectives: The relationship between external exposure and internal dose (i.e., toxicokinetics) should be considered in developing a POD and should be included here. If relevant data are not available, this should be discussed as an important uncertainty.

EPA Response: Thank you for the comment. EPA’s default methodology was followed in accounting for toxicokinetic differences by using $BW^{3/4}$ allometric scaling (EPA, 2011) as internal dose information is limited for GenX chemicals. Text has been added to section 3.2 stating this (EPA, 2021a). Additionally, EPA has added summaries of data relevant to internal dose from studies that were published after the public comment draft (Blake et al., 2020; Conley et al., 2021) to sections 2.3.3 and 8.6 of the GenX toxicity assessment (EPA, 2021a).

- NJDEP stated that on p. 23. Bullets at top of page: The explanations of subchronic and chronic durations need to be clarified. The durations for humans (up to 10% of a lifetime; greater than 10% of a lifetime) are not distinguished from the durations for laboratory animals (30 days to 90 days; 90 days to 2 years). It should be stated that a subchronic duration of 30 to 90 days is up to about 10% of the lifespan of the laboratory animals and is intended to reflect human exposure of up to about 10% of the human lifespan. Similarly, exposure to animals of 90 days to 2 years is intended to reflect chronic/lifetime human exposure.

EPA Response: No revision needed because EPA used the subchronic and chronic study duration definitions as outlined in *Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002). This information is included in section 3.2.

Specifically, the following recommended definitions are presented:

- Subchronic: Repeated exposure by the oral, dermal, or inhalation route for more than 30 days, up to approximately 10% of the life span in humans (more than 30 days, up to approximately 90 days in typically used laboratory animal species).
 - Chronic: Repeated exposure by the oral, dermal, or inhalation route for more than approximately 10% of the life span in humans (more than approximately 90 days to 2 years in typically used laboratory animal species).
- NJDEP stated that on p. 23. First full paragraph, last line – carcinogenicity descriptor: The phrase “...suggestive evidence of tumor formation...” should be revised to “Suggestive Evidence of Carcinogenic Potential in humans” as stated on p. 47. There is no doubt that GenX caused tumors in animals, but these tumor data have been interpreted as providing “suggestive evidence” for human carcinogenicity. Additionally, the

description of the carcinogenic potential of GenX should be included in the Executive Summary, as in the perfluorobutane sulfonic acid (PFBS) document.

EPA Response: Thank you for the comment. To respond to this comment, the final assessment was revised to include language consistent with the 2005 U.S. EPA Cancer Guidelines throughout the document, including section 3.4 (EPA, 2005). Additionally, the Executive Summary has been revised to include the description of carcinogenic potential consistent with the PFBS document.

- NJDEP stated that on p. 27. First full paragraph, last line: It is not clear what is meant by “the tumor data failed to demonstrate a direct response to dose.” Statistically significant increases in pancreatic tumors in males and liver tumors in females were observed at the highest doses, but not lower doses. The highest doses were 50-fold and 10-fold greater than the next lowest doses in males and females, respectively.

EPA Response: Thank you for the comment. EPA has clarified the statement in section 3.5 of the assessment (EPA, 2021a). A more complete discussion of the cancer endpoints can be found in sections 4.4 and 5.6 of the draft and final assessments (EPA, 2018a, 2021a).

- NJDEP stated that on p. 36. Last two paragraphs: As above, it is notable and should be mentioned that GenX increased the incidence of hepatic carcinomas, as well as adenomas in female rats and the incidence of combined pancreatic acinar cells adenomas and carcinomas in male rats, while PFOA increased only the incidence of benign hepatic and pancreatic tumors in rats (Biegel et al., 2001).

EPA Response: No changes are needed because this assessment provides the hazard identification and dose-response assessment for HFPO dimer acid and its ammonium salt. Thus, EPA has presented the available cancer data for the GenX chemicals but not for PFOA.

- NJDEP stated that on p. 37. First full paragraph: Although the incidence of testicular interstitial cell adenomas was not statistically significant compared to controls, the authors of the study conclude that “a relationship to treatment for these findings in the 50 mg/kg (i.e., high dose) group cannot be ruled out” (Caverly Rae et al., 2015). This conclusion should be noted in the USEPA document.

EPA Response: Thank you for the comment. This information has been added to the study description in section 4.4 (EPA, 2021a).

- NJDEP stated that in section 5.6 on page 47, it should be mentioned in the EPA document that PFOA increased only benign tumors (adenomas) while GenX increased both malignant (carcinomas) and benign tumors of the liver and pancreas in rats (Caverly Rae et al. 2015, DuPont-18405-1238, 2013). They further note that the following statement on page 52 appears to be inaccurate:
 - “Conversely, male and female rats exhibited no subchronic hepatocellular necrosis in the 90-day study (DuPont-17751-1026, 2009), yet hepatocellular necrosis is observed in the chronic study at **much higher doses** [bold added by commenter] (DuPont-18405-1238, 2013).”

NJDEP mentioned that in the 90-day study, hepatic necrosis was not reported in male rats at up to 100 mg/kg/day or in female rats at up to 1000 mg/kg/day. Also, they noted that in the 2-year study, statistically significant increases in hepatic necrosis occurred in males at 50 mg/kg/day and in females at 500 mg/kg/day; a non-significant dose-related increase occurred in females at 50 mg/kg/day. Therefore, the LOAELs for hepatic necrosis in the chronic study (50 mg/kg/day in males; 500 mg/kg/day in females) were lower, not much higher, than the NOAELs for this effect in the subchronic study (100 mg/kg/day in males; 1000 mg/kg/day in females).

EPA Response: Thank you for the comments. This assessment provides the hazard identification and dose response assessment for HFPO dimer acid and its ammonium salt. EPA has presented the available cancer data for the GenX chemicals only and not for other PFAS.

Text has been added in section 6.0 (EPA, 2021a) to clarify that the necrosis observed in the rats occurred at much higher doses than in the mice in the developmental/reproductive mouse study. Text has also been added to section 5.1 (Hepatic) to more thoroughly describe the liver necrosis observed in the rat studies.

1.2 ALLOMETRIC SCALING OF DOSES BETWEEN TEST SPECIES

1.2.a Comment: Several commenters, including NYSDOH, MDEQ in collaboration with MDHHS, NJDEP, TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that in the absence of available human half-life data, it is unclear whether humans may demonstrate greater sensitivity compared with animals than is currently accounted for in the proposed POD_{HED} development method (i.e., $BW^{3/4}$ allometric scaling) and applied interspecies UF.

NYSDOH and ACC noted that chemical-specific information on the serum half-life in humans and physiologically based pharmacokinetic (PBPK) models are currently not available for GenX; thus, EPA's choice of using default $BW^{3/4}$ scaling appears reasonable. The ACC supports allometric scaling because the information available for GenX suggests that these substances are eliminated from the body relatively rapidly and will not accumulate in contrast with PFOA, perfluorooctane sulfonate (PFOS) and other legacy PFAS.

NYSDOH further noted that GenX (like many PFAS) might have a longer serum half-life in humans than in rodents and that without human half-life data, it is unknown whether cross-species pharmacokinetic differences are adequately covered by this approach or whether co-exposure to multiple PFAS chemicals would change GenX clearance. NYSDOH recommended that EPA conduct additional research to improve the understanding of GenX cross-species differences and GenX clearance when part of a PFAS mixture as a key refinement of EPA's draft RfD derivation for GenX.

MDEQ suggested that EPA consider additional discussion regarding the uncertainties of using an allometric scaling approach in the absence of adequate half-life information in the animal model and human being compared.

TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health, noted that the Netherland's National Institute for Public Health and the

Environment (RIVM) concluded that although the elimination rates for GenX are faster than PFOA in animal models, without data in humans it is not possible to make assumptions about the toxicokinetics of GenX chemicals in humans (Beekman et al., 2016 (cited as RIVM, 2016 in comments)). The group of commenters noted that it is unclear how the human equivalent dose (HED) based on liver effects in adults would compare to the HED based on developmental effects in infants and children.

NJDEP noted that RIVM did calculate and apply a toxicokinetic factor of 66 based on the ratio of half-lives in humans (1378 days) and cynomolgus male monkeys (20.9 days) for PFOA (Butenhoff et al., 2012, Olsen et al., 2009) to account for the potential kinetic difference between nonhuman primates and humans; this is an example of an alternative approach to extrapolating animal doses to human doses for PFAS that do not yet have human toxicokinetic data. It is unclear whether the dosimetric adjustment factor (DAF) of 0.14 - 0.15, equivalent to a factor of 6-7, is sufficient to account for the higher internal dose in humans compared to mice from the same administered dose.

EPA Response: EPA recognizes the data gap regarding the human half-life and clearance of GenX chemicals. EPA agrees that in the absence of publicly available, adequate human half-life data, it is unclear whether humans might demonstrate differential sensitivity (greater or lesser) than is currently accounted for in the draft POD_{HED} calculation (i.e., $BW^{3/4}$ allometric scaling) and applied interspecies UF (UF_A). However, in data-poor situations such as this, EPA relies on its established risk assessment methods (EPA, 2011). While there is some indication that elimination rates for GenX chemicals are faster than for PFOA in animal models (Gannon et al., 2016), use of half-life information for other PFAS has the potential to introduce additional uncertainty in the analysis. It is not clear whether the biochemistry associated with PFOA is representative of GenX chemicals' human clearance time (Beekman et al., 2016). As pointed out by RIVM, EPA agrees that it is not possible to draw a conclusion on the bioaccumulation potential of FRD-902 (a synonym for HFPO dimer acid ammonium salt) in the absence of data on the human clearance time, based on the results of Gannon et al. (2016) and Beekman et al. (2016).

Moreover, preliminary, unpublished human half-life data that have not been peer-reviewed suggest that the human half-life for GenX chemicals is shorter and more closely aligned with available animal half-life estimates than the case for PFOA. These data have been described in the document (Clark, 2021; see section 8.4 of the final assessment for additional details (EPA, 2021a)). Given the preliminary nature of the human half-life study, more research is needed to confirm these results.

The GenX chemicals' half-life estimates in mice and rats range from 24.2-72.2 hours (Gannon et al., 2016), a range that is comparable to the preliminary half-life findings reported for humans. In contrast, PFOA half-life values for the monkey, rat, and mouse are 20.8 days, 11.5 days, and 15.6 days, respectively, while the PFOA human half-life value is 2.3 years among members of the general population (EPA, 2016). Finally, limited biomonitoring data from residents living near a manufacturing facility that produces GenX chemicals reported that GenX chemicals were not detected in the blood or urine of any individuals but PFOA and other legacy PFAS were detected (Kotlarz et al., 2020; Pritchett et al., 2019). Taken together, these data suggest that toxicokinetic differences exist between the GenX chemicals and PFOA in humans.

EPA practice states clearly that, in the absence of adequate data to support the development of human equivalent oral exposures from laboratory animal species:

“...body weight scaling to the $\frac{3}{4}$ power (i.e., $BW^{3/4}$) is endorsed as a general default procedure to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purposes of deriving an oral Reference Dose (RfD)” (EPA, 2011).

EPA strongly agrees with the comment that further study is needed to better understand the differences in GenX toxicokinetics between males and females as well as between mice and humans. Further, collection of such information is broadly needed across the entire class of PFAS chemicals. However, the GenX toxicity assessment can be conducted with the available data and EPA risk assessment methods without addressing these knowledge gaps.

1.2.b Comment: Green Toxicology LLC notes that EPA’s default approach for allometric scaling “is designed to approximate (at least) the toxicokinetic portion of the interspecies extrapolations (USEPA, 2002, Section 4.4.3.4): that is, for the chronic studies, an approximation for extrapolation of the area under the concentration-time curve (the AUC) for the active chemical between and among species”. They noted that there were direct measurements of AUC for three mammalian species (mouse, rat, monkey) that would allow comparison of the allometric scaling with scaling of AUC. Green Toxicology LLC performed this comparison and found that allometric scaling between male rat and male monkey does correspond well with AUC/dose scaling. They suggest that a more defensible, evidence-based approach than default allometric scaling from male mouse to human would be to use the measured AUC/dose scaling from mouse to monkey and then assume allometric scaling (i.e., $BW^{3/4}$) from monkey to human, resulting in an increase in the estimated RfD by a factor of 7.07.

EPA Response: EPA’s *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (EPA, 2014) states that “half-life is not an acceptable basis for [Data-Derived Extrapolation Factors] calculation because it is related to neither body weight nor volume of distribution.” The commenters’ proposed approach using scaling of the AUC makes several assumptions related to differences between monkeys, rodents, and humans that would introduce significant uncertainty. The calculations underlying the statistics provided by the commenter (i.e., the AUC/dose statistics) are not fully described and are therefore unclear. The source of the data, Gannon et al. (2016), does not present AUC statistics for the mouse, rat, or monkey, and the comment does not include the methodology for the calculations presented in the provided spreadsheet. The commenter’s method appears to propose the use of half-life data from Gannon et al. (2016) to derive AUC-based DAFs for two-thirds of the conversion but relies on a $BW^{3/4}$ conversion for the final conversion from cynomolgus monkeys to humans because half-life data are unavailable for humans.

EPA does not support the commenters’ proposed AUC scaling approach because it is not supported by a cogent biological rationale and the analysis provided is unclear. EPA has applied the $BW^{3/4}$ allometric scaling approach as described in EPA’s guidance (EPA, 2011). In instances in which chemical-specific data are unavailable, it is EPA policy to use a BW scaling to the $\frac{3}{4}$ power approach to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans (EPA, 2011). This scaling addresses some aspects

of cross-species extrapolation of toxicokinetic and toxicodynamic processes (UF_A), but some residual uncertainty remains. Thus, in the absence of chemical-specific data for GenX chemicals to quantify this uncertainty, a UF_A of 3 is justified per EPA guidance.

1.2.c Comment: The Silent Spring Institute notes that physiologically-based toxicokinetic (PBTK) data and models should be used instead of $BW^{3/4}$ to account for toxicokinetic variability.

EPA Response: There are currently no published peer-reviewed PBPK models for GenX chemicals. Limited information is available on the toxicokinetics of GenX chemicals across different species of animals and in humans. The available data for GenX chemicals suggest that these chemicals might behave differently than other legacy PFAS. Due to the lack of clarity in this area, it is premature to use models based on data derived from more data-rich PFAS. Therefore, no changes were made. However, further discussion of this point can be found in section 7.3 of the assessment (EPA, 2021a).

1.3 UNCERTAINTY AND DATA QUALITY

1.3.1 Uncertainty Factors—Database Uncertainty

1.3.1.a Comments: Several commenters (EWG [individually], PADEP and PADOH, MDEQ and MDHHS, and a group of organizations, including TEDX, the Natural Resources Defense Council, the Sierra Club, EWG [as a member of this coalition], and the Center for Environmental Health) recommended increasing the UF_D from 3 to 10 for several reasons, including lack of human, immunotoxicity, and reproductive and developmental data. One commenter (ACC) recommended lowering the UF_D . Specific comments received include:

- PADEP and PADOH noted that an UF_D of 3 is not adequate to protect human health and an UF_D /modifying factor of 10 should instead be applied for database limitations/deficiencies. PADEP and PADOH noted that in the development of a chronic oral RfD for GenX chemicals, USEPA applied an UF_D of 3 for database deficiencies, including immune effects and additional developmental studies. They mentioned that Agency for Toxic Substances and Disease Registry (ATSDR) recently applied an UF_D /modifying factor of 10 for the development of oral intermediate duration (15 to 364 days) Minimum Risk Levels (MRLs) for PFOS, PFHxS, and perfluorononanoic acid (PFNA) because of data limitations, but not for PFOA. PADEP and PADOH further noted that ATSDR considered immune effects as a more sensitive health effect for the development of intermediate oral MRLs. Lastly, they mentioned that ATSDR could not develop oral chronic MRLs for any of the PFAS chemicals such as PFOA, PFOS, PFHxS, and PFNA stating that “there are insufficient data for derivation of a chronic oral MRL.”
- MDEQ in collaboration with MDHHS stated that EPA should apply the full UF_D of 10 given the number and significance of data gaps:
 - There are no human data from epidemiological studies in the general population or worker cohorts-evaluating the effects of exposure to GenX chemicals. Human data has significantly improved our understanding of the toxicological profile of many PFAS (ATSDR, 2018a). Human data is especially important considering

the difference in elimination rates for PFAS between animal models and humans. A lack of human data to complement and compare to animal toxicological data is a critical data gap.

- There is no chronic study in the mouse. The only chronic toxicity study was conducted using rats, which appear to be less sensitive than mice to GenX exposure. An additional limitation of this study is that there were higher than normal early deaths across all study groups (DuPont-18405-1238, 2013; DuPont-18405-1307, 2010 (cited as Dupont Chem 2010b in comment)).
- There is limited testing of developmental toxicity (an identified sensitive endpoint for other PFAS) from GenX exposure.
- There is limited testing of immunological responses including immune function assays, histopathology, and antibody levels (identified sensitive endpoints for other PFAS and supported for GenX by findings from Rushing et al. [2017]).
- There is no full two-generation reproductive toxicity study for GenX in any animal species.
- A group of organizations including TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health similarly stated that EPA should apply the UF_D of 10 because:
 - Developmental toxicity and immunotoxicity are common health effects associated with PFAS exposure, both of which can occur after extremely low levels of exposure (ATSDR 2018a, 2018b). Two developmental toxicity studies, only one of which was in mice, and a single study that specifically assesses immune effects is a serious database limitation.
 - One critical data gap is the lack of a full 2-generation toxicity study evaluating exposures during early organogenesis. Additionally, there are many developmental and immune effects that have yet to be assessed, including reproductive system development (e.g., mammary gland development and function), neurodevelopment, autoimmunity, infectious disease resistance, and immune hypersensitivity (e.g., asthma and allergies).
 - There are very few experimental studies in laboratory animals and no data on non-oral exposure routes for GenX.

1.3.1.b Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted the lack of toxicity data from inhalation and dermal exposure routes: Both the HFPO dimer acid and its salt can be transported through air (DuPont CCAS 2009). Inhalation could be a significant exposure route, especially in areas where GenX processing occurs. In 2017 the North Carolina Division of Air Quality estimated that despite some cutback in emissions, the Chemours Fayetteville Works plant emitted approximately 2,700 pounds of GenX chemicals per year (NC DEQ, 2018a) and GenX chemicals have been found in rainwater up to 7 miles from the Chemours Fayetteville Works plant (NC DEQ, 2018b). Minimal dermal absorption of the HFPO dimer acid ammonium salt has also been demonstrated (Dupont-25292, 2008a), however, there is a lack of information on the dermal absorption potential or toxicity of the HFPO dimer acid.

1.3.1.c Comment: The ACC stated that the database UF of 3 should not be applied because it is reasonable to conclude that toxicity values generated from the liver effects observed in the 90-day subchronic study in mice (DuPont 18404-1307) will provide sufficient protection against potential developmental and immunotoxic effects. The ACC notes that this is because the available evidence suggests that any developmental and immune effects are likely to occur at exposure levels that are comparable to the liver effects that are the basis of the draft toxicity value for GenX chemicals. They further state that while the 90-day subchronic study in mice (DuPont 18404-1307) and a prenatal development toxicity study in rats (DuPont 18405-841) have reported developmental effects, the LOAELs and NOAELs for the most sensitive effect (i.e., pup BW in mice) are consistent with the liver results. They also assert that a study of immunological effects in mice treated with 100 mg/kg/day (Rushing et al., 2017) observed TDAR suppression well above the NOAEL/LOAEL reported in the liver studies. Lastly, the ACC notes that other studies report decreases in spleen weight after 28 days but only when treated with concentrations of 100 mg/kg/day.

EPA Response to Comments 1.3.1.a–1.3.1.c: EPA follows its guidance to select the appropriate UFs to apply when deriving an RfD (EPA, 2002). The intended purpose of the UF_D is to address the possibility that a lower reference value might result if additional data were available (EPA, 2002). EPA bases its decisions on the specific toxicity information that is lacking from the database at the time of the assessment. As the science on health effects of these GenX chemicals evolves, EPA will continue to evaluate the new evidence. EPA agrees that the database for other more data-rich PFAS, such as PFOA and PFOS, has grown over time and resulted in changes to toxicity values. EPA guidance does not allow generalized speculation of how the GenX chemicals database will change in the future to serve as justification for assigning the UF_D in the present.

EPA agrees that there is limited information identifying health effects from inhalation or dermal exposures to GenX chemicals in animals. As described in the problem formulation, consideration of dermal or inhalation routes of exposure are not among the objectives of this hazard assessment and are thus not considered when determining the UF_D.

The GenX chemicals database does not include a two-generation reproductive and developmental toxicity study. The potential for additional developmental health outcomes at doses similar to or below doses in the selected critical study cannot be surmised based on non-developmental endpoints observed in other, shorter duration studies. Moreover, recent publications on the reproductive/developmental toxicity of GenX chemicals (Blake et al., 2020; Conley et al., 2019, 2021) raise additional concern related to impacts on pregnancy that might lead to additional effects later in life. Significant adverse effects observed following exposure to HFPO dimer acid ammonium salt include changes in maternal GWG, placental lesions, early delivery of pups, decreased pup BW, decreased pup survival, and delays in the attainment of balanopreputial separation and vaginal patency in mice. The available reproductive/developmental studies report that most of these effects occur at doses higher than those resulting in liver effects; however, increases in GWG and placental lesions in mice occurred at doses in a similar range to those observed in the liver (Blake et al., 2020; Conley et al., 2019; DuPont-18405-841, 2010; DuPont-18405-1037, 2010). Blake et al. (2020) specifically reported that HFPO dimer acid exposure increased maternal GWG and histopathological lesions in the

placenta in pregnant mice at doses greater than 2 mg/kg/day (the lowest tested dose). This increase in GWG in mice was also observed in DuPont-18405-1037 (2010) at 0.5 mg/kg/day.

The study by Cannon et al. (2020) demonstrated that HFPO dimer acid has the ability to modify the activity of transporters at the blood-brain barrier. Specifically, data indicate that HFPO dimer acid inhibited P-glycoprotein and breast cancer resistance protein transport in rat brain capillaries. The potential neural effects after GenX exposure that might result from inhibition of transport activity are unknown and require additional investigation. Furthermore, Conley et al. (2019, 2021) and Blake et al. (2020) observed alterations in thyroid hormones in the pregnant dam after gestational exposure to GenX chemicals. Conley et al. (2019, 2021) demonstrated significant decreases in maternal serum total triiodothyronine and T4 levels in the pregnant rat, while Blake et al. (2020) reported a significant increase in mouse placental total T4 levels relative to control. Taken together, these studies raise the possibility that neurodevelopmental effects might result from the disruption of these thyroid hormones after GenX exposure. However, additional investigations at lower doses are needed.

Finally, Blake et al. (2020) demonstrated accumulation of HFPO dimer acid in whole mouse embryos from embryonic day (E) 11.5 to E17.5. This study highlights the need for further studies to evaluate developmental toxicity (i.e., a full two-generation reproductive toxicity study evaluating early organogenesis and additional developmental endpoints). The lack of studies evaluating these endpoints at or below doses included in the critical study is a significant gap in the understanding of the developmental toxicity of GenX chemicals.

As for the available immunotoxicity information, EPA concluded that the results of the Rushing et al. (2017) TDAR assay in combination with the supportive findings of decreased globulin levels and spleen weight provide some evidence that GenX chemicals can induce immune suppression in female mice. Without additional studies investigating endpoints such as measures of immunopathology, humoral immunity, cell-mediated immunity, nonspecific immunity, or host resistance, the immunotoxicity database is also incomplete and warranted application of a UFD.

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

1.3.2 Uncertainty Factors—Subchronic-to-Chronic Extrapolation

1.3.2.a Comments: Two commenters (NJDEP, and MDEQ in collaboration with MDHHS)) recommended increasing the uncertainty factor addressing the extrapolation from a UFs from 3 to 10. One commenter (Green Toxicology LLC) suggested that EPA should reduce the UFs to 1. Specific comments received include:

- NJDEP noted that the rationale for using a duration of exposure UF of 3 instead of the default value of 10 in the chronic RfD is unclear. NJDEP noted that on p. 56 of the GenX draft toxicity value document that: “The NOAELs for the [mouse] oral reproductive/developmental toxicity study and the [rat] chronic study are within one order of magnitude of each other, suggesting consistency in dose-response relationships between these studies. The combined chronic toxicity/oncogenicity study was conducted, however, in rats that appear to be less sensitive than mice. For these reasons, a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD.” Specifically, the commenter noted:
 - The RfD is based on hepatic single-cell necrosis in male mice exposed for 84-85 days (DuPont-18405-1037, 2010). The NOAEL for hepatic single-cell necrosis was 0.1 mg/kg/day and the LOAEL was 0.5 mg/kg/day in males.
 - In the chronic rat study, the doses were widely spaced (0, 0.1, 1, and 50 mg/kg/day in males; 0, 1, 50, 500 mg/kg/day in females), and the highest doses (males – 50 mg/kg/day; females – 500 mg/kg/day) were identified as LOAELs. The LOAEL in males for the chronic rat study (50 mg/kg/day) is therefore 100-fold higher than the LOAEL in males exposed for 84-85 days (0.5 mg/kg/day) in DuPont-18405-1037, 2010. The actual level at which no effects occur may be substantially higher than the lowest dose level (1 mg/kg/day) in the chronic study, particularly in males for which there is a 50-fold difference between the LOAEL and the NOAEL. Accordingly, the no effect levels in the mouse subchronic and rat chronic studies are not within one order of magnitude of each other.
 - As stated in the peer-reviewed publication of the chronic rat study (Caverly Rae et al., 2015): “The no-observed-adverse-effect-level in this study lies between 1 and 50 mg/kg for males and between 50 and 500 mg/kg for females.” Finally, comparison of the rat subchronic (DuPont-17751-1026, 2009) and chronic studies (Caverly Rae et al., 2015; DuPont-18405-1238, 2013) indicates that hepatic necrosis occurred after chronic exposure at doses below the subchronic NOAEL for this effect.
- NJDEP suggested that a UFs of 10 be considered by USEPA.
- MDEQ in collaboration with MDHHS noted that EPA selected a partial UFs value of 3 to represent the uncertainty of using a subchronic study to extrapolate a chronic RfD. The EPA starting point for this UFs is 10 and can be reduced through consideration of supporting information. MDEQ stated that the results of a chronic study conducted using a less sensitive animal species are solely provided in support of selecting the reduced UFs value. EPA should consider applying the entire UFs of 10 until additional data become available. The uncertainty of whether steady state tissue levels had been achieved in the subchronic study and whether the most sensitive endpoint had been identified forms the basis of the recommendation.
- Green Toxicology LLC notes that in developing its draft RfD, EPA used a factor of 3 to extrapolate from its chosen subchronic study to a lifetime (or chronic) exposure; however, there is no evidence that such a factor is needed because the 28-day studies in mice show that the endpoint is the same as seen in the 90-day study, and the dose-response curve for these studies is consistent. They further note that the 28-day studies

with 28-day recovery show that the endpoint is eliminated after the recovery period, strongly suggesting that this endpoint is a continuing steady-state process rather than a cumulative one. Thus, they suggest removing the UFs used to extrapolate from a subchronic to a chronic toxicity study.

EPA Response: The UFs is applied to account for use of a critical study with less than chronic studies in the derivation of chronic reference values. Its application addresses the possibility that, with additional exposure duration, adverse effects might be observed at lower doses. Therefore, application of a UFs is appropriate and consistent with EPA guidance (EPA, 2002).

EPA's justification of the application of the UFs of 3 in the public comment draft assessment was based on comparison of PODs, specifically NOAELs, between the chronic rat study to the available subchronic studies in mice (EPA, 2018a). Following public comment, EPA further considered the impact of duration on progression of the liver effects observed, taking into account duration of exposure and species sensitivity. Though the liver effects observed in the 2-year chronic rat study are consistent with the liver effects observed in the subchronic oral reproductive/developmental study in mice, a comparison of the LOAELs for liver effects between studies found a difference of two orders of magnitude (50 mg/kg/day in rats versus 0.5 mg/kg/day in mice). Therefore, EPA concluded that the comparison of study NOAELs is not appropriate and has removed this point from the current assessment.

Additionally, there are two key differences in the analysis that was presented in the draft assessment 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) (see 1.1.j for additional details). This is important because the males and females were exposed for different durations in the DuPont-18405-1037 (2010) study. The female mice were dosed well below the 90-day exposure window typically employed in a subchronic study; F0 females that delivered were dosed daily starting 14 days prior to pairing and were dosed through LD20 for a total of 53 to 64 days of exposure, depending on delivery date. By contrast, F0 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 to 85 days of exposure. The critical effect in female mice was observed after a shorter exposure duration than the males experienced, providing support for increasing the subchronic-to-chronic duration uncertainty factor.

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 were 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 (0%) in control versus 11/70 (16%) 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., ALP, ALT, or 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 (40%) 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, consistent with a progression of liver effects with increasing duration of treatment.

For these reasons, EPA increased the UF from a 3 to 10 to account for duration of exposure for the chronic RfD. The rationale for the UFs of 10 is described in section 7.3 (EPA, 2021a). This change in UFs does not affect the subchronic RfD because the critical study is of subchronic duration.

Although the commenters raise uncertainty concerns associated with whether steady state tissue levels had been achieved and whether the most sensitive endpoint in the subchronic study had been identified, these uncertainties are addressed by the application of the UF_A and UF_D , respectively. See the interspecies uncertainty discussion in section 1.3.3, the database uncertainty discussion in section 1.3.1, and section 7.3 of the assessment (EPA, 2021a) for additional details.

1.3.3 Uncertainty Factors—Interspecies Uncertainty

1.3.3.a Comments: The Silent Spring Institute, MDEQ in collaboration with MDHHS, and NJDEP all provided comments for clarification and in support of increasing the UF_A . Specific comments received include:

- The Silent Spring Institute stated that EPA should increase the UF_A from 3 to 10, a possible value as outlined in EPA’s own guidance document, or possibly higher in order to account for the uncertainty in toxicokinetics and toxicodynamics and based on a careful evaluation of all available data. As an example, they indicated that immune effects have been reported at lower exposures than EPA’s health advisory level for PFOS, indicating that EPA’s risk assessment approaches are not adequately health protective.
- MDEQ in collaboration with MDHHS noted that it is unclear whether EPA, in the absence of any human GenX toxicity, toxicokinetic, or exposure data, considered and eliminated any other candidate interspecies UF values or simply selected the default value of 3. Given that differences in elimination of PFOA have been attributed to modulation of organic anion transporters in male and female rats (ATSDR, 2018a) and differences in these transporters have been found between human and rat cells (Zhao et al., 2017), PFAS might not match the assumption of this default approach.
- NJDEP mentioned that the EPA chronic RfD for GenX (80 ng/kg/day) in the draft assessment is only four-fold higher than for PFOA (20 ng/kg/day) but the PFOA RfD considers the much longer half-life in humans versus mice while the GenX RfD is based on the default approach for interspecies extrapolation. NJDEP noted that the default approach recommended in EPA (2011) guidance is $BW^{3/4}$ scaling to account for toxicokinetic (and some toxicodynamic) differences in HED, with an application of UF of 3 to account for other interspecies differences. However, when chemical-specific data are available, EPA (2011) recommends other approaches (e.g., toxicokinetic modeling, “intermediate approaches” based on what is known about species differences and toxicokinetic and toxicodynamics of the chemical) be used to derive an appropriate cross-species adjustment (i.e., a data supported scaling function, a different UF, or a combination of the two). NJDEP noted that although the human half-life of GenX is not available, it is likely much longer than in mice, based on relative human and mouse half-

lives for other PFAS for which data are available (Michigan PFAS Science Advisory Panel (2018), Table 2, updated from Lau (2015)). They further noted that the Netherlands National Institute for Public Health and the Environment (Beekman et al., 2016 (cited as RIVM, 2016 in the comment)) considered the potentially much longer half-life in humans than in rodents with an interspecies toxicokinetic factor of 66, which is 10-fold greater than the factor of 6-7 based on the DAF of 0.14-0.15. If the human versus mouse half-life ratio for GenX is similar to the ratio for other PFAS, the commenter concluded that a chronic RfD that considers the relative half-lives would be lower for GenX than PFOA based on the LOAELs identified by EPA.

EPA Response: The default interspecies scaling approach outlined in EPA’s guidance (EPA, 2011) is applied specifically to address uncertainty in the absence of chemical-specific data. PFOA, PFOS, and GenX chemicals are evaluated based on their own chemical-specific database available at the time of assessment. Although GenX chemicals are part of the PFAS class, “chemical-specific” in this scenario is interpreted as the equivalent of “GenX chemical-specific data.” In the absence of data to support similarities, assumptions that GenX chemicals behave similarly to other in-class chemicals cannot be made. Additional data are needed to verify side-by-side comparisons of toxicokinetics/toxicodynamics.

As EPA guidance states, chemical-specific data are preferred (i.e., PBPK models) over the $BW^{3/4}$ default (EPA, 2011); however, until sufficient human half-life (see response to comment 1.2.a) and supporting absorption, distribution, metabolism, and excretion data become available for GenX chemicals, EPA will rely on the prescribed $BW^{3/4}$ methodology. Thus, in the absence of sufficient GenX chemical-specific toxicokinetic/toxicodynamic information, EPA followed the $BW^{3/4}$ guidance and historical agency precedent that indicates a UF_A value of 3 when $BW^{3/4}$ allometric scaling is employed (EPA, 2011).

1.3.4 Uncertainty Factors—Total Uncertainty

1.3.4.a Comments: The TEDX, Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health request that EPA increase the total uncertainty on the GenX chemicals RfDs for a variety of reasons including: their persistence in the environment, difficulty in removal from groundwater, soil and sediments, and the lack of data for GenX chemicals especially in comparison to other PFAS. Specific comments received include:

- A group of organizations, including TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that EPA proposes a total UF of 100 for the chronic RfD in the draft GenX assessment. By comparison, the group points out that EPA used a combined UF of 300 for PFOA (a chemical with a much larger toxicological database), and despite the relatively complete databases for PFOA and PFOS, and the use of UFs to account for extrapolations from laboratory studies to human health, the available evidence suggests that EPA’s practices of quantitative risk assessment were not fully protective of human health. The group is concerned that the GenX quantitative toxicity assessment is premature and that several major research efforts (e.g., *in vitro* studies by EPA and the NTP and *in vivo* studies by Blake and Fenton (2010) and Cope et al. (2019)) are underway that will provide more information about these chemicals. They ask EPA to commit to updating these toxicity

assessments and incorporating new studies on additive or synergistic effects, including any data published before the draft documents are finalized. The group also noted that the total UF for GenX chemicals used by North Carolina’s Department of Environmental Quality was 1000 and that the total UF used by the RIVM was 1088.

- Because GenX is persistent in the environment and cannot be efficiently removed from groundwater, soil and sediments, a group of organizations that includes TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health requests a larger margin of safety for GenX to add assurance of protection in the event future evidence proves a greater potency than EPA currently estimates.

EPA Response: To clarify, the comments mischaracterized the total uncertainty factor (UF_{TOT}) applied in the public comment draft (EPA, 2018a). The total UF for the chronic RfD was 300, and the total UF for the subchronic RfD was 100. The revised GenX chemicals assessment now uses a total UF of 300 for the subchronic RfD and a total UF of 3,000 for the chronic RfD. One of the reasons for the increase in uncertainty is due to the results of the NTP PWG and studies published after the public comment draft (Blake et al., 2020; Conley et al., 2019, 2021) which were reviewed and addressed during document finalization.

The GenX chemicals toxicity values reflect EPA’s analysis of the best available peer-reviewed science using EPA guidance. States might issue different values based on their own analyses, using different approaches and assumptions. RIVM’s approach was based on using PFOA clearance data as a surrogate for GenX. EPA determined that, given the minimal understanding of whether the biochemistry associated with PFOA is representative of GenX chemicals, this approach was not appropriate in the application of UFs.

Finally, EPA agrees with the commenters that GenX chemicals are expected to persist in the environment, making remediation a challenge. However, EPA interprets consideration of remediation options to be part of the risk management process and not part of the toxicological assessment process by which RfDs are calculated. As a result, EPA cannot consider an additional “margin of safety” that would increase the UF_{TOT} to account for risk management concerns because EPA guidance does not include risk management or remediation considerations among the criteria factored into the assignment of UFs.

1.3.5 Uncertainty Factors—Consideration of Other PFAS Data in Assigning Uncertainty

1.3.5.a Comment: EWG stated that EPA should review what was learned from PFOA and PFOS in recent studies in comparison with previously assumed “safety factors”. In addition, EPA should reevaluate the use of these safety factors if future scientific research supports a need for a greater safety factor, especially for children’s health protection from this class of toxic chemicals. EWG mentioned that this factor of 3 does not sufficiently capture the full extent of PFAS toxicity as has been demonstrated for chemically related compounds, nor is it protective of human health. They noted that filling critical data gaps about toxicity for PFOA and PFOS (which are better-studied chemicals than GenX) has supported a reduction in guideline values of tenfold to a hundredfold or greater. EWG noted that the changes in relative RfD levels calculated

from recent studies on PFOA and PFOS as compared to studies from decades ago should be used to set UFs for data gaps in the GenX.

EPA Response: EPA human health risk assessment practices include the evaluation of the risks associated with exposure to a given chemical based on the completeness of the database at the time of the assessment. The science describing chemical toxicity evolves over time, and EPA will continue to evaluate new literature as it becomes available. Indeed, new toxicokinetic and toxicological information resulting from multiple studies published after the public comment draft (Blake et al., 2020; Conley et al., 2019, 2021) served to heighten concern regarding the impact of GenX chemicals exposure on reproduction, development, and neurotoxicity. As a result, EPA has increased the UF_D from 3 to 10 in the final assessment. See the UF_D response above and section 7.3 of the assessment (EPA, 2021a) for additional details.

1.3.5.b Comment: A group of organizations that includes TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health stated that most people with risk of exposure to GenX chemicals will generally have greater than typical exposures to legacy PFAS chemicals as well and that these chemicals will impact the same body systems as other, better-studied PFAS. They therefore recommended that EPA should use a UF_D of 10 to account for the high likelihood of additive effects from exposure to GenX with other legacy PFAS.

EPA Response: In the draft and final assessments (EPA, 2018a, 2021a), EPA evaluated the human health hazards associated with exposures to GenX chemicals. EPA followed its guidance in making determinations as to the appropriate UFs to apply when deriving the RfDs for GenX chemicals (EPA, 2002). The MOAs underlying the effects associated with GenX chemicals exposure are unknown, as are the MOAs for PFOA, PFOS, and other PFAS. Therefore, it is also unknown whether effects from exposure to multiple PFAS are additive. It is inappropriate and a deviation from EPA guidance to increase the UF_D based on the possibility of additive effects after exposure to multiple PFAS. However, new toxicokinetic and toxicological information (i.e., Blake et al., 2020; Conley et al., 2019, 2021) provides hazard information regarding the impact of GenX chemicals exposure on reproduction, development, and neurotoxicity. As a result, EPA has increased the UF_D from 3 to 10 in the final assessment (EPA, 2021a). See the UF_D response above in section 1.3.1 and section 7.3 of the assessment for additional details. Additionally, future EPA actions on PFAS chemicals could draw on the present understanding of GenX chemicals toxicity, as well as for other PFAS chemicals, when conducting a cumulative assessment of risk for a broader group of PFAS. Similar toxicity assessments of human health hazards for other PFAS chemicals are underway at EPA (EPA, 2020a).

1.3.5.c Comment: A group of organizations that includes TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health stated that:

- The data EPA reviewed suggest that GenX chemicals share many of the same toxicity endpoints as the legacy PFAS chemicals they replaced, including harm to the liver, thyroid, and kidney.

EPA Response: EPA agrees that GenX chemical exposures can lead to adverse effects on the liver, kidney, and immune system; adverse developmental effects; and cancer, and that these health effects have been associated with exposure to other PFAS (e.g., PFOA).

In this assessment, EPA is assessing the human health hazards associated only with exposure to GenX chemicals and not a cumulative assessment of risk due to exposure to multiple PFAS.

- People with GenX exposure undoubtedly have exposures to legacy and other PFAS chemicals as evidenced by the studies in drinking water in the Cape Fear river which found most tap water had GenX, Nafion byproduct 2, 2,2-difluoro-2-(trifluoromethoxy)-acetic acid (PFMOAA), 2-[difluoro(trifluoromethoxy)methoxy]-2,2-difluoroacetic acid (PFO2HxA), and 3,5,7,9-tetraoxadecanoic perfluoro acid (PFO4DA) (NCSU CHHE 2018a).

EPA Response: The assessment is for the defined GenX chemicals; it is not a cumulative assessment of risk associated with exposure to multiple or all PFAS. Future EPA efforts could focus on cumulative risk. See reference to cumulative risk comment above.

1.3.6 Quality of the Data Used in the Assessment

1.3.6.a Comments: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that the database includes limited peer-reviewed, independently funded studies for HFPO dimer acid and its ammonium salt: Of the studies that assess health effects of GenX, only three were peer-reviewed. Of these three, one was independently funded (Rushing et al., 2017), one was funded by DuPont (Caverly Rae et al., 2015), and one was independently funded but excluded from the assessment (Wang et al., 2017). The group strongly urges EPA to update and strengthen its GenX assessment by ensuring that it relies upon a more robust data set. Similarly, MDEQ in collaboration with MDHHS noted that the majority of the data for the GenX draft toxicity value document was submitted to EPA by DuPont under the Toxic Substances Control Act (TSCA). As such, these studies and data therein did not undergo the robust scientific peer review typical of studies in the published literature. MDEQ in collaboration with MDHHS further stated the need for additional toxicology and epidemiological studies that would provide the necessary information to more adequately evaluate GenX exposure, including laboratory animal studies examining mixtures of PFAS, as well as having EPA conduct a focused literature review to identify recently published studies that address these data gaps before finalization of the toxicity value documents.

EPA Response: Most of the available data for HFPO dimer acid and its ammonium salt, including premanufacture notices (PMNs), were submitted to EPA by DuPont (now Chemours), the manufacturer of GenX chemicals, under TSCA (Title 15 of the United States Code (U.S.C.) § 2601 et seq.), as required pursuant to a consent order (EPA, 2009) or as required under TSCA reporting requirements (15 U.S.C. § 2607.8(e)). Submitted test data on HFPO dimer acid and its ammonium salt were available for numerous endpoints such as acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity in mice and rats with dosing durations of up to 2 years. Most of these submitted studies were conducted according to Organization for Economic Cooperation and Development (OECD) TGs and/or EPA health effects TGs for pesticides and toxic substances, which “are generally intended to meet testing requirements for human health impacts of chemical substances under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. § 136 et seq.) and TSCA.” The majority of the TSCA-submitted industry studies included in this assessment adhered to the principles of Good Laboratory Practices, and

full study reports were submitted for agency review. EPA included all of the available data on GenX chemicals that were either peer-reviewed or submitted to TSCA.

In addition to the 27 studies identified through literature searches and included in the public comment draft (EPA, 2018a), updated literature searches were conducted in February 2019, October 2019, and on March 3, 2020; these resulted in 48 additional studies from the peer-reviewed literature that were identified since the 2018 public comment draft. Of the 48 studies, three studies provided dose-response toxicity information and were incorporated into the assessment. All studies containing mechanistic information were summarized in section 4.6.2 of the assessment (EPA, 2021a). Included in the mechanistic study summaries is a summary for the Wang et al. study (2017). Additionally, another peer-reviewed study providing dose-response toxicity information (Conley et al., 2021) was identified after the final literature search and was added to the assessment.

The studies submitted under TSCA and literature identified by the search of publicly available sources are available to the public through EPA's Health & Environmental Research Online (HERO) website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

In accordance with EPA's Office of Research and Development (ORD) systematic review practices, relevancy screenings were conducted on all the studies submitted from DuPont/Chemours and the publicly available, peer-reviewed literature resulting from the literature searches mentioned above (EPA, 2021a).

The 12 industry or peer-reviewed studies providing dose-response information were then evaluated for study quality using an approach consistent with the draft ORD Handbook for developing Integrated Risk Information System (IRIS) assessments (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; EPA, 2020b; Rushing et al., 2017; Thompson et al., 2019). This study quality evaluation method is an in-depth process using three independent reviewers with expertise in toxicological studies and results in quality rating of low, medium, or high. Study quality was determined by two independent reviewers who assessed risk of bias and sensitivity in the following domains: reporting quality, risk of bias (selection or performance bias, confounding/variable control, and reporting or attrition bias), and study sensitivity (exposure methods sensitivity, and outcome measures and results display) using EPA's version of the health assessment workspace collaborative (HAWC). A third reviewer made the final decision on the quality ratings based on the primary and secondary reviewer ratings. Importantly, all of the studies considered for dose-response received a quality score of medium or high. The results of the study quality evaluation are provided in Figure 3 of the assessment (EPA, 2021a), and an interactive version of the heatmap can be found here:

<https://hawcprd.epa.gov/summary/visual/assessment/100500273/GenX-SQE-Heatmap/>.

1.3.6.b Comment: NJDEP noted that the prenatal and developmental study in rats did not assess postnatal mortality but an abstract for the 2019 Society of Toxicology (SOT) meeting (Conley et al., 2019) reports that maternal doses of 10-250 mg/kg/day GenX on GD8-PND3 “resulted in significant, dose-response neonatal mortality at ≥ 62.5 mg/kg/d and reduced body weight of surviving pups at all doses (≥ 10 mg/kg/d).”

EPA Response: EPA is aware of the cited meeting presentation, and the recent publication of this study (Conley et al., 2019) has been included in the updates to sections 4.5 and 5.4 in the assessment (EPA, 2021a).

1.4 MODE OF ACTION (MOA)—PPAR α

1.4.a Comment: The American Water Works Association (AWWA) and National Association of Water Companies (NAWC) point out that the GenX document includes a possible MOA but does not indicate a high degree of confidence that the actual MOA is known. They further argue that a basic understanding of why toxicity may be observed is needed to establish the biological plausibility and toxicity values used in risk assessment and communication.

EPA Response: The MOA(s) for GenX chemicals are currently unknown. There is some evidence that supports PPAR α as one potential MOA for these chemicals. The document has been revised to include additional clarifying language in the MOA discussion (section 6.0 of EPA, 2021a), confirming that PPAR α is a potential MOA (see EPA response to comment 1.1.k, above). Language has also been added to describe other plausible MOAs. Toxicity data, not MOA data, is necessary to perform a toxicity assessment.

1.4.b Comment: Dr. James Klaunig, in comments submitted on behalf of Chemours, commented that the studies on the GenX compounds cited in the draft document (section 4.7 and elsewhere) overwhelmingly support a PPAR α MOA for GenX compounds. The commenter states that there is a misunderstanding of the concept of the MOA in general and of the PPAR α MOA specifically. In the case of the PPAR α MOA for liver, taking the established key events in temporal order are:

- Key Event 1, Activation of PPAR α (activation of the receptor)
- Key Event 2, Induction of cell growth genes
- Key Event 3, Increase in cell growth (this can be achieved via increased cell proliferation and/or a decrease in apoptosis)
- Key Event 4, Selective clonal expansion of the preneoplastic focal lesions
- Key Event 5, Formation of hepatic neoplasms

The commenter stated that the demonstration of these key events can be through the measurement of the key event itself or through the use of surrogate markers (associated events). The commenter explained that surrogate markers for PPAR activation are the induction of palmitoyl-coenzyme A oxidation and peroxisome proliferation; both of these associated events have been demonstrated in multiple studies examining GenX compounds in rodents, confirming Key Event 1. Key Events 2 and 3 (involving induction of growth genes and resulting increase in cell growth) have been demonstrated for GenX compounds by observed increased mitosis. The commenter mentioned that once the activation of PPAR α has been demonstrated by a compound, then the PPAR α MOA is established. Further, the commenter argued that additional evidence for PPAR α MOA with GenX compounds is the observed targeted effects of the GenX compounds on the pancreas, liver and Leydig cells in the rat (these tissues are uniquely linked to many PPAR α activators in the rat). In addition, where the serum cholesterol was measured in rodent

studies cited in the draft GenX document, a reduction in the serum cholesterol, an established attribute of PPAR α activating compounds, was noted.

EPA Response: The revised final toxicity document includes additional clarifying language in the MOA discussion (section 6.0 in EPA, 2021a); those data indicate that the PPAR α MOA and other MOAs are likely operative in the liver. Section 6.0 outlines the supporting data and the data gaps that exist for each key event outlined in the comment. EPA disagrees that Key Events 2 and 3 have been demonstrated by observed increased mitosis after exposure to GenX chemicals because no increases in mitosis or decrease in apoptosis were observed at the LOAEL in the developmental and reproductive study in mice, yet necrosis is observed. Additional language has also been added to describe other plausible MOAs.

1.4.c Comment: Dr. James Klaunig, in comments submitted on behalf of Chemours, provided the following specific corrections to the MOA section of the draft GenX document:

1. “The Draft Document states that the lack of the demonstration of steatosis is a data gap for the establishment of the PPAR α MOA. “Other indicators such as steatosis were not assessed in any of the DuPont/Chemours studies.” Steatosis (fatty change) involves the abnormal accumulation of lipids within the hepatocyte. PPAR α in the liver cell regulates the uptake, utilization, and catabolism of fatty acids by upregulating the genes involved in fatty acid transport. Some hypolipidemic drugs (fibrate drugs) are PPAR α activators (for example bezafibrate) and they are used clinically to reduce steatosis in humans. The requirement for steatosis as a demonstration of PPAR activation MOA is incorrect and this statement should be removed from the document.”

EPA Response: Thank you for the comment. References to steatosis have been removed from the MOA section of the assessment.

2. “The reference to PPAR α agonism needs to be changed to PPAR α activation. Agonism infers the requirement for binding to the PPAR α receptor. Many PPAR α activators do not bind to the PPAR α receptor but activate the receptor producing downstream effects.”

EPA Response: Thank you for the comment. References to PPAR α agonism have been changed to PPAR α activation where appropriate in the assessment (EPA, 2021a).

3. The commenter notes that important peer reviewed literature on the PPAR α MOA for liver tumors is missing from the discussion on the MOA in the draft GenX document and has provided a list of these references for inclusion.

EPA Response: Thank you for the comment and for providing additional references. The Elmore et al. publication (2016) is included in the final assessment. The MOA section in the assessment (section 6.0 in EPA, 2021a) addresses all observed toxicity resulting from exposure to GenX chemicals (e.g., liver necrosis) that is considered for the development of an RfD. The development of liver tumors is outside the scope of this discussion, so the other references provided on PPAR α and liver tumors have not been added.

1.4.d Comment: Several commenters, including NJDEP, Dr. Damian Shea, and Green Toxicology LLC, noted that EPA states that increased liver weight or hepatocellular hypertrophy (in the absence of other liver toxicity) may result from PPAR α activation, which may be more relevant to rodents than humans. NJDEP notes, however, that multiple lines of evidence

demonstrated that increased liver weight or hepatocellular hypertrophy caused by other PFAS are partially or primarily independent of PPAR α . NJDEP requested that EPA provide clarification on whether the more severe hepatic effects (e.g., necrosis, inflammation, fibrosis) that accompany the increased liver weight or hepatocellular hypertrophy considered relevant to humans by EPA are potentially related to PPAR α . NJDEP noted that it is well documented that PPAR α activation is not required for the increased liver weight caused by PFOA, PFNA, and PFOS (reviewed in DWQI, 2015; DWQI, 2017; DWQI, 2018; NJDEP, 2018; Post et al., 2017).

EPA Response: To address this comment, EPA has revised this discussion in sections 6 and 7 of the assessment (EPA, 2021a). The available data indicate that multiple MOAs might be involved in the liver toxicity resulting from exposure to GenX chemicals. PPAR α is one MOA that is likely a contributor to the observed toxicity; however, the degree to which PPAR α drives the toxicity is unknown. The commenter correctly notes that PPAR α activation is not required for liver effects observed with other PFAS. Following exposure to GenX chemicals, the degree to which the observed necrosis is related to PPAR α is unclear. Because EPA does not have a well-established MOA or multiple MOAs for the rodent liver effects, EPA relied on the Hall criteria to guide the determination of what effects should be considered adverse when determining the NOAELs/LOAELs summarized in Table 12 (Hall et al., 2012). This is consistent with recommendations from the panel peer review of the EPA Office of Water PFOA and PFOS health effects support documents (see the response to peer review [here](#) for more details). Additionally, the NTP PWG indicated that the constellation of observed liver lesions (i.e., cytoplasmic alteration (which includes hypertrophy), apoptosis, single-cell necrosis, and focal necrosis) are adverse in DuPont-18405-1307 (2010) and DuPont-18405-1037 (2010).

1.4.e Comment: NJDEP indicated that on page 43 of the GenX document, hepatic steatosis is mentioned as an indicator that is consistent with PPAR α agonism; however, this is not necessarily the case. The commenter noted that perfluoroalkyl acids (PFAAs) induce hepatic steatosis, while strong PPAR α activators do not, and that some PFAAs cause hepatic steatosis in PPAR α -null mice (Das et al., 2017).

EPA Response: Thank you for the comment. This reference to hepatic steatosis has been removed (see EPA response to 1.4.c, above).

1.4.f Comment: NJDEP notes that the following text is unclear in section 5.1 on page 43 of the GenX document:

“Hepatocellular hypertrophy and an increased liver-to-BW ratio are common findings in rodents but are considered nonadverse and less relevant to humans when there is evidence for PPAR α activation. The increased relative liver weight and hepatocellular hypertrophy are only considered adverse when they are accompanied by effects such as necrosis, fibrosis, inflammation, steatosis, and significantly increased serum levels for enzymes indicative of liver tissue damage (Hall et al., 2012).”

They note that the statements (above) raise the following questions about the consistency of EPA’s approach to liver toxicity:

- Are hepatocellular hypertrophy and increased liver-to-BW ratio, in the absence of other effects, always considered non-adverse and less relevant to humans by USEPA, or only when there is evidence for PPAR α activation?
- Does USEPA consider the other more severe effects mentioned (necrosis, etc.) to be indicative of a non-PPAR α MOA that is more relevant to humans?

EPA Response: Because for some PFAS (e.g., PFOA), PPAR α activation in rodents has been proposed as a potential MOA for the observed liver endpoints, specifically liver cancer (Klaunig et al., 2003, 2012; Maloney and Waxman, 1999), EPA evaluated liver effects using the Hall et al. criteria (2012). Hepatocellular hypertrophy and an increased liver-to-BW ratio are common findings in rodents when PPAR α activation leads to peroxisome proliferation. Hepatic necrosis, effects on bile ducts, and other signs of liver damage that are unrelated to PPAR α activation when observed in conjunction with the increased liver weight and hepatocellular hypertrophy are sufficient to justify the liver weight and hypertrophy as adverse (Hall et al., 2012). No changes were made.

1.4.g Comment: ToxStrategies, Inc. noted that an *in vivo* study of exposure of mice to peroxisomal proliferator Wy-14,643 has been shown to increase apoptosis in the liver of wild-type mice but not PPAR α null mice (Xiao et al., 2006), thus indicating that increased apoptosis *in vivo* is part of PPAR α signaling. The commenter states that the increase in apoptosis in mice treated with GenX supports the involvement of PPAR α in the MOA for GenX in the mouse liver, and the updated diagnosis of “single cell necrosis” in male mice as apoptosis.

EPA Response: Please see the response to comment 1.1.j and the discussion on potential MOAs in section 6.0 of the assessment (EPA, 2021a).

1.4.h Comment: ToxStrategies, Inc. and Dr. James Klaunig (in comments submitted on behalf of Chemours) asked that EPA reevaluate its conclusion that GenX does not act through a MOA consistent with peroxisome proliferators. Specifically, ToxStrategies, Inc. note that several of the OECD TG studies on GenX reported effects consistent with peroxisome proliferators (histopathology indicative of increased peroxisomes, increased liver weight, dose-dependent increases in liver peroxisomal enzyme activity); mice administered GenX were shown to exhibit significant enrichment of genes related to signaling related to PPAR pathways in the liver (Wang et al., 2017), and it is well-recognized that perfluorinated chemicals cause liver effects via the PPAR α pathway (Klaunig et al., 2012; Rosen et al., 2008; Vanden Heuvel et al., 2006).

EPA Response: EPA describes the data supporting activation of the peroxisome proliferator-activated receptor pathways in detail in section 6.0 (EPA, 2021a). EPA has revised the document to indicate that, at this time, the findings regarding the PPAR α MOA are not adequate to conclude that a PPAR α MOA is solely operative for HFPO dimer acid and/or ammonium salt. Contrary to the commenter’s assertion, there is uncertainty about the MOA(s) for GenX chemicals even though some of the available data are consistent with a peroxisome proliferation MOA.

EPA acknowledges that activation of PPAR α could be one of multiple possible MOAs for GenX chemicals. At this time, there are insufficient data to conclude that PPAR α activation is the sole mechanism underlying the liver effects associated with exposure to GenX chemicals. For example, there are no studies investigating GenX chemical exposure in PPAR α -null mice.

Further, it is worth noting that exposure to PFOA has been demonstrated to induce liver effects in PPAR α -null mice, including hepatocellular hypertrophy (Minata et al., 2010). Liver necrosis was consistently observed in rodent toxicity studies after exposure to HFPO dimer acid ammonium salt and was reaffirmed by the NTP PWG's review of the 90-day subchronic study in mice (DuPont 18405-1307, 2010) and the reproductive/developmental study in mice (DuPont 18405-1037, 2010) (see appendix E in EPA, 2021a), which is consistent with cytotoxicity as a possible MOA. Recent studies have also demonstrated that GenX exposure in rats and in HEK 293 embryonal kidney cells activates genes associated with the PPAR γ signaling pathway, indicating that liver toxicity might extend beyond a single PPAR-based MOA (Conley et al., 2019; Li et al., 2019). See EPA response to comment 1.1.k.

1.5 LITERATURE SEARCH AND SCREENING

1.5.1 Identification of New Literature

1.5.1.a Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health mentioned that new toxicity data on GenX chemicals is expected to be available soon, as there are several studies abstracts submitted for presentation at the upcoming SOT meeting in March 2019. In one study of gestationally exposed mice, puberty delays were evident in female pups exposed to PFOA or 10 mg/kg GenX. Mammary gland development was also stunted in all dose groups of GenX and PFOA, with mammary glands from exposed mice displaying limited branching, lack of ductal growth, and fewer terminal end buds (Cope et al., 2019).

EPA Response: Thank you for communicating this important information. EPA has included literature published through March 2021 in the assessment (EPA, 2021a); these were the data available at the time the systematic review was conducted. The science describing chemical toxicity is constantly evolving. EPA will continue to evaluate new literature as it becomes available.

1.5.2 Systematic Literature Review

1.5.2.a Comment: Several commenters, including the Environmental Protection Network (EPN), NJDEP, TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health, noted that the systematic review process (i.e., the draft 2018 guidance entitled "Application of Systematic Review in TSCA Risk Evaluations") used to derive RfDs is inconsistent with best practices in systematic review and should not be used. The commenters had submitted detailed criticisms of the draft systematic review process, including what they characterize as EPA's failures to follow necessary internal and external peer review procedures in developing that process, serious flaws throughout the TSCA systematic review process, and critical flaws in evaluating individual studies for use in toxicity assessments.

NJDEP also noted that EPA used two separate approaches to systematic review in the GenX and PFBS documents and urged EPA to ensure that the two approaches were not in conflict and to maximize the best of both approaches. NJDEP noted that the criteria and scoring systems for these two approaches were different and this could result in differing conclusions in the level of confidence in a study and potentially impacting the overall outcome of the toxicity evaluations.

In addition, the PFBS document discusses the level of confidence in the RfDs while the GenX document does not. The comments from TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health urged EPA to reformat and reevaluate the data in the GenX assessment so that it, like the PFBS assessment, adheres to best practice guidelines for systematic review; they noted that this would help avoid confusion as to why these two assessments were conducted using different methods. A commenter requested increased transparency regarding the inclusion and exclusion criteria that were used during the study screening process.

EPA Response: Thank you for your comments. The OPPT systematic review protocol that was used in the draft GenX toxicity assessment for public comment (EPA, 2018a) underwent an external peer review by the National Academies of Science, Engineering, and Medicine following the release of the public comment draft. As a result of the National Academy review and recommendations, OPPT stated that “EPA is not using, and will not again use, the systematic review approach” that was used in the draft for public comment (see link for additional detail: <https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/application-systematic-review-tsca-risk-evaluations>). Therefore, EPA subsequently performed the systematic review for the GenX chemicals database in accordance with EPA’s ORD systematic review practices (EPA, 2021a) and removed reference to the findings from the TSCA systematic review methods used in the draft toxicity assessment. Specifically, relevancy screenings were conducted on all the studies submitted from DuPont/Chemours and the publicly available, peer-reviewed literature resulting from the literature searches. These studies were subjected to title and abstract screening to determine relevancy according to the population, exposure, comparator, and outcome (PECO) criteria statement/inclusion and exclusion criteria outlined in Table A-6 in appendix A of the assessment (EPA, 2021a). The title and abstract of each study were independently screened by two screeners using Distiller SR. The studies that met the PECO criteria were tagged as having relevant human data, animal data in a mammalian model, or a PBPK model. A study was included as relevant if it was unclear from the title and abstract whether it met the inclusion or exclusion criteria. Studies that did not meet the inclusion criteria but provide supporting information were categorized as supplemental, relative to the type of supporting information they provided. When two screeners did not agree if a study should be included, excluded, or tagged as supplemental, a third reviewer made the final decision. The title and abstract screening resulted in 12 studies tagged as relevant (i.e., containing dose-response information). The relevancy of these studies was confirmed by a full-text review.

The 12 studies providing dose-response information were then evaluated for study quality using an approach consistent with the draft ORD Handbook for developing IRIS assessments (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; EPA, 2020b; Rushing et al., 2017; Thompson et al., 2019). Study quality was determined by two independent reviewers who assessed risk of bias and sensitivity for the following domains: reporting quality, risk of bias (selection or performance bias, confounding/variable control, and reporting or attrition bias), and study sensitivity (exposure methods sensitivity, and outcome measures and results display) using EPA’s version of HAWC. A third reviewer made the final decision on the quality ratings based on the primary ratings. Importantly, all of the studies considered for dose-response received a quality score of medium or high. The results of the study quality evaluation are provided below, and an

interactive version of the heatmap can be found here:

<https://hawcprd.epa.gov/summary/visual/assessment/100500273/GenX-SQE-Heatmap/>.

1.5.2.b Comment: The Toxics Use Reduction Institute (TURI) noted that it was important that EPA collected and summarized peer-reviewed studies and conducted a systematic literature review because information in the GenX document provided essential information to begin a state-level review.

EPA Response: Thank you for your comment. Additional searches of the peer-reviewed literature were completed in February 2019 and October 2019, and on March 3, 2020; the relevant studies identified from those searches are summarized in the assessment. EPA also identified Conley et al. (2021) after the final literature search and added it to the assessment (EPA, 2021a). The resulting studies are publicly available in EPA's HERO database (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2627).

1.5.2.c Comment: The ACC stated that incorporation of more thoughtfully developed exclusion criteria gives the EPA GenX assessment greater transparency compared to the PFBS assessment. Additional strengths of the GenX assessment were noted including the predesignated mathematical evaluation of included studies (which helps smooth the implications of skewed evaluations due to potential bias in selection) and consistent and thoughtful designation of weighting factors to account for the fact that some domains are more important to the quality and utility of study findings than others. The ACC argued that a method for truly integrating the various data streams in determining the most appropriate basis for defining a toxicity value was not provided because a single study defines the health outcome for regulatory purposes. They further suggested that eliminating data because it does not produce the most conservative value, is by definition, not considering all available information in the final assessment, and concluded that this is a major flaw in most systematic review processes.

EPA Response: Thank you for your comment. EPA updated the study quality evaluation for the GenX chemicals studies containing dose-response information in accordance with EPA's ORD systematic review practices (EPA, 2021a) in the revised the assessment. See EPA response to comment 1.5.2.a.

1.5.2.d Comment: The ACC argued that EPA's decision to reject the liver results from the 90-day subchronic study (DuPont-18405-1307) raises concerns about the approach EPA has taken in integrating data from the various studies as part of its systematic review. Both this study and the reproductive/developmental study used in the assessment were assigned an overall quality level of "High" in EPA's data evaluation tables and both received the best possible weighted score of "1" in relation to the number of animals per group. The ACC notes that any concern about the number of animals in the 90-day study should have been reflected in the data evaluation and scoring as opposed to it being an arbitrary decision to choose one study over another based solely on generating a lower value.

EPA Response: EPA considered studies that observed adverse liver effects at the lowest dose tested in the selection of the critical study for derivation of the RfDs. Liver effects observed in the 90-day study in mice (DuPont-18405-1307, 2010) were observed at higher doses (greater than or equal to 5 mg/kg/day) than in the oral reproductive/developmental toxicity study in mice (0.5 mg/kg/day), as noted by the NTP PWG (see appendix E in EPA, 2021a).

EPA disagrees with the comment; the results in the 90-day toxicity study in mice were included. EPA sent the 90-day study in mice (DuPont-18405-1307, 2010) to the NTP PWG for reanalysis because it is a key study supplying important dose-response information about exposure to GenX chemicals. EPA is transparent about the number of animals in each study in order to highlight a possible explanation for why liver effects are observed in the developmental/reproductive study (DuPont-18405-1037, 2010) in the 0.5-mg/kg/day dose group but are not observed in the 90-day study (DuPont-18405-1307, 2010) in the 0.5-mg/kg/day dose group. In fact, liver damage was observed at 0.5 mg/kg/day in the 90-day toxicity study in mice, although these effects did not reach a statistically significant difference from the control group. Specifically, absolute and relative liver weight increased in males by 12% and 11%, respectively, relative to control mice at 0.5 mg/kg/day. In males dosed with 0.5 mg/kg/day, 4/10 (40%) livers were observed to be discolored, compared to 0/10 (0%) for control mice. Increases in serum liver proteins were observed at 0.5 mg/kg/day in males, although they did not differ significantly from control. AST, ALP, and ALT increased 35%, 40%, and 35%, respectively, compared to the control. Finally, the NTP PWG reported that 10 out of 10 (100%) male mice exhibited cytoplasmic alteration at the 0.5-mg/kg/day dose, compared to 0 in the controls in this study. Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions considered adverse, 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. In agreement with the Hall criteria (Hall et al., 2012), EPA did not consider the cytoplasmic alteration alone as an adverse effect in this dose group, resulting in the constellation of liver lesions in the male mice being a high-dose group effect. Additionally, the female mice in this study did not exhibit a dose response for the constellation of liver lesions, although focal necrosis was observed in the 0.5-mg/kg/day dose group. Thus, the difference in the NOAEL for liver effects between the 90-day study and the reproductive/developmental study likely reflects the difference in animal number per dose group. No changes were made.

1.5.2.e Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health note that a numerical scoring system is not in line with current best practices for systematic review methodology. The U.S. Institute of Medicine recommends standards for conducting high quality systematic reviews that specifically warn against scoring systems (Institute of Medicine, 2011).

EPA Response: Thank you for the comment. Please see the response to comment 1.5.2.a. EPA's evaluation demonstrates that, in the case of GenX chemicals, systematic review methodologies used for GenX chemicals and for PFBS provided similar results.

1.5.2.f Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that one portion of the literature search for HFPO dimer acid was completed in July 2017 and had not been updated; EPA should have performed the updated search for HFPO dimer acid when the search for HFPO dimer ammonium salt was conducted in January/February 2018. The group asks that EPA ensure that all literature searches are conducted within 6 months of final publication and that the cut-off date is reported in the assessments. In addition, future health assessments should consider the solvent used for preparation and storage of the chemicals because it could have an effect on the stability of the chemicals.

EPA Response: Thank you for the comment. EPA conducted literature searches for HFPO dimer acid and its ammonium salt in February 2019, October 2019, and a final literature search in

March 2020 prior to publication of the final toxicity values. All relevant literature has been incorporated into the assessment. Any studies containing dose-response data were subject to the systematic review process outlined in the assessment (EPA, 2021a). Additionally, all studies containing mechanistic information are summarized in section 4.6.2.

1.5.2.g Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health mentioned that “GenX chemicals” has been too narrowly defined by the literature search terms used and this information should be provided in a protocol made available before the assessment was conducted.

EPA Response: This toxicity assessment was for HFPO dimer acid and its ammonium salt. The search terms reflect the subject of the assessment (EPA, 2018a, 2021a). As EPA states in the assessment, the assessment is specific to the two of the chemicals used in the GenX processing aid technology. There could be future assessments that may also fall into the GenX chemicals category, but this assessment is specific to HFPO dimer acid and its ammonium salt.

1.6 PFAS USES AND TSCA

1.6.a Comment: The Silent Spring Institute noted that because of the extreme persistence, mobility and toxicity of PFAS, EPA should ban all production and limits on importing PFAS to prevent further environmental PFAS contamination.

EPA Response: TSCA is a risk-based statute. Before EPA can exercise its regulatory authorities under TSCA sections 5 or 6 to, for example, prohibit or restrict manufacture or import of a chemical, EPA must first conduct an assessment to determine whether that chemical presents risks to health or the environment. Where unreasonable risks are identified through that process, TSCA mandates that EPA take action to address those risks. In other words, for both new and existing chemicals, a risk assessment/evaluation and determination must precede any risk management action under TSCA.

While some PFAS were in commerce when the TSCA inventory was established and were not subject to new chemical review, EPA has conducted risk assessment on new PFASs introduced as new chemicals since the 1970s.

1.6.b Comment: EWG requested that EPA block any new PFAS chemicals from the market until complete toxicity testing information for these chemicals becomes available. EPA’s draft GenX toxicity assessment reinforces a major concern of scientists that a PFAS chemical that is not either PFOA or PFOS does not indicate that such a chemical is safe. EPA should assume that any new PFAS has the potential to be as toxic as the most potent PFAS studied to date.

EPA Response: Please see the response to comment 1.6.a. TSCA does not require any specific toxicity testing to be conducted for submission of a premanufacture notice for any new chemical. In review of new PFAS chemicals, EPA relies on read-across from other PFAS chemicals, including PFOA, PFOS, and other chain-length PFAS for which toxicity data exist. Because it is known that the nature and extent of toxicity of PFAS vary depending on structural features and physical-chemical properties, the existing PFAS with the most structurally and biologically similarity is most likely to be used to indicate the toxicity potential in assessing the new PFAS. When similarity to an existing PFAS is insufficient, EPA can require testing be conducted on the

new PFAS. In fact, when GenX chemicals were reviewed as new chemicals, EPA did exercise its authority to require including toxicity studies in multiple species via multiple routes of exposure and durations, including a 2-year cancer bioassay.

1.6.c Comment: TURI noted that in the case of PFAS chemicals, a prevention-oriented approach is relevant because many communities face contaminated drinking water and cleanup costs from past PFAS contamination. Toxics use reduction can help prevent additional contamination for occurring in the future.

EPA Response: The comment is noted; however, it addresses potential risk management and regulatory approaches that might be employed for addressing PFAS contamination. As stated elsewhere, the scientific objective of this particular hazard assessment is solely to “provide the health effects basis for the development of oral RfDs for subchronic and chronic durations for GenX chemicals.” Risk management and any subsequent regulatory approaches for addressing risk are beyond the stated goals of this hazard assessment.

1.6.d Comment: NJDEP mentioned that it cannot be assumed that short-chain PFAS with non-PFAA structures, such as GenX, are “safe” replacements for phased out long-chain PFAAs. An example of the potential toxicity of a replacement PFAS already approved by EPA based on minimal toxicity data is a substance referred to as “Solvay’s Product” (CAS 329238-24-6) (Wang et al., 2013), a mixture of fluorinated polyether congeners with greater than 7 carbons; multiple congeners of the product have been tentatively identified in environmental media in New Jersey. NJDEP noted that the toxicological effects of GenX are similar to those of PFOA and subsequent to EPA’s approval, GenX was found to cause reproductive, developmental and carcinogenic effects. They further noted that GenX has been found in ground water, surface water, and drinking water in the United States and overseas. NJDEP noted that it is unfortunate that EPA did not develop toxicity values for GenX until after this widespread contamination was discovered and became a public concern.

EPA Response: Section 1.1 of the assessment (EPA, 2021a) describes the history of EPA’s assessment of GenX chemicals. As part of the TSCA process for new chemicals, EPA completed a PMN assessment, which included a hazard assessment of the toxicity data submitted to EPA by DuPont/Chemours which was used to conduct the risk assessment for GenX chemicals. EPA also exercised its TSCA authority to require additional toxicity studies in multiple species via multiple routes of exposure and durations, including a 2-year cancer bioassay. The 28-day study in mice (DuPont-24459, 2008), which was submitted with the original PMN, was selected as the primary basis for the POD in the PMN assessment. Additional submitted studies were also used, in concert with information on other PFAS chemicals, to inform the decision for further testing included in the consent order that concluded the PMN review (EPA, 2009). Furthermore, EPA issued an enforceable consent order for the GenX chemicals, which required significantly limited releases of GenX chemicals to air and water from Chemours/DuPont facilities.

2 POLICY QUESTIONS

2.1 POTENTIAL RISK MANAGEMENT AND REGULATORY APPROACHES

2.1.a Comment: MDEQ in collaboration with MDHHS, several anonymous citizens, and EPN requested that EPA develop a regulatory standard for GenX, especially to protect babies and children. Several of these commenters indicated that they had concerns about being exposed to these chemicals and wanted EPA to set the GenX standard to 0 ppt to protect contaminated communities.

EPA Response: EPA is committed to following the Safe Drinking Water Act (SDWA) process for evaluating and establishing drinking water standards for PFAS chemicals (42 U.S.C. § 300f et seq.). This process is designed to ensure public participation, transparency, and the use of the best-available science and other technical information. Please see the response to comment 2.1.b for clarity on how an RfD might be used in an assessment of risk for GenX chemicals and response to comment 2.3.b for a description of the scope of the GenX toxicity assessment.

With respect to protecting sensitive subpopulations, the RfDs are based on adverse effects observed at the lowest tested dose and applied a UF_H of 10 to account for differences in response/susceptibility among humans. The critical effects selected are adverse liver effects observed in the pregnant dam, a susceptible lifestage.

2.1.b Comment: EPN stated that it was irresponsible for EPA to not provide exposure assessments or address the legal, political, social, economic and technical considerations involved in risk management of these chemicals. EPN further noted that EPA is failing to exercise its authority under the SDWA to publish drinking water health advisories for unregulated contaminants. They noted that it was unreasonable to expect each state to assess all the available data and decide whether to base health advice for these chemicals on a bottle fed infant, a pregnant woman, a lactating woman, or an adult male.

EPA Response: EPA develops toxicity assessments as individual products. These assessments may be used as part of the risk assessment process. Specifically, the draft GenX chemicals (EPA, 2018a) covers the first two steps (Step 1. Hazard Identification and Step 2. Dose-Response) of the four-step risk assessment process developed by the National Academy of Sciences (National Research Council, 1983). Risk assessment and characterization, which is not included in these toxicity assessments, requires additional consideration of exposure. Toxicity information, when combined with specific information on potential exposures, could be used by federal, state, tribal, and local partners to help characterize the public health risks of these chemicals, which completes the risk assessment process. For more details about this process, visit <https://www.epa.gov/risk/conducting-human-health-risk-assessment>.

EPA has completed toxicity assessments for PFOA, PFOS, and PFBS and is working to develop additional PFAS toxicity values for PFBA, PFHxA, PFHxS, PFNA, and perfluorodecanoic acid (PFDA) through EPA's IRIS Program. EPA researchers are also applying computational and high-throughput toxicology tools to PFAS toxicity testing on a larger scale to enable faster understanding of potential toxicity for the universe of thousands of PFAS, for most of which little or no published toxicity data exists. EPA will continue to work with our state, tribal, and

local partners to provide technical assistance, as appropriate, including providing information on PFAS toxicity as it becomes available.

2.1.c Comment: TURI noted that the allowable PFAS levels developed by Minnesota and other states are more protective than the levels proposed by EPA. In addition, The Silent Spring Institute and a group of commenters, including TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health, noted that the European Union through REACH and the Norwegian Environmental Agency are acting to restrict further emissions for GenX through designating it as a Substance of Very High Concern even though they determined that a threshold concerning the level of risk cannot be derived with any certainty (ECHA, 2018). An anonymous citizen has requested that EPA take a precautionary path and set low standards to protect public health and the environment until more scientific studies on GenX have been performed.

EPA Response: The RfDs derived in this assessment are not enforceable “standards;” rather, they are toxicity values that represent an estimate, with uncertainty spanning perhaps an order of magnitude, of daily oral exposure to the human population including sensitive subgroups that is likely to be without an appreciable risk of deleterious effects during a designated period of time. EPA is making this final toxicity assessment (EPA, 2021a) available to provide states, tribes, and local governments with the subchronic and chronic RfDs to help inform whether further actions are needed to protect public health. Please see the response to comment 1.1.f for clarity on how an RfD might be used in an assessment of risk for GenX chemicals.

EPA appreciates the concern expressed by the commenters and would like to assure the public that all available dose-response studies of sufficient study quality specific to GenX chemicals was considered in the derivation of the RfDs. EPA’s human health risk assessment best practices documents, guidance, and systematic review documents ensure that the best available science will be employed in the derivation of the toxicity values.

2.1.d Comment: The Association of State Drinking Water Administrators (ASDWA) noted that some states might have the authority, ability and resources to conduct feasibility analyses, technical evaluations, and cost/benefit evaluations and develop and implement an action level, health advisory or regulatory standard for GenX; however, other states do not have the ability to do this without a federal health advisory or standard and are unable to take actions to protect public health and the environment based on the GenX human health toxicity assessment. The ASDWA noted that this leads to variations in state actions and creates public confusion about what levels are safe in drinking water and what states should be doing to appropriately address risks. They further noted that states will likely derive different drinking water action levels, guidelines, or standards using the RfDs and toxicity values used by different states. States then are having to take primary responsibility for ensuring that water systems respond to and address high levels of unregulated contaminants. Further concerns provided by ASDWA are listed below.

- EPA toxicity values create *de facto* maximum contaminant levels (MCLs) where states must ask water systems to monitor for these contaminants and treat for them without state regulatory enforcement authority.

EPA Response: Toxicity values are one piece of information used to inform regulatory decisions. They provide information about the potential hazard to human health and the environment posed by the chemical, which is often combined with information about exposure to support a regulatory decision. In making any determination to regulate a contaminant in drinking water under the SDWA, EPA must consider three criteria: (1) adverse human health effects, (2) occurrence in public drinking water systems with a frequency and at levels of health concern, and (3) in the sole judgement of the Administrator, a meaningful opportunity for health risk reduction through regulation. Toxicity values inform the first of those three criteria and are not *de facto* drinking water MCLs.

- Without the certainty of having federal regulations and knowing about implementation and compliance dates, states are unprepared to respond to detections of these compounds at significant levels and would not have adequate resources to respond to contamination incidents.

EPA Response: In February 2021, EPA made a final determination to regulate PFOS and PFOA (EPA, 2021b) and outlined avenues that the agency is considering to further evaluate additional PFAS chemicals and provide flexibility for the agency to consider groups of PFAS as supported by the best available science. EPA is committed to following the SDWA process for evaluating and establishing drinking water standards for PFAS chemicals. This process is designed to ensure public participation, transparency, and the use of the best available peer-reviewed science and other technical information.

- Development of the GenX chemicals and PFBS assessments is a first step in addressing risk to these PFAS. With the publication of this final toxicity assessment, the GenX chemicals and PFBS RfDs provide information on health effects and may be used to inform health-based national standards, cleanup levels at local sites, and non-regulatory Health Advisory levels. RfDs can be applied in a variety of exposure scenarios to characterize potential risk from chemical exposure and develop health protective levels for chemicals in water, soil, and other media.
- The Department of Defense does not recognize this type of assessment as an Applicable or Relevant Requirement (ARAR) under Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) and will not act to modify new or existing cleanup activities to reflect these levels of concern.

EPA Response (to both bulleted points above): Thank you for these comments about the ways that the GenX toxicity assessment may be used. While the specific comments are beyond the scope of the GenX toxicity assessment, EPA continues to work with our federal, state, tribal, and local partners and the regulated community to address PFAS contamination and provide support and assistance as appropriate.

2.1.e Comment: Several commenters, including the Silent Spring Institute, TURI, the Cape Fear Public Utility Authority, and two anonymous citizens, have noted that it is expensive and difficult to clean up GenX contamination. Some of these commenters have requested that the chemical companies responsible for causing GenX contamination be financially responsible for the costs of remediation and providing safe drinking water to impacted populations.

EPA Response: The comment is noted; however, this comment pertains to potential risk management and regulatory approaches that might be employed for addressing GenX contamination. As stated elsewhere, the scientific objective of this particular hazard assessment is solely to “provide the health effects basis for the development of oral RfDs for subchronic and chronic durations for GenX chemicals.” Risk management and any subsequent regulatory approaches for addressing risk are beyond the stated goals of this hazard assessment.

2.2 ASSESSMENT/REGULATION OF PFAS AS A GROUP OR AS A MIXTURE

2.2.a Comment: TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that biomonitoring studies (Centers for Disease Control and Prevention’s National Health and Nutrition Examination Survey [CDC NHANES], Ye et al., 2018) indicate that Americans have chronic exposure to multiple PFAS chemicals throughout their lifetimes and it is therefore impossible to be exposed to GenX and no other PFAS chemicals. The commenter suggested that the toxicity assessment should account for simultaneous exposure to other PFAS chemicals that impact the same target organs and notes that EPA does this for its RfD used to establish the present drinking water guideline for the sum of PFOA and PFOS. Further, the commenter noted that the European Food Safety Authority allows for the consideration of additive effects for chemicals that target the same health endpoint, even when MOA is unknown (EFSA, 2014) as does the National Academy of Sciences (National Research Council, 2008, 2009). The commenter mentioned that the Netherlands used a relative potency estimate for liver hypertrophy using experimental data for 11 perfluoroalkyl sulfonates and perfluoroalkyl carboxylates and read across assumptions for 7 additional PFAS. The commenter asked that EPA promote similar assessments for other PFAS related health outcomes with potential for additive toxicity, including kidney toxicity, lipid metabolism, birth outcomes, immunotoxicity and developmental effects.

EPA Response: Understanding cumulative risk requires an understanding of the toxicity and MOA along with exposures associated with each individual chemical in the mixture. At this point in time, we are just beginning to understand the toxicities associated with individual PFAS chemicals based on the available scientific information. EPA has released this final toxicity assessment for GenX chemicals. Previously, EPA released the toxicity assessment for PFBS and announced the initiation of assessments for five additional PFAS (PFBA, PFHxS, PFHxA, PFNA, and PFDA) via EPA’s IRIS Program (EPA, 2018a, 2018b, 2021a, 2020a, 2020b). EPA researchers are also applying computational and high-throughput toxicology tools to a large number of PFAS. The final GenX and PFBS toxicity assessments provide a risk assessor with some of the information needed to make risk assessment and management decisions, including for cumulative assessment.

It is important to clarify that, because the 2016 RfDs for both PFOA and PFOS are based on similar developmental effects and are numerically identical, EPA recommended a conservative and health-protective approach to compare the sum of the concentrations when these two chemicals co-occur at the same time and location in a drinking water source. Additional information can be found here: <https://www.epa.gov/ground-water-and-drinking-water/supporting-documents-drinking-water-health-advisories-pfoa-and-pfos>.

2.2.b Comment: Several anonymous citizens requested that EPA set a health advisory of 0 ppt for PFAS as a chemical class (especially for communities that are chronically exposed) because citizens deserve clean drinking water.

EPA Response: Development of the GenX chemical and PFBS assessments is the first step in addressing risk from these PFAS. These final RfDs provide information on health effects and can be used to inform health-based national standards, cleanup levels at local sites, and non-regulatory advisory levels. RfDs can be applied in a variety of exposure scenarios to characterize potential risk from chemical exposure and develop health-protective levels for chemicals in water, soil, and other media.

2.2.c Comment: The Silent Spring Institute, EWG (individually), PADEP and PADOH, TEDX, the Natural Resources Defense Council, the Sierra Club, EWG (as a member of this coalition), and the Center for Environmental Health requested that EPA prioritize and expedite the regulation PFAS as a class because conducting individual risk assessments for almost 5,000 chemicals in the class would take hundreds of years and therefore cannot protect human health. The group noted that production of PFAS creates GenX as a byproduct and production of GenX creates other PFAS byproducts. Thus, regulating PFAS as a class is the only approach that is matched to the scope of the problem. PADEP and PADOH recommended that EPA prioritize PFAS efforts for developing toxicity values, RfDs, health advisory levels or regulatory maximum contaminant levels, as well as develop risk communication messaging, to address multiple PFAS compounds holistically.

EPA Response: In February 2021, EPA announced that it made a final determination to regulate PFOS and PFOA (EPA, 2021b) and outlined avenues that the agency is considering to further evaluate additional PFAS chemicals and provide flexibility for the agency to consider groups of PFAS as supported by the best available science. EPA is committed to following the SDWA process for evaluating and establishing drinking water standards for PFAS chemicals. This process is designed to ensure public participation, transparency, and the use of the best available peer-reviewed science and other technical information. Please see response to comment 2.2.a above.

EPA has initiated research to understand how known PFAS can inform our knowledge of many PFAS chemical subclasses. EPA researchers are currently applying computational and high-throughput toxicology tools for PFAS toxicity testing on a larger scale to determine potential toxicity for the universe of thousands of PFAS, most of which have little or no published toxicity data. EPA will continue to work with our state, tribal, and local partners to provide technical assistance, as appropriate, including providing information on PFAS toxicity as it becomes available. EPA will also work collaboratively to develop a risk communication toolbox that includes multimedia materials and messaging for federal, state, tribal, and local partners to use with the public.

2.2.d Comment: EWG noted that all humans across the world are exposed to a mixture of PFAS from water, food, food wares, dust, textiles, consumer products, and other sources; therefore, developing toxicity assessments of and exposure regulations about this class of chemicals, including considerations of a class-based approach based on extrapolations from the most toxic member, will more efficiently and effectively protect human health and stop the chemical industry from transitioning from one chemical to another without providing substantiating data.

EPA Response: The comment is noted; however, it addresses potential risk management approaches that might be employed for addressing health concerns that arise from exposure to the entire class of PFAS chemicals. The scientific objective of this particular hazard assessment is solely to “provide the health effects basis for the development of oral RfDs for subchronic and chronic durations for GenX chemicals.” Risk assessment and any subsequent regulatory approaches for addressing risk are beyond the stated goals of this hazard assessment. Please see response to comment 2.2.c.

2.2.e Comment: Several commenters, including the Cape Fear Public Utility Authority and a group of commenters that includes TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental Health noted that EPA should be considering the whole mixture involved in the GenX process and associated byproducts when assessing the toxicity of GenX chemicals. The commenters mentioned that communities exposed to GenX chemicals will likely be concurrently exposed to other PFAS chemicals involved in the process and the resulting byproducts, as found in a non-targeted analysis of wastewater discharge into the Cape Fear River in North Carolina. The estimated concentrations of three additional PFAS (PFMOAA, PFO2HxA, and 2-[[difluoro(trifluoromethoxy)methoxy]difluoromethoxy]-2,2-difluoro-acetic acid (PFO3OA)) dropped significantly after Chemours stopped discharging GenX chemicals; thus it is believed that these three PFAS were part of the same wastewater discharge that included GenX chemicals (NC DEQ, 2017a, 2017b). The GenX Exposure Study, set in the Lower Cape Fear River Basin, recently reported to study participants that there were 4 new PFAS found in participants’ blood (Nafion2, PFO4DA, perfluoro-3,5,7,9,11-pentaoxadodecanoic acid (PFO5DoDA) and Hydro-EVE) (NCSU CHHE, 2018b; Smart, 2018).

EPA Response: This toxicity assessment is specific to HFPO dimer acid and its ammonium salt as they are the major chemicals associated with this technology. The available toxicity information for GenX chemicals is the result of studies conducted using exposure to HFPO dimer acid and the ammonium salt. Additional toxicity studies on other chemicals involved in or byproducts of the GenX process are not publicly available. Please see responses to comments 2.2.a, 2.2.c, and 2.2.d above.

2.2.f Comment: In regard to the description on page vii of the GenX document, the New York State Department of Environmental Conservation (NYSDEC) asked whether the toxicology assessment applies to other salts of the HFPO dimer acid, including CASRN 67963-75-1 (sodium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate) and CASRN 67118-55-2 (potassium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propionate), or only the dimer acid and the ammonium salt. NYSDEC requested that EPA consider inclusion of other GenX process related chemicals as part of the toxicity assessment for HFPO dimer acid, due to similar chemical structures as well as co-mingled occurrence in the environment (Cape Fear River, surface waters near fluoropolymer facilities) (NC DEQ, 2018b; Pan et al., 2017; Song et al., 2018; Strynar et al., 2015; Sun et al., 2016). Specifically they asked EPA to consider HFPO because it is used by the manufacturer to create HFPO dimer acid, as well as other process related PFAS (Hogue, 2018). NYSDEC also asked EPA to consider additional HFPO oligomers, specifically the trimer acid (HFPO-TrA) and tetramer acids (HFPO-TeA) because these chemicals have been found to show greater toxic effects on cell viabilities as compared with PFOA and PFOS (Sheng et al., 2018).

EPA Response: This final toxicity assessment is specific to HFPO dimer acid and its ammonium salt as they are the major chemicals associated with this technology. The available toxicity

information for GenX Chemicals is the result of studies using exposure to HFPO dimer acid and the ammonium salt. This assessment may be applicable to other HFPO salts with solubilities similar to the ammonium salt such as sodium and potassium salts, but not necessarily all other HFPO salts. Additional toxicity studies on other chemicals involved in or byproducts of the GenX process are not publicly available.

2.2.g Comment: ASDWA indicated that additional PFAS toxicity assessments should be prioritized through a stakeholder process based on prevalence of compounds throughout the entire U.S. and potential health impacts and stakeholder engagement.

EPA Response: In May 2018, EPA convened a 2-day National Leadership Summit on PFAS in Washington, DC, that brought together more than 200 federal, state, tribal, and local leaders from across the country to discuss steps to address PFAS. Following the Summit, EPA hosted a series of visits during the summer of 2018 in communities directly impacted by PFAS. EPA interacted with more than 1,000 people during community engagement events in Exeter, NH; Horsham, PA; Colorado Springs, CO; Fayetteville, NC; and Leavenworth, KS, as well as through a roundtable in Kalamazoo, MI, and events with tribal representatives in Spokane, WA.

EPA is committed to understanding the toxicity of all PFAS using available toxicity data and estimating toxicity using New Approach Methods. EPA initiated efforts in 2018 to develop toxicity assessments for GenX chemicals and PFBS (EPA, 2018a, 2018b, 2019a, 2021a) in the near term and toxicity assessments for five additional PFAS (PFBA, PFHxS, PFHxA, PFNA, and PFDA) via EPA's IRIS Program (EPA, 2020a). In November 2019, EPA announced the availability of the Systematic Review Protocol for the PFAS IRIS Assessment for a 45-day public comment period (EPA, 2019a).

2.2.h Comment: PADEP and PADOH mentioned that EPA should work collaboratively with ATSDR to develop consensus standards that can be used to support a regulatory determination for PFAS.

EPA Response: Federal agencies have a variety of tools that provide federal, state, tribal, and local governments; health professionals; and the public with information about how a chemical might impact human health. These tools can be used together to assess health risks and protect people from exposure to these contaminants. EPA and the CDC's ATSDR have different missions, as reflected by each agency's work establishing their own health-based contaminant values for PFAS. Following through on its commitment to work in close collaboration with our federal and state partners to develop draft toxicity assessments for GenX chemicals and PFBS (EPA, 2019b), EPA has engaged with federal, tribal, and state partners, including ATSDR, throughout the development of the draft toxicity assessments. EPA looks forward to continuing to collaborate with ATSDR and all our federal partners as we work together to protect public health. EPA and ATSDR share information during the development of their respective PFAS toxicity products.

2.3 RISK COMMUNICATION

2.3.a Comment: TURI noted that it was useful to have the data available for GenX chemicals in combination with the results of peer-reviewed studies through the EPA HERO site. TEDX, the Natural Resources Defense Council, the Sierra Club, EWG, and the Center for Environmental

Health requested that EPA continue to make this information publicly available and transparent through this site or the HAWC when finalizing the GenX assessment and for future PFAS chemical assessments.

EPA Response: Thank you for the comment. The submitted studies and literature identified by the search of publicly available sources are available through EPA's HERO website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

2.3.b Comment: An anonymous citizen requested that EPA include an appendix identifying all known consumer products that contain these compounds or list materials that were tested so that consumers can decide whether to purchase or continue using the identified materials. EWG requested that EPA provide information about the uses of GenX substances encompassed in the assessment, including whether the assessments cover legacy compounds or compounds currently in routine use, and provide a sense of scale with respect to the use of these specific compounds.

EPA Response: The toxicity assessment for GenX chemicals is a scientific and technical report that includes toxicity values associated with potential non-cancer health effects following oral exposure (in this case, oral RfDs for HFPO dimer acid and HFPO dimer acid ammonium salt). These chemicals are also known as "GenX chemicals" because they are the two major chemicals associated with GenX processing aid technology. This assessment evaluates human health hazards. The scope of this assessment is presented in section 3.0 (Problem Formulation) of that document (EPA, 2018a, 2021a). Identification of all known consumer products or materials that contain these compounds is outside the scope of this assessment. Section 1.2 of the assessment (EPA, 2021a) does provide details related to the uses of GenX chemicals under TSCA.

2.3.c Comment: EWG requested that EPA provide a publicly available listing and map of locations that have used GenX chemicals or potentially released them as a byproduct, and all ground and drinking monitoring locations at which these contaminants have been detected.

EPA Response: As a separate activity from this assessment, EPA is partnering with the Environmental Council of the States (ECOS) to build an interactive map to provide users with easy access to publicly available data on potential PFAS sources and occurrence (EPA, 2019b). EPA will provide updates on actions outlined in the plan on the agency's website as they occur.

2.3.d Comment: NJDEP noted that the conceptual model and diagram on pages 20-21 of the GenX human health toxicity values document should indicate that the information on organ systems comes from animal studies and that human epidemiological data is lacking. They further indicated that the diagram should include fields for toxicokinetic information in humans and laboratory animals (i.e., how external exposures translate into internal exposures) and it should indicate that toxicokinetic data for GenX in humans are not available.

EPA Response: In section 3.0 (Problem Formulation) of the assessment (EPA, 2018a, 2021a), the conceptual model is described in detail. Specifically, this section states that there are no epidemiological studies for GenX chemicals available at the time of the systematic literature review. Moreover, the boxes for potential receptors (adults, children, pregnant women and fetuses, and lactating women) are white, indicating there were no available data for humans. The assessment explains that the available data are from oral exposure studies of acute, subchronic, and chronic duration available in rodent species, including rats and mice. The conceptual model

for a toxicity assessment typically presents the stressor of interest, sources of exposure, potential receptors, and endpoints of concern. Toxicokinetic information for GenX chemicals is described in detail in section 2.3 of the assessment (EPA, 2018a, 2021a).

2.3.e Comment: AWWA and NAWC requested that EPA put the physiological responses described in the GenX human health toxicity values document in context (e.g., indicate the anticipated degree of disturbance in hormone levels in context of typical hormone ranges and typical levels of variability in hormone levels).

EPA Response: For the selected critical effect in this health assessment (i.e., liver single cell necrosis), adversity was defined as a 10% change from control. There is no clinically defined degree of change for this endpoint; thus, putting the selected toxicological endpoint in the context of the clinical setting is a difficult task. Consistent with EPA's Benchmark Dose Technical Guidance (EPA, 2012), the BMD and the benchmark dose lower limit (BMDL) were estimated using a BMR of 10% extra risk for dichotomous data, in the absence of information regarding the level of change considered biologically important, and to facilitate a consistent basis for comparison across endpoints, studies, and assessments.

2.3.f Comment: PADEP and PADOH indicated that EPA should work collaboratively with ATSDR to develop and deliver a clear and consistent public message regarding risks from PFAS, including considerations for special populations such as pregnant women, infants, breastfeeding mothers, children, immunocompromised and the elderly. AWWA and NAWC noted that CDC and other federal agencies should reconcile with EPA any differences that might exist with respect to GenX toxicity values to avoid confusion and better aid decisions by public health agencies and water systems and help them explain risk posed by GenX chemicals.

EPA Response: Thank you for the comment. Please see the response to comment 2.2.h.

2.3.g Comment: AWWA and NAWC suggested the following detailed opportunities to improve risk communication associated with the GenX toxicity values:

- Provide adequate context for how toxicity values are used
 - Expand the technical fact sheet to clearly illustrate the compounding effect of assumptions and uncertainty/safety factors used in EPA's analysis; include a diagram in the fact sheet illustrating the relationship between the initial toxicity assessment, risk characterization, and risk management.
 - Develop additional communication materials to improve risk communication with the general public (e.g., public notice language), including describing how toxicity values are used in different contexts, and auxiliary water uses beyond direct consumption.
 - Describe the RfD in the context of existing body burden from various sources, taking into consideration relative importance varying with life stage.
 - Communicate about actions that inform making incremental changes in exposure, including making judgments as to the need for immediate mitigation measures.

- Explain additional precautionary assumptions for using the RfD for calculating a public health goal (e.g., typical sources of exposure, significance of short-term exposure, populations at risk) for calculating RfDs.
- Provide a more complete understanding of decision-making processes to reduce unnecessary public fear where exposure factors are limited and in other limited circumstances, inform prompt corrective action where exposure factors justify.
- Consider providing a case of an exposed individual experiencing a cumulative body burden exceeding levels of exposure where effects have been observed in epidemiology studies.
- Provide a relative risk comparison to known health issues from other drinking water contaminants or to more general health hazards such as smoking.

EPA Response: Thank you for your suggestions. EPA is committed to working collaboratively to develop a risk communication toolbox that includes multimedia materials and messaging for federal, state, tribal, and local partners to use to work with the public (EPA, 2019b). The toxicity assessment also includes a discussion of uncertainty and variability in section 8.1 (EPA, 2021a).

2.3.h Comment: AWWA and NAWC noted that EPA did not engage in any outreach to states, local government, or water systems before release of the draft GenX human health toxicity assessment but the EPA fact sheet directed the public to reach out to those entities; consequently, the EPA fact sheet directed the public to individuals who do not have the information needed to help them. They requested that EPA actively engage with the water utility and local government associations to develop communication resources to which water systems can direct customers interested in PFAS chemicals, including GenX, prior to the release of the toxicity values.

EPA Response: EPA is following through on its commitment to work with our federal and state partners as we develop toxicity assessments for GenX chemicals. EPA has engaged with federal, tribal, and state partners throughout the development of the toxicity assessments, including before and after an external peer review.

Federal and tribal partners included:

- U.S. Department of Defense (DoD)
- U.S. Department of Energy (DOE)
- U.S. Geological Survey (USGS)
- U.S. Department of Health and Human Services (HHS), including the Food and Drug Administration (FDA), ATSDR, and NIEHS, including NTP
- U.S. Department of Veterans Affairs (VA)
- National Aeronautics and Space Administration (NASA)
- National Toxics Tribal Council (NTTC)
- Office of Management and Budget (OMB)

EPA also engaged extensively with the ASDWA and five state partners recommended by ECOS: Colorado, Michigan, Minnesota, New Hampshire, and Ohio. EPA has also communicated regularly with North Carolina.

EPA discussed the assessment process, available data, and methods to be used to derive toxicity values (in this case, RfDs) for GenX chemicals with federal, tribal, and state partners. After the first external peer review, EPA also discussed the comments received and how EPA planned to address those comments with the partners.

EPA held additional detailed discussions with North Carolina's Department of Health and Human Services and Department of Environmental Quality to continue the agency's efforts to provide technical assistance as the state develops its own technical assessment for GenX chemicals for North Carolina Secretaries' Science Advisory Board (SAB) review. EPA also presented its available data and approaches to North Carolina Secretaries' SAB.

The GenX and PFBS draft assessments were released for a 60-day public comment period on November 14, 2018 and the public comment period closed on January 22, 2019.

2.4 IMPLEMENTATION TOOLS

2.4.a Comment: The Cape Fear Public Utility Authority noted that to ensure public health is protected, drinking water providers need testing capabilities, regulatory guidance and treatment goals for comprehensive PFAS reduction.

EPA Response: Thank you for the comment. Please see the response to comment 2.2.c.

2.4.b Comment: PADEP and PADOH mentioned that EPA should work collaboratively with ATSDR to develop guidance for state drinking water programs, public water systems, and the public regarding Health Advisory Levels (HALs), MRLs, toxicity values, and RfDs so that the public understands how the values are used.

EPA Response: ATSDR has published key messages related to their Toxicological Profile for PFAS that includes a discussion of the differences between EPA's drinking water health advisory levels and ATSDR's MRLs. The messages are available at https://www.atsdr.cdc.gov/docs/PFAS_Public_KeyMessages_June20_Final-508.pdf. Please see the response to comment 2.2.h for additional detail.

2.4.c Comment: ASDWA requested that EPA provide additional information on water system recommendations for sampling and confirmation of results, as well as timeliness or response.

EPA Response: The agency is developing new risk communication materials; continuing to coordinate with our federal, state, local, and tribal partners to ensure consistent messaging; and adding training opportunities for the agency's workforce. EPA's Council on PFAS, established in April 2021, is working to better understand and ultimately reduce the potential risks caused by PFAS, including GenX chemicals, and will be working with our partners to ensure effective and consistent communications.

EPA is exploring the development of drinking water health advisories or enforceable levels for additional PFAS (beyond PFOA and PFOS) in drinking water. The agency is actively working to better understand potential health risks, exposure pathways, and options for treatment and removal (EPA, 2019b).

3 GENERAL

3.1 GENERAL SUPPORT OF THE TOXICITY ASSESSMENT

Several commenters, including the Cape Fear Public Utility Authority, MDEQ and MDHHS, AWWA, and NAWC noted that they appreciated EPA's efforts to develop human health toxicity values for GenX chemicals. PADEP and PADOH appreciated that the document was clearly written, consolidated study results, and integrated suitable evidence to support judgments of health hazards. AWWA and NAWC indicated that this document will help provide a full understanding of the ways these chemicals operate in humans and in the environment, ensuring that manufacturers, wastewater dischargers, groundwater clean-up programs, and water systems can better evaluate their actions to control exposure to these chemicals.

EPA Response: Thank you for the comment. No response needed.

4 PUBLIC COMMENT PERIOD

4.1 REQUEST FOR EXTENSION

Several commenters, including the ACC, Arnold & Porter, Legal Counsel to Chemours Company on behalf of Chemours Company, Dr. James Klaunig (in comments submitted on behalf of Chemours), and a group of organizations (the Natural Resources Defense Council, TEDX, the Sierra Club, EWG, Clean Water Action/Clean Water Fund, Alaska Community Action on Toxics, and Safer States) requested an extension of the public comment period to allow them to provide meaningful input on the science and technical approaches used in the derivation of the draft toxicity assessment for GenX chemicals, provide relevant data and analyses, and to ensure that the draft assessment incorporates recently implemented principles for systematic review of the available data consistently and comprehensively. Several commenters indicated that the draft toxicity documents were sizable, and the original 60-day comment period spanned two holiday periods. These commenters requested extension times ranging from 30 to 120 days past the original January 22, 2019 public comment period end date.

EPA Response: EPA considered the requests for an extension of the comment period and responded to the requestors via letter. EPA considered the 60-day public comment period appropriate and, therefore, denied the requests for an extension. To develop the draft and final toxicity assessments (EPA, 2018a, 2021a), EPA relied on the best available science on the health effects of these chemicals. EPA also engaged extensively with federal and state partners prior to and after the initial draft assessments underwent independent, external expert peer review in June 2018. The comment period was 60 days, as EPA moved forward quickly to provide final assessments to states and local communities, conveying important public health information about these chemicals to inform their decisions and actions.

5 REFERENCES

- Allendorf, F., U. Berger, K. Goss, and N. Ulrich. 2019. Partition coefficients of four perfluoroalkyl acid alternatives between bovine serum albumin (BSA) and water in comparison to ten classical perfluoroalkyl acid. *Environmental Science: Processes & Impacts* 21:1852–1863. doi:10.1039/C9EM00290A.
- ATSDR (Agency for Toxic Substances and Disease Registry). 2018a. *Toxicological Profile for Perfluoroalkyls. (Draft for Public Comment)*. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA. Accessed July 2020. <https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=1117&tid=237>.
- ATSDR (Agency for Toxic Substances and Disease Registry). 2018b. *PFAS Toxicological Profile Key Messages*. U.S. Department of Health and Human Services, Agency for Toxic Substances and Disease Registry, Atlanta, GA. Accessed July 2020. https://www.atsdr.cdc.gov/docs/PFAS_Public_KeyMessages_June20_Final-508.pdf.
- Beekman, M., P. Zweers, A. Muller, W. de Vries, P. Janssen, and M. Zeilmaker. 2016. *Evaluation of Substances Used in the GenX Technology by Chemours, Dordrecht*. RIVM Letter Report 2016-0174. National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport, The Netherlands. Accessed May 2018. <https://www.rivm.nl/bibliotheek/rapporten/2016-0174.pdf>.
- Biegel, L.B., M.E. Hurtt, S.R. Frame, J.C. O'Connor, and J.C. Cook. 2001. Mechanisms of extrahepatic tumor induction by peroxisome proliferators in male CD rats. *Toxicological Sciences* 60:44–55. doi:10.1093/toxsci/60.1.44.
- Blake, B., and S.E. Fenton. 2010. 1719: An in Vitro Screen of a Panel of Perfluoroalkyl Substances and an in Vivo Assessment of Effects on Placental and Fetal Growth. In *Proceedings of the Society of Toxicology*, Baltimore, MD, March 10–14, 2019.
- Blake, B.E., H.A. Cope, S.M. Hall, R.D. Keys, B.W. Mahler, J. McCord, B. Scott, H.M. Stapleton, M.J. Strynar, S.A. Elmore, and S.E. Fenton. 2020. Evaluation of maternal, embryo, and placental effects in CD-1 Mice following gestational exposure to perfluorooctanoic acid (PFOA) or hexafluoropropylene oxide dimer acid (HFPO-DA or GenX). *Environmental Health Perspectives* 128(2):027006. doi:10.1289/EHP6233.
- Butenhoff, J.L., G.L. Kennedy Jr., S. Chang, and G.W. Olsen. 2012. Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats. *Toxicology* 298:1–13. doi:10.1016/j.tox.2012.04.001.
- Cannon, R.E., A.C. Richards, A.W. Trexler, C.T. Juberg, B. Sinhra, G.A. Knudsen, and L.S. Birnbaum. 2020. Effect of GenX on p-glycoprotein, breast cancer resistance protein, and multidrug resistance-associated protein 2 at the blood–brain barrier. *Environmental Health Perspectives* 128(3):037002. doi:10.1289/EHP5884.

- Caverly Rae, J.M., L. Craig, T.W. Slone, S.R. Frame, L.W. Buxton, and G.L. Kennedy. 2015. Evaluation of chronic toxicity and carcinogenicity of ammonium 2,3,3,3-tetrafluoro-2 (heptafluoropropoxy)-propanoate in Sprague-Dawley rats. *Toxicology Reports* 2:939–949. doi:10.1016/j.toxrep.2015.06.001.
- Clark, Dawn, The Chemours Company. 2021, March 17. Letter to EPA, Office of Pollution Prevention and Toxics regarding propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-CAS RN 13252-13-6 (also known as HFPO-DA). https://heronet.epa.gov/heronet/index.cfm/reference/details/reference_id/8631852.
- Conley, J.M., C.S. Lambright, N. Evans, M.J. Strynar, J. McCord, B.S. McIntyre, G.S. Travlos, M.C. Cardon, E. Medlock-Kakaley, P.C. Hartig, V.S. Wilson, and L.E. Gray, Jr. 2019. Adverse maternal, fetal, and postnatal effects of hexafluoropropylene oxide dimer acid (GenX) from oral gestational exposure in Sprague-Dawley rats. *Environmental Health Perspectives* 127(3):037008. doi:10.1289/EHP4372.
- Cope H., B. Blake, and S.E. Fenton. 2019. 1186: Effects of PFOA and GenX on Early Developmental Outcomes and Puberty in CD-1 mice. In *Proceedings of the Society of Toxicology*, Baltimore, MD, March 10–14, 2019.
- Das, K.P., C.R. Wood, M.T. Lin, A.A. Starkov, C. Lau, K.B. Wallace, J.C. Corton, and B.D. Abbott. 2017. Perfluoroalkyl acids-induced liver steatosis: effects on genes controlling lipid homeostasis. *Toxicology* 378:37–52. doi:10.1016/j.tox.2016.12.007 .
- D’eon, J.C., A.J. Simpson, R. Kumar, A.J. Baer, and S.A. Mabury. 2010. Determining the molecular interactions of perfluorinated carboxylic acids with human sera and isolated human serum albumin using nuclear magnetic resonance spectroscopy. *Environmental Toxicology and Chemistry* 29(8):1678–1688. doi:10.1002/etc.204.
- DuPont-17751-1026: E.I. du Pont de Nemours and Company. 2009. *A 90 Day Oral (Gavage) Toxicity Study of H-28548 in Rats with a 28-Day Recovery*. OECD Test Guideline 408. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: October 5, 2009), Ashland, OH.
- DuPont-18405-841: E.I. du Pont de Nemours and Company. 2010. *An Oral (Gavage) Prenatal Developmental Toxicity Study of H-28548 in Rats*. U.S. EPA OPPTS 850.3700; OECD Test Guideline 414. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: July 2, 2010), Ashland, OH.
- DuPont-18405-1017 RV1: E.I. du Pont de Nemours and Company. 2011. *H-28548: Absorption, Distribution, Metabolism, and Elimination in the Rat*. U.S. EPA OPPTS 870.7485. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: November 3, 2010; Report Revision 1 Completed: April 21, 2011), Newark, DE, and Wilmington, DE.
- 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-18405-1238: E.I. du Pont de Nemours and Company. 2013. *H-28548: Combined Chronic Toxicity/Oncogenicity Study 2-Year Oral Gavage Study in Rats*. U.S. EPA OPPTS 870.4300; OECD Test Guideline 453. Study conducted by MPI Research, Inc. (Study Completion Date: March 28, 2013), Mattawan, MI.
- 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.
- DuPont-24447: E.I. du Pont de Nemours and Company. 2008. *A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery*. OECD Test Guideline 407. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: August 22, 2008), Ashland, OH.
- DuPont-25292: E.I. du Pont de Nemours and Company. 2008. *Determination of a Permeability Coefficient (Kp) for H-28308 Using Human and Rat Skin Mounted in an in Vitro Static Diffusion Cell*. Test guideline not identified. Testing laboratory not identified (Study Completion Date: February 27, 2008). Testing laboratory location not identified.
- DuPont-24459: E.I. du Pont de Nemours and Company. 2008. *A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery*. OECD Test Guideline 407. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: August 29, 2008), Ashland, OH.
- Dupont CCAS D. 2009. *Sublimation of Processing Aids FRD-903k and FRD-902*. DuPont Corporate Center for Analytical Sciences, Wilmington, DE.
- DWQI (New Jersey Drinking Water Quality Institute). 2015. *Health-Based Maximum Contaminant Level Support Document: Perfluorononanoic Acid (PFNA)*. DWQI, Health Effects Subcommittee, Trenton, NJ.
<http://www.nj.gov/dep/watersupply/pdf/pfna-health-effects.pdf>.
- DWQI (New Jersey Drinking Water Quality Institute). 2017. *Health-Based Maximum Contaminant Level Support Document: Perfluorooctanoic Acid (PFOA)*. DWQI, Health Effects Subcommittee, Trenton, NJ.
<https://www.state.nj.us/dep/watersupply/pdf/pfoa-appendixa.pdf>.
- DWQI (New Jersey Drinking Water Quality Institute). 2018. *Health-Based Maximum Contaminant Level Support Document: Perfluorooctane Sulfonate (PFOS)*. DWQI, Health Effects Subcommittee, Trenton, NJ.
<https://www.state.nj.us/dep/watersupply/pdf/pfos-recommendation-appendix-a.pdf>.
- ECHA (European Chemicals Agency). 2018. *Risk Management Option Analysis Conclusion Document for Perfluorobutane Sulfonic Acid (PFBS), Its Salts and Related Substances*. ECHA, Norway.

- EFSA (European Food Safety Authority). 2014. *Scientific Opinion on the Identification of Pesticides to be included in Cumulative Assessment Groups on the Basis of their Toxicological Profile*. EFSA-Q-2009-00860. EFSA, Parma, Italy.
- Elmore, S.A., D. Dixon, J.R. Hailey, T. Harada, R.A. Herbert, R.R. Maronpot, T. Nolte, J.E. Rehg, S. Rittinghausen, T.J. Rosol, and H. Satoh. 2016. Recommendations from the INHAND apoptosis/necrosis working group. *Toxicologic Pathology* 44(2):173–188. doi:10.1177/0192623315625859.
- EPA (Environmental Protection Agency). 2002. *A Review of the Reference Dose and Reference Concentration Processes*. EPA/630/P-02/002F. EPA, Risk Assessment Forum, Washington, DC. <http://www.epa.gov/osa/review-reference-dose-and-reference-concentration-processes>.
- EPA (Environmental Protection Agency). 2005. *Guidelines for Carcinogen Risk Assessment*. EPA/630/P-03/001B. EPA, Risk Assessment Forum, Washington, DC. Accessed May 2018. <https://www.epa.gov/risk/guidelines-carcinogen-risk-assessment>.
- EPA (Environmental Protection Agency). 2009. *Consent Order and Determinations Supporting Consent Order for Premanufacture Notice Numbers: P-08-508 and P-08-509*. EPA, Office of Pollution Prevention and Toxics, Washington, DC. Accessed May 2018. https://chemview.epa.gov/chemview/proxy?filename=sanitized_consent_order_p_08_0508c.pdf
- EPA (Environmental Protection Agency). 2011. *Recommended Use of Body Weight^{3/4} as the Default Method in Derivation of the Oral Reference Dose*. EPA/100/R11/0001. EPA, Office of the Science Advisor, Risk Assessment Forum, Washington, DC. Accessed May 2018. <https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf>.
- EPA (Environmental Protection Agency). 2012. *Benchmark Dose Technical Guidance*. EPA/100/R-12/001. EPA, Risk Assessment Forum, Washington, DC. Accessed May 2018. https://www.epa.gov/sites/production/files/2015-01/documents/benchmark_dose_guidance.pdf.
- EPA (Environmental Protection Agency). 2014. *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation*. EPA/R-14/002F. EPA, Office of the Science Advisor, Risk Assessment Forum, Washington, DC. Accessed May 2018. <https://www.epa.gov/sites/production/files/2015-01/documents/ddef-final.pdf>.
- EPA (Environmental Protection Agency). 2016. *Health Effects Support Document for Perfluorooctanoic Acid (PFOA)*. EPA/822/R-16/003. EPA, Office of Water, Health and Ecological Criteria Division, Washington, DC. Accessed May 2018. https://www.epa.gov/sites/default/files/2016-05/documents/pfoa_hesd_final-plain.pdf

- EPA (Environmental Protection Agency). 2018a. *Public Comment Draft: Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). Also Known as “GenX Chemicals”*. EPA/823/P-18/001. EPA, Office of Water, Health and Ecological Criteria Division, Washington, DC. Accessed July 2020. https://www.epa.gov/sites/production/files/2018-11/documents/genx_public_comment_draft_toxicity_assessment_nov2018-508.pdf.
- EPA (Environmental Protection Agency). 2018b. *Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3)*. EPA/823/R-18/307. EPA, Office of Research and Development, Washington, DC. Accessed July 2020. https://www.epa.gov/sites/production/files/2018-11/documents/pfbs_public_comment_draft_toxicity_assessment_nov2018-508.pdf
- EPA (Environmental Protection Agency). 2019a. *Systematic Review Protocol for the PFAS IRIS Assessment*. EPA/635/R-19/050. EPA, Washington, DC.
- EPA (Environmental Protection Agency). 2019b. *EPA’s Per- and Polyfluoroalkyl Substances (PFAS) Action Plan*. EPA/823/R-18/004. EPA. Accessed July 2020. https://www.epa.gov/sites/production/files/2019-02/documents/pfas_action_plan_021319_508compliant_1.pdf.
- EPA (Environmental Protection Agency). 2020a. *IRIS Program Outlook*. EPA. Accessed July 2020. <https://www.epa.gov/iris/iris-program-outlook>.
- EPA (Environmental Protection Agency). 2020b. *ORD Staff Handbook for Developing IRIS Assessments (Public Comment Draft, Nov 2020)*. EPA/600/R-20/137. EPA, Washington, DC.
- EPA (Environmental Protection Agency). 2021a. *Human Health Toxicity Values for Hexafluoropropylene Oxide (HFPO) Dimer Acid and Its Ammonium Salt (CASRN 13252-13-6 and CASRN 62037-80-3). Also Known as “GenX Chemicals.”* EPA/822/R-21/010. EPA, Office of Water, Health and Ecological Criteria Division, Washington, DC.
- EPA (Environmental Protection Agency). 2021b. *EPA Takes Action to Address PFAS in Drinking Water*. EPA. Accessed March 2021. <https://www.epa.gov/newsreleases/epa-takes-action-address-pfas-drinking-water>.
- Gannon, S.A., W.J. Fasano, M.P. Mawn, D.L. Nabb, R.C. Buck, L.W. Buxton, G.W. Jepson, and S.R. Frame. 2016. Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. *Toxicology* 340(18):1–9. doi:10.1016/j.tox.2015.12.006.
- Hall, A.P., C.R. Elcombe, J.R. Foster, T. Harada, W. Kaufmann, A. Knippel, K. Küttler, D.E. Malarkey, R.R. Maronpot, A. Nishikawa, T. Nolte, A. Schulte, V. Strauss, and M.J. York. 2012. Liver hypertrophy: A review of adaptive (adverse and non-adverse) changes—Conclusions from the 3rd International ESTP Expert Workshop. *Toxicologic Pathology* 40(7):971–994. doi:10.1177/0192623312448935.

- Han, X., T.A. Snow, R.A. Kemper, and G.W. Jepson. 2003. Binding of perfluorooctanoic acid to rat and human plasma proteins. *Chemical Research in Toxicology* 16(6):775–781. doi:10.1021/tx034005w.
- Health Canada. 2016. *Perfluorooctanoic Acid (PFOA) in Drinking Water: Document for Public Consultation*. Prepared by the Federal-Provincial-Territorial Committee on Drinking Water, Ottawa, Ontario.
- Hogue, C. 2018. What's GenX still doing in the water downstream of a Chemours plant? *Chemical and Engineering News* 96(7):2–8. <https://cen.acs.org/articles/96/i7/whats-genx-still-doing-in-the-water-downstream-of-a-chemours-plant.html>.
- Institute of Medicine. 2011. *Finding What Works in Health Care: Standards for Systematic Reviews*. The National Academies Press, Washington, DC.
- Klaunig, J.E., M.A. Babich, L.P. Baetcke, J.C. Cook, J.C. Corton, R.M. David, J.G. DeLuca, D.Y. Lai, R.H. McKee, J.M. Peters, R.A. Roberts, and P.A. Fenner-Crisp. 2003. PPAR α agonist-induced rodent tumors: modes of action and human relevance. *Critical Reviews in Toxicology* 33(6):655–780. doi:10.1080/713608372.
- Klaunig, J.E., B.A. Hocevar, and L.M. Kamendulis. 2012. Mode of action analysis of perfluorooctanoic acid (PFOA) tumorigenicity and human relevance. *Reproductive Toxicology* 33(4):410–418. doi:10.1016/j.reprotox.2011.10.014.
- Kotlarz, N., J. McCord, D. Collier, C.S. Lea, M. Strynar, A.B. Lindstrom, A.A. Wilkie, J.Y. Islam, K. Matney, P. Tarte, M.E. Polera, K. Burdette, J. DeWitt, K. May, R.C. Smart, D.R.U. Knappe, and J.A. Hoppin. 2020. Measurement of novel, drinking water-associated PFAS in blood from adults and children in Wilmington, North Carolina. *Environmental Health Perspectives* 128(7):077005. doi:10.1289/EHP6837.
- Lau, C. 2015. Perfluorinated Compounds: An Overview. Chapter in *Toxicological Effects of Perfluoroalkyl and Polyfluoroalkyl Substances*, ed. J. DeWitt, pp. 1–21, Humana Press, Totawa, NJ.
- Li, C.H., Ren, X.M. and Guo, L.H. 2019. Adipogenic activity of oligomeric hexafluoropropylene oxide (perfluorooctanoic acid alternative) through peroxisome proliferator-activated receptor γ pathway. *Environmental Science & Technology* 53(6):3287–3295. doi:10.1021/acs.est.8b06978.
- Maloney, E.K., and D.J. Waxman. 1999. Trans-activation of PPAR α and PPAR γ by structurally diverse environmental chemicals. *Toxicology & Applied Pharmacology* 161(2):209–218. doi:10.1006/taap.1999.8809.
- Michigan PFAS Science Advisory Panel. 2018. *Scientific Evidence and Recommendations for Managing PFAS Contamination in Michigan*. Prepared for the Michigan PFAS Action Response Team, Lansing, MI, by Michigan PFAS Science Advisory Panel. https://www.michigan.gov/documents/pfasresponse/Science_Advisory_Board_Report_64_1294_7.pdf.

- Minata, M., Harada, K.H., Kärrman, A., Hitomi, T., Hirosawa, M., Murata, M., Gonzalez, F.J. and Koizumi, A. 2010. Role of peroxisome proliferator-activated receptor- α in hepatobiliary injury induced by ammonium perfluorooctanoate in mouse liver. *Industrial Health* 48(1):96–107. doi:10.2486/indhealth.48.96.
- National Research Council. 1983. *Risk Assessment in the Federal Government. Managing the Process*. National Research Council (U.S.) Committee on the Institutional Means for Assessment of Risks to Public Health. The National Academies Press, Washington, DC. doi:10.17226/366.
- National Research Council. 2008. *Phthalates and Cumulative Risk Assessment: The Tasks Ahead*. National Research Council (U.S.) Committee on the Health Risks of Phthalates. The National Academies Press, Washington, DC. doi:10.17226/12528.
- National Research Council. 2009. *Science and Decisions: Advancing Risk Assessment*. The National Academies Press, Washington, DC.
- NC DEQ (North Carolina Department of Environmental Quality). 2017a. *60-Day Notice of Intent to Suspend Permit nc0003573: The Chemours Company, Fayetteville Works*. NC DEQ, Fayetteville, NC.
- NC DEQ (North Carolina Department of Environmental Quality). 2017b. *State Seeks to Stop Additional Chemical Discharges into the Cape Fear River*. NC DEQ, Raleigh, NC. Accessed July 2020. <https://deq.nc.gov/state-seeks-stop-additional-chemical-discharges-cape-fear-river>.
- NC DEQ (North Carolina Department of Environmental Quality). 2018a. *60-Day Notice of Intent to Modify Air Quality Permit No. 03735t43: The Chemours Company, Fayetteville Works*. NC DEQ, Fayetteville, NC.
- NC DEQ (North Carolina Department of Environmental Quality). 2018b. *Groundwater and Rainwater Sample Testing Results*. NC DEQ, Fayetteville, NC.
- NCSU CHHE (NC State University Center for Human Health and the Environment). 2018a. *The GenX Exposure Study*. Accessed July 2020. <https://chhe.research.ncsu.edu/the-genx-exposure-study/>.
- NCSU CHHE (NC State University Center for Human Health and the Environment). 2018b. *GenX Exposure Study: PFAS Blood Sample Results*. Accessed July 2020. <https://chhe.research.ncsu.edu/wordpress/wp-content/uploads/2018/11/Community-event-BLOOD-slides.pdf>.
- NJDEP (New Jersey Department of Environmental Protection). 2018. *Comments on ATSDR 2018 Draft Toxicological Profile for Perfluoroalkyls*. NJDEP, Division of Science, Research and Environmental Health, Trenton, NJ. <https://www.regulations.gov/document?D=ATSDR-2015-0004-0047>.

- Olsen, G.W., S.C. Chang, P.E. Noker, G.S. Gorman, D.J. Ehresman, P.H. Lieder, and J.L. Butenhoff. 2009. A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. *Toxicology* 256:65–74. doi:10.1016/j.tox.2008.11.008.
- Pan, Y., H. Zhang, Q. Cui, N. Sheng, L.W. Yeung, Y. Guo, Y. Sun, and J. Dai. 2017. First report on the occurrence and bioaccumulation of hexafluoropropylene oxide trimer acid: an emerging concern. *Environmental Science & Technology* 51(17):9553–9560. doi:10.1021/acs.est.7b02259.
- Peters, T. 1995. *All About Albumin: Biochemistry, Genetics, and Medical Applications*. Academic Press, San Diego, CA.
- Post, G.B., J.A. Gleason, and K.R. Cooper. 2017. Key scientific issues in developing drinking water guidelines for perfluoroalkyl acids: contaminants of emerging concern. *PLoS Biology* 15(12):e2002855. doi:10.1371/journal.pbio.2002855.
- Pritchett, J.R., J.L. Rinsky, B. Dittman, A. Christensen, R. Langley, Z. Moore, A.T. Fleischauer, K. Koehler, A.M. Calafat, R. Rogers, and L. Esters., 2019. Notes from the field: Targeted biomonitoring for GenX and other per- and polyfluoroalkyl substances following detection of drinking water contamination—North Carolina. *Morbidity and Mortality Weekly Report* 68(29):647–678. doi:10.15585/mmwr.mm6829a4.
- Rosen, M.B., J.S. Lee, H. Ren, B. Vallanat, J. Liu, M.P. Waalkes, B.D. Abbott, C. Lau, and J.C. Corton. 2008. Toxicogenomic dissection of the perfluorooctanoic acid transcript profile in mouse liver: evidence for the involvement of nuclear receptors PPAR alpha and CAR. *Toxicological Sciences* 103:46–56. doi:10.1093/toxsci/kfn025.
- Rushing, B., Q. Hu, J. Franklin, R. McMahan, S. Dagnio, C. Higgins, M. Strynar, and J. DeWitt. 2017. Evaluation of the immunomodulatory effects of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate in C57BL/6 mice. *Toxicological Sciences* 156(1):179–189. doi:10.1093/toxsci/kfw251.
- Sheng, N., R. Cui, J. Wang, Y. Guo, J. Wang, and J. Dai. 2018. Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid binding protein. *Archives of Toxicology* 92(1):359–369. doi:10.1007/s00204-017-2055-1.
- Smart, B. 2018. “I was livid all over again”: Blood tests in GenX exposure study come back positive for PFAS. WECT News 6, Wilmington, NC. <https://www.wect.com/2018/11/14/i-was-livid-all-over-again-blood-tests-genx-exposure-study-come-back-positive-pfas-pfoa-is-four-times-national-average/>.
- Song, X., R. Vestergren, Y. Shi, J. Huang, and Y. Cai. 2018. Emissions, transport, and fate of emerging per- and polyfluoroalkyl substances from one of the major fluoropolymer manufacturing facilities in China. *Environmental Science & Technology* 52(17):9694–9703. doi:10.1021/acs.est.7b06657.

- Strynar, M., S. Dagnino, R. McMahan, S. Liang, A. Lindstrom, E. Andersen, L. McMillan, Thurman, I. Ferrer, and C. Ball. 2015. Identification of novel perfluoroalkyl ether carboxylic acids (PFECAs) and sulfonic acids (PFESAs) in natural waters using accurate mass time-of-flight mass spectrometry (TOFMS). *Environmental Science & Technology* 49(19):11622–11630. doi:10.1021/acs.est.5b01215.
- Sun, M., E. Arevalo, M. Strynar, A. Lindstrom, M. Richardson, B. Kearns, A. Pickett, C. Smith, and D.R.U. Knappe. 2016. Legacy and emerging perfluoroalkyl substances are important drinking water contaminants in the Cape Fear River watershed of North Carolina. *Environmental Science & Technology Letters* 3:415–419. doi:10.1021/acs.estlett.6b00398.
- Thompson, C.M., S.E. Fitch, C. Ring, W. Rish, J.M. Cullen, and L.C. Haws. 2019. Development of an oral reference dose for the perfluorinated compound GenX. *Journal of Applied Toxicology* 39(9):126–1282. doi:10.1002/jat.3812.
- Thoolen, B., R.R. Maronpot, T. Harada, A. Nyska, C. Rousseaux, T. Nolte, D.E. Malarkey, W. Kaufmann, K. Küttler, U. Deschl, D. Nakae, R. Gregson, M.P. Vinlove, A.E. Brix, B. Singh, 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.
- Vanden Heuvel, J.P., J.T. Thompson, S.R. Frame and P.J. Gillies. 2006. Differential activation of nuclear receptors by perfluorinated fatty acid analogs and natural fatty acids: a comparison of human, mouse, and rat peroxisome proliferator-activated receptor-alpha, -beta, and -gamma, liver X receptor-beta, and retinoid X receptor-alpha. *Toxicological Sciences* 92(2):476–489. doi:10.1093/toxsci/kfl014.
- Wang, J., X. Wang, N. Sheng, X. Zhou, R. Cui, H. Zhang, and J. Dai. 2017. RNA-sequencing analysis reveals the hepatotoxic mechanism of perfluoroalkyl alternatives, HFPO2 and HFPO4, following exposure in mice. *Journal of Applied Toxicology* 37(4):436–444. doi:10.1002/jat.3376.
- Wang, Z., I.T. Cousins, M. Scheringer, and K. Hungerbühler. 2013. Fluorinated alternatives to long-chain perfluoroalkyl carboxylic acids (PFCAs), perfluoroalkane sulfonic acids (PFASAs) and their potential precursors. *Environment International*. 60:242–248. doi:10.1016/j.envint.2013.08.021.
- Xiao, S., S.P. Anderson, C. Swanson, R. Bahnemann, K.A. Voss, A.J. Stauber, and J.C. Corton. 2006. Activation of peroxisome proliferator-activated receptor alpha enhances apoptosis in the mouse liver. *Toxicological Sciences* 92:368–377. doi:10.1093/toxsci/kfl002.
- Ye, X., Kato, K., Wong, L.Y., Jia, T., Kalathil, A., Latremouille, J. and Calafat, A.M., 2018. Per- and polyfluoroalkyl substances in sera from children 3 to 11 years of age participating in the National Health and Nutrition Examination Survey 2013–2014. *International Journal of Hygiene and Environmental Health* 221(1):9–16. doi:10.1016/j.ijheh.2017.09.011.

Zhao, W., J.D. Zitzow, Y. Weaver, et al. 2017. Organic anion transporting polypeptides contribute to the disposition of perfluoroalkyl acids in humans and rats. *Toxicological Sciences* 156(1):84–95. doi:10.1093/toxsci/kfw236.