Human Health Toxicity Values for

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

Also Known as “GenX Chemicals”

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Prepared by:

U.S. Environmental Protection Agency
Office of Water (4304T)
Health and Ecological Criteria Division
Washington, DC 20460

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- Office of Policy
- Office of Children's Health Protection
- Office of Research and Development
- Regions 1–10

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Acronyms and Abbreviations

ºC	degree Celsius
3D	three dimensional
A/G	albumin-to-globulin
AAALAC	American Association for Accreditation of Laboratory Animal Care
ADME	absorption, distribution, metabolism, and excretion
AGD	anogenital distance
AIC	Akaike information criterion
ALD	approximate lethal dose
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AOP	adverse outcome pathway
AR	androgen receptor
AST	aspartate aminotransferase
atm-m^3/mol	atmosphere cubic meter per mole
ATP	adenosine triphosphate
BAF	bioaccumulation factor
BBDR	biologically based dose-response
BCF	bioconcentration factor
BCRP	breast cancer resistance protein
BMD	benchmark dose
BMD_{10}	dose level corresponding to the 95% lower confidence limit for a 10% response level
BMDL	benchmark dose lower limit
$BMDL_{10}$	lower bound on the BMD_{10}
BMDS	Benchmark Dose Software
BMR	benchmark response
BOD	biochemical oxygen demand
BUN	blood urea nitrogen
BW	body weight
BW_a	animal body weight
BW_h	human body weight
CASRN	Chemical Abstracts Service Registry Number
CFR	Code of Federal Regulations
cm/hr	centimeter per hour
CoA	coenzyme A
COV	coefficient of variation
Crl:CD(SD)	Sprague Dawley
DAF	dosimetric adjustment factor
DMEM/F-12	Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DWEL	drinking water equivalent level
DWTP	drinking water treatment plant
E	embryonic day
E1	heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether
E2	estradiol
ELISA	enzyme-linked immunosorbent assay
EPA	U.S. Environmental Protection Agency
ERα	estrogen receptor alpha
ERβ	estrogen receptor beta
F_0	parent generation
F_1	offspring of the F_0 generation
FABP	fatty acid-binding protein

FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FRD-902	synonym for HFPO dimer acid ammonium salt
FRD-903	synonym for HFPO dimer acid
g	gram
g/L	gram per liter
g/mol	gram per mole
GenX chemicals	hexafluoropropylene oxide dimer acid and its ammonium salt
GD	gestation day
GLP	Good Laboratory Practices
GWG	gestational weight gain
H_3O+	hydronium ion
H&E	hematoxylin and eosin
HAWC	Health Assessment Workspace Collaborative
HDL	high-density lipoprotein
HED	human equivalent dose
HERO	Health & Environmental Research Online
HFPO	hexafluoropropylene oxide
HFPO-DA	HFPO dimer acid
HFPO dimer acid	2,3,3,3-tetrafluoro-2-(heptafluoropropoxy) propanoic acid
HFPO-TA	HFPO trimer acid
HFPO-TeA	hexafluoropropylene oxide tetramer acid
hL-FABP	human liver fatty acid-binding protein
HPLC	high-performance liquid chromatography
HPLC/MS/MS	high-performance liquid chromatography-tandem mass spectrometry
IC_{50}	concentration at which 50% inhibition is observed
ICR	Institute of Cancer Research
IgM	immunoglobulin M
INHAND	International Harmonization of Nomenclature and Diagnostic Criteria
IUPAC	International Union of Pure and Applied Chemistry
i.v.	intravenous
K_{OC}	soil-water partition coefficient for organic compounds
K_{OW}	octanol-water partition coefficient
kPa	kilopascal
L/kg	liter per kilogram
LC_{50}	median lethal concentration
LD	lactation day
LD_{50}	median lethal dose
LDL	low-density lipoprotein
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantification
µg/g	microgram per gram
µg/L	microgram per liter
µg/mL	microgram per milliliter
µL	microliter
µM	micromolar
mg	milligram
mg/kg	milligram per kilogram
mg/kg/day	milligram per kilogram per day
mg/L	milligram per liter
mg/m^3	milligram per cubic meter

mg/mL	milligram per milliliter
mL	milliliter
mM	millimolar
mm Hg	millimeter of mercury
MOA	mode of action
MMAD	mass median aerodynamic diameter
mRNA	messenger ribonucleic acid
MRP2	multidrug resistance-associated protein 2
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
N/A	not applicable
NAM	new approach methodology
NC DHHS	North Carolina Department of Health and Human Services
ND	not detected
ng/g	nanogram per gram
ng/mL	nanogram per milliliter
NHANES	National Health and Nutrition Examination Survey
NIEHS	National Institute of Environmental Health Sciences
NLM	National Library of Medicine
nM	nanomolar
nm	nanometer
NOAEL	no-observed-adverse-effect level
NQ	not quantified
NR	not rated
NTP	National Toxicology Program
OECD	Organization for Economic Cooperation and Development
OPPT	Office of Pollution Prevention and Toxics
ORD	Office of Research and Development
P-gp	P-glycoprotein
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PCR	polymerase chain reaction
PECO	population, exposure, comparator, and outcome
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutanesulfonic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonic acid
PFO4DA	3,5,7,9-tetraoxadecanoic perfluoro acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PK	pharmacokinetic
Pk_a	acid dissociation constant
Pk_b	base dissociation constant
pM	picomolar
pmol	picomole
PMN	premanufacture notice
PMOH	ammonium perfluoro(2-methyl-3-oxahexanoate)
PMPP	3H-perfluoro-3-(3-methoxypropoxy) propanoic acid
PND	postnatal day
POD	point of departure
POD_{HED}	point of departure human equivalent dose
PPAR	peroxisome proliferator-activated receptor
PPARα	peroxisome proliferator-activated receptor alpha

PPAR-β/δ	peroxisome proliferator-activated receptor beta/delta
PPARγ	peroxisome proliferator-activated receptor gamma
ppm	parts per million
PWG	Pathology Working Group
RBC	red blood cell
RfD	Reference dose
RIVM	National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
RNA	ribonucleic acid
rT3	reverse triiodothyronine
SD	standard deviation
SDH	sorbitol dehydrogenase
TEM	transmission electron microscopy
TG	Test Guideline
TK	toxicokinetic
ToxRTool	Toxicological Data Reliability Assessment Tool
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
UF	uncertainty factor(s)
UF_A	interspecies uncertainty factor
UF_D	database uncertainty factor
SE	standard error
SM	Standard Model
$T_{1/2}$	half-life
T3	triiodothyronine
T4	thyroxine
TDAR	T cell-dependent antibody response
UF_H	intraspecies uncertainty factor
UF_L	LOAEL to NOAEL extrapolation uncertainty factor
UF_S	extrapolation from subchronic to a chronic exposure duration uncertainty factor
UF_{TOT}	total uncertainty factor
VTG	vitellogenin
WOS	Web of Science

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing final subchronic and chronic oral toxicity values (i.e., reference doses, or RfDs) 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. These chemicals are also known as "GenX chemicals" because they are the two major chemicals associated with GenX processing aid technology. The toxicity assessment for GenX chemicals is a scientific and technical report that provides an assessment of all available toxicity and carcinogenicity data and includes toxicity values associated with potential noncancer health effects following oral exposure (in this case, oral RfDs). This toxicity assessment evaluates human health hazards. It is not a risk assessment as it does not include an exposure assessment nor an overall risk characterization. Further, the toxicity assessment does not address the legal, political, social, economic, or technical considerations involved in risk management. The GenX chemicals toxicity assessment can be used by EPA, states, tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, if, and when, it is necessary to take action to address potential risk associated with human exposures to GenX chemicals.

These GenX chemicals are organic fluorinated ether chemicals that are part of a larger group of chemicals referred to as "per- and polyfluoroalkyl substances" or PFAS. In 2006, EPA initiated a stewardship program with the goal of eliminating chemical emissions of perfluorooctanoic acid (PFOA) and related chemicals by 2015. GenX chemicals are replacements for PFOA. Specifically, GenX is a trade name for a processing aid technology that enables the creation of fluoropolymers without the use of PFOA. Information on specific products containing these chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. Fluoropolymers are used in many applications, including the manufacture of nonstick coatings for cookware, water repellent garments, and other specialty agrochemical and pharmaceutical applications.

For HFPO dimer acid and its ammonium salt, acute, short-term, subchronic, chronic, and reproductive and developmental oral animal toxicity studies are available in rats and mice. Limited information identifying health effects in animals from inhalation of or dermal exposures to GenX chemicals is available. Repeated-dose toxicity data are available for oral exposure, but not for the other exposure routes (inhalation and dermal exposures). Thus, this assessment applies only to the oral route of exposure. These studies report liver toxicity (increased relative liver weight, hepatocellular hypertrophy, apoptosis, and single-cell/focal necrosis), kidney toxicity (increased relative kidney weight), immune effects (antibody suppression), hematological effects (decreased red blood cell count, hemoglobin, and hematocrit), reproductive/developmental effects (increased early deliveries, placental lesions, changes in maternal gestational weight gain, and delays in genital development in offspring), and cancer (liver and pancreatic tumors). Overall, the available toxicity studies demonstrate that the liver is particularly sensitive to HFPO dimer acid- and HFPO dimer acid ammonium salt-induced toxicity. Consistent with the *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a), EPA

concluded that there is *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans, based on the female hepatocellular adenomas and hepatocellular carcinomas and male combined pancreatic acinar adenomas and carcinomas observed in the chronic 2-year study in rats.

EPA followed the general guidelines for risk assessment set forth by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a) in determining the point of departure (POD) for the derivation of the RfDs for these chemicals. Consistent with the recommendations presented in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002), EPA applied uncertainty factors (UFs) to address intraspecies variability, interspecies variability, and extrapolation from a subchronic to a chronic exposure duration.

The critical study chosen for determining the subchronic and chronic RfDs for HFPO dimer acid and/or its ammonium salt was the oral reproductive/developmental toxicity study in mice with a no-observed-adverse-effect level (NOAEL) of 0.1 milligram per kilogram per day (mg/kg/day) based on liver effects (a constellation of lesions, including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) in females (DuPont-18405-1037, 2010; NTP, 2019). EPA determined that the constellation of liver lesions observed in the rodent are relevant to human health and not a result of PPARα-induced cell proliferation unique to rodents. Using EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012), EPA conducted benchmark dose modeling to empirically model the dose-response relationship in the range of observed data. Additionally, EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans. Allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies UFs). The resulting POD human equivalent dose is 0.01 mg/kg/day. UFs applied include a 10 for intraspecies variability, 3 for interspecies differences, and 10 for database deficiencies, including immune effects and additional developmental studies, to yield a subchronic RfD of 0.00003 mg/kg/day or 0.03 µg/kg/day. In addition to those above, a UF of 10 was also applied for extrapolation from a subchronic to a chronic duration in the derivation of the chronic RfD of 0.000003 mg/kg/day or 0.003 µg/kg/day.

1.0 Introduction and Background

1.1 History of Assessment of GenX Chemicals

In 2008, DuPont de Nemours, Inc. (hereinafter DuPont) submitted premanufacture notices (PMNs) to the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) (Title 15 of the United States Code § 2601 *et seq*.) for two chemicals—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—which are part of the GenX processing aid technology they developed.

Note: In July 2015, DuPont announced it had separated its Performance Chemicals segment through the creation of The Chemours Company. As a result, the GenX processing technology and associated chemicals are now products of The Chemours Company (Chemours, 2018). Because the submitted studies were conducted prior to the 2015 separation, however, the studies are referenced with DuPont identifiers.

Upon receipt, EPA assigned these PMNs case numbers P-08-0508 and P-08-0509, and they were reviewed by the New Chemicals Program in the Office of Pollution Prevention and Toxics (OPPT) and posted in the Federal Register (73 FR 46263, August 8, 2008) for public comment (EPA, 2008). A PMN assessment was completed and included a hazard assessment based on EPA review of test data submitted to the agency with the PMNs (including two 28-day oral (gavage) toxicity studies in mice (DuPont-24459, 2008) and rats (DuPont-24447, 2008)), as well as publicly available literature and TSCA confidential business information on other per- and polyfluoroalkyl substances (PFAS). Submitted test data on HFPO dimer acid and/or 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 28 days.

EPA OPPT evaluated the methods and data submitted and deemed the studies acceptable to the agency. The studies submitted in 2008 with the PMNs formed the primary basis of EPA's hazard assessment at that time. The 28-day toxicity study in mice, from which EPA OPPT derived the point of departure (POD) of 0.1 milligrams per kilogram per day (mg/kg/day), was conducted according to Organization for Economic Cooperation and Development (OECD) Test Guideline (TG) 407 (OECD, 2008a) and followed Good Laboratory Practices (GLP) (DuPont-24459, 2008; OECD, 2008a). The submitted studies were also used, in concert with information on other PFAS chemicals, to inform the decision to require further testing, as described in the Consent Order that concluded the PMN review (EPA, 2009).

The Consent Order included, among other things, additional testing pertaining to human health. The tests were identified in the Consent Order according to OECD TG numbers and/or EPA health effects TGs for pesticides and toxic substances numbers. Following are the studies included in the Consent Order relevant to human health and this assessment:

- Repeated dose metabolism and pharmacokinetics studies (OPPTS 870.7485) in mice and rats (Dupont-18405-1017, 2011)

- Modified Oral (Gavage) Reproduction/Developmental Toxicity Study in Mice (OECD TG 421) (Dupont-18405-1037, 2010; OECD, 2016a)
- 90 Day Oral (Gavage) Toxicity Study (OECD, 1998) (species not specified): Both mice (DuPont-18405-1307, 2010) and rats (Dupont-17751-1026, 2009) were submitted
- Combined Chronic Toxicity/Oncogenicity Study in Rats (OECD, 2009) (Dupont-18405-1238, 2013)

The OECD TGs are accepted internationally as standard methods for safety testing and:

> …are covered by the Mutual Acceptance of Data, implying that data generated in the testing of chemicals in an OECD member country, or a partner country having adhered to the Decision, in accordance with OECD Test Guidelines and Principles of GLP, be accepted in other OECD countries and partner countries having adhered to the Decision, for the purposes of assessment and other uses relating to the protection of human health and the environment (OECD, 2018a).

Specifically, for the required oral reproductive/developmental toxicity test, EPA OPPT included requirements for specific modifications to the test to increase the robustness of the study for this class of chemicals (DuPont-18405-1037, 2010; OECD, 2016a). These modifications are stated in the Consent Order (EPA, 2009) and were followed by the testing laboratory as outlined in the study report (DuPont-18405-1037, 2010). For the required combined chronic toxicity/oncogenicity study, EPA reviewed and concurred with protocols submitted to the agency prior to the study being conducted (DuPont-18405-1238, 2013). In addition, the submitter consulted with EPA on study findings to determine the need for additional data (e.g., further toxicokinetic testing based on results of the first tier OPPTS 870.7485 study). Finally, while not specifically required under the Consent Order, DuPont conducted and submitted results for additional OECD TG studies for Agency review (e.g., the prenatal and developmental toxicity study in rats (OECD, 2001b) (DuPont-18405-841, 2010).

1.2 Uses of GenX Chemicals under TSCA

GenX is a trade name for a processing aid technology developed by DuPont to make high-performance fluoropolymers without the use of perfluorooctanoic acid (PFOA) (Chemours, 2018). Transition to GenX processing aid technology began in 2009 as part of the company's commitment under the 2010/2015 PFOA Stewardship Program to work toward the elimination of these chemicals from emissions and products by 2015. Although production of most long-chain PFAS has been phased out in the United States and has been generally replaced by production of shorter chain PFAS, EPA is aware of ongoing use of long-chain PFAS by companies that did not participate in the PFOA Stewardship Program and ongoing use of the chemicals available in existing stocks or being newly introduced via imports.

Fluoropolymers are used in many applications because of their unique physical properties such as resistance to high and low temperatures, resistance to chemical and environmental degradation, and nonstick characteristics. Fluoropolymers also have dielectric and fire-resistant properties that have a wide range of electrical and electronic applications, including architecture, fabrics, automotive uses, cabling materials, food processing, electronics, pharmaceutical and biotech manufacturing, and semiconductor manufacturing (Gardiner, 2014).

One of the two PMNs EPA received in 2008, P-08-0508, was for HFPO dimer acid, a chemical used as an intermediate to make the polymerization aid HFPO dimer acid ammonium salt. The PMN for HFPO dimer acid ammonium salt was received by EPA under PMN P-08-0509 and is used as a replacement for PFOA in the manufacture of fluoropolymers. The GenX resin manufacturing process includes the thermal transformation of the HFPO dimer acid ammonium salt processing aid into a hydrophobic hydride. HFPO is used in the manufacture of HFPO dimer acid, HFPO dimer acid ammonium salt, other HFPO dimer acid derivatives, fluoropolymers (including polyethers), and other specialty agrochemical and pharmaceutical applications. Information on specific products containing GenX chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. GenX chemicals may also be generated as a byproduct of fluoromonomer production. When in water, both HFPO dimer acid and HFPO dimer acid ammonium salt dissociate to form the HFPO dimer acid anion ($HFPO^-$) as a common analyte. HFPO is manufactured from hexafluoropropene. HFPO dimer acid can react with additional HFPO to form the HFPO trimer acid and longer polymer fluorides. Other PFAS chemicals might be part of the GenX processing aid technology, but HFPO dimer acid and its ammonium salt are the major chemicals associated with this technology.

1.3 Occurrence

GenX chemicals were identified in North Carolina's Cape Fear River and its tributaries in the summer of 2012 (Strynar et al., 2015). Following this discovery, between June and December 2013, Sun et al. (2016) sampled source water at three drinking water treatment plants (DWTPs) (identified as DWTPs A, B, and C) treating surface water from the Cape Fear River watershed. The mean concentration of HFPO dimer acid in the finished drinking water treated by DWTP C was 0.631 microgram per liter (µg/L) (Sun et al., 2016). In a separate experiment to look at removal efficiency of DWTP C, water samples were taken during August 2014 from the raw water intake and after each treatment process step used by DWTP C (i.e., coagulation/ flocculation/sedimentation, raw and settled water ozonation, biological activated carbon filtration, and disinfection by medium-pressure ultraviolet lamps and free chlorine). GenX chemicals were found at concentrations of 0.4–0.5 µg/L at all steps of the treatment process, indicating that the concentrations of HFPO dimer acid were only slightly decreased by the conventional and advanced water treatment processes used at this DWTP.

The publication of these data prompted the North Carolina Department of Environmental Quality to sample sites for GenX chemicals along the Cape Fear River and in private wells close to the Chemours facility. In certain samples of surface water, groundwater, and finished drinking water, GenX chemicals were detected above 0.140 µg/L, which is North Carolina's drinking water health goal for GenX chemicals (NCDEQ, 2018c). Chemours has indicated that GenX chemicals have been discharged into the Cape Fear River for several decades as a byproduct of other manufacturing processes (NCDEQ, 2017). Petre et al. (2021) quantified the mass transfer of PFAS from contaminated groundwater to five tributaries of the Cape Fear River, including GenX chemicals. HFPO dimer acid and another fluoroether accounted for 61% of the total quantified PFAS. The study authors calculated that 32 kg/year of PFAS discharges from the groundwater to the five tributaries and the movement of these fluoroethers from the groundwater through the subsurface and into the streams occurred in less than the past 50 years. These data indicate that

the discharge of contaminated groundwater has led to long-term contamination of surface water and could lead to subsequent impacts on downstream drinking water (Petre et al, 2021).

Community concern over the detection of GenX chemicals in the Cape Fear Watershed led to the initiation of the GenX exposure study in Wilmington, North Carolina[1]. Blood samples from 344 Wilmington residents were collected between November 2017 and May 2018 and repeated blood samples from 44 of the participants were collected 6 months after the first sample collection. The blood sampling coincided with source control of GenX chemicals, and it is unknown whether study participants were drinking tap water at the time of collection. GenX chemicals were not detected above the analytical reporting limit of 2 ng/mL in any of the blood samples collected (Kotlarz et al., 2020).

GenX chemicals and other PFAS were also analyzed in 2682 urine samples from 2013–2014 National Health and Nutrition Examination Survey (NHANES) participants ≥ 6 years of age (Calafat et al., 2019). GenX chemicals were one of the few tested PFAS to be detected in the urine and was detected in approximately 1.2% of the population. The limit of detection was 0.1 µg/L. Importantly, this study demonstrated that the urine does not appear to be a good biomarker for PFAS. For example, PFOA and PFOS were detected in serum samples for > 98% of this study population, yet PFOA and PFOS were only detected in paired urine samples for < 0.1% of the same population.

In a report submitted by The Chemours Company to EPA, 24 human plasma samples were analyzed for HFPO dimer acid and were found at concentrations ranging from 1.0 ng/mL – 51.2 ng/mL. In seven of the samples, HFPO dimer acid was not detected above the analytical reporting limit of less than 1.0 ng/mL. No additional information about the study participants was provided in the report (DuPont- C30031_516655, 2017). GenX chemicals have been identified in other media, including rainwater and air emissions. North Carolina Department of Environmental Quality estimates for the Chemours Fayetteville Works plant (in the North Carolina Cape Fear watershed) indicate that Chemours' annual emissions of GenX chemicals could have exceeded 2,700 pounds per year during the reporting period (2017–2018) (NCDEQ, 2018a). Additional details on air emissions of GenX chemicals at the Fayetteville Works plant can be found at https://files.nc.gov/ncdeq/GenX/2018_April6_Letter_to_Chemours_DAQ_FINAL_signed.pdf. Rainwater samples were collected between February 28 and March 2, 2018 up to 7 miles from the North Carolina plant (NCDEQ, 2018b). The highest concentration of GenX chemicals in a rainwater sample (0.810 µg/L) was detected 5 miles from the Fayetteville Works facility center. The three samples collected 7 miles from the plant ranged from 0.045 to 0.060 µg/L (NCDEQ, 2018b). GenX chemicals also have been detected in three on-site production wells and one on-site drinking water well at the Chemours Washington Works facility in Parkersburg, WV. EPA subsequently requested that Chemours test for GenX chemicals in both raw and finished water at four public drinking water systems and 10 private drinking water wells. Chemours agreed to the testing and completed sampling during February 2018. The results from these samples are available at https://www.epa.gov/sites/production/files/2018-04/documents/hfpo_chemours_wash_works_sampling_2018.pdf and range before treatment from less than 0.010–0.081 µg/L in the public drinking water systems and less than 0.010–0.052

[1] https://genxstudy.ncsu.edu/

µg/L in the private drinking water wells (EPA, 2018a). All samples were below the limit of detection (0.010 µg/L) after treatment (EPA, 2018a).

Additionally, between the summer of 2016 and March 2018, GenX chemicals were identified in surface water and some soil samples collected upstream and downwind of a fluoropolymer production facility in Parkersburg, WV (Galloway et al., 2020). The highest concentrations of HFPO dimer acid in surface water samples (37–227 ng/L) were found in the direction of prevailing winds, directly across the Ohio River to the north and upstream to the northeast of the plant on the East Fork of the Little Hocking River. HFPO dimer acid was found in surface water samples up to 24 kilometers north of the facility, close to Beverly, OH. HFPO dimer acid was also detected in soil samples from Drag Strip Road, Veto Lake, and the Little Hocking Water Association at concentrations ranging from 3.09 nanograms per gram (ng/g) to 8.14 ng/g. These data reveal the downwind atmospheric transport of HFPO dimer acid.

Low concentrations of HFPO dimer acid (0.003–0.004 µg/L) were detected in the Delaware River, as reported in the recent publication by Pan et al. (2018).

The Kentucky Department of Environmental Protection (2019) reported detecting HFPO dimer acid in 11 samples from DWTPs at concentrations ranging from more than 1.32 ng/L to 29.7 ng/L. The study analyzed DWTPs using both surface water and ground water as sources and found the most frequent and highest detections of HPFO dimer acid at plants that use the Ohio River and ground water from the Ohio River alluvial aquifer as sources. For HFPO dimer acid, 10 detections were from surface water DWTPs and one detection was from a ground water DWTP. The ground water DWTP reported the highest concentration of HFPO dimer acid of all detections.

Globally, GenX chemical occurrence has been reported in Germany (Heydebreck et al., 2015; Pan et al., 2018), China (Heydebreck et al., 2015; Pan et al., 2017, 2018; Song et al., 2018), the Netherlands (Heydebreck et al., 2015; Gebbink et al., 2017; Pan et al., 2018), the United Kingdom (Pan et al., 2018), South Korea (Pan et al., 2018), and Sweden (Pan et al., 2018). HFPO dimer acid was also detected with a mean concentration of 30 pg/L in Artic surface water samples, suggesting long range transport (Joerss et al., 2020).

1.4 Other Assessments of GenX Chemicals

1.4.1 North Carolina Assessment

The North Carolina Department of Health and Human Services (NC DHHS) released a health assessment and provisional drinking water health goal for GenX chemicals in July 2017, which was finalized in October 2018 (NCDEQ, 2018c). North Carolina defines “health goal” as a nonregulatory, non-enforceable level of contamination below which no adverse health effects would be expected over a lifetime of exposure. The provisional health goal for exposure to GenX chemicals in drinking water is 0.140 µg/L, which is intended to protect the most sensitive population, namely bottle-fed infants. The state selected bottle-fed infants as the most sensitive population because they drink the largest volume of water per body weight (BW).

North Carolina’s provisional health goal is based on a reference dose (RfD) derived from a NOAEL of 0.1 mg/kg/day for liver effects (single-cell necrosis) in mice (DuPont-24459, 2008; DuPont-18405-1037, 2010). The total UF applied was 1,000, including individual factors to

account for interspecies variability (10), intraspecies variability (10), and extrapolation from a subchronic to a chronic exposure duration (10). This RfD of 0.0001 mg/kg/day was used to derive a drinking water equivalent level (DWEL), which considers exposure. The DWEL was calculated using BW and drinking water intake for bottle-fed infants and a relative source contribution of 20% to account for potential exposure to GenX chemicals from other media and routes, including air, soil, dust, and food (NCDEQ, 2018c). Additional details are available at NC DHHS.

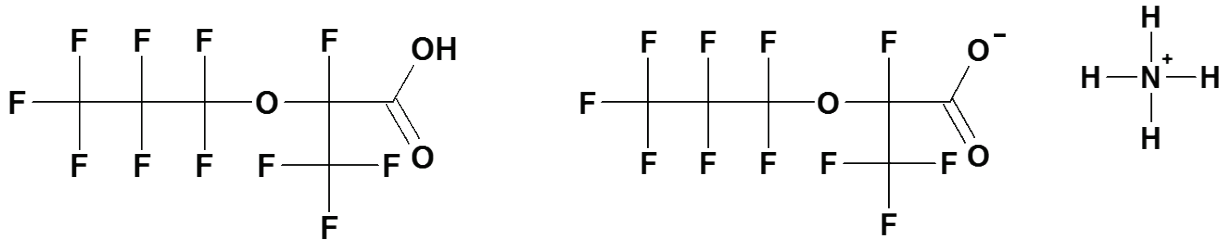
1.4.2 Report by the Netherlands National Institute for Public Health and the Environment

The National Institute for Public Health and the Environment (RIVM) in the Netherlands evaluated the data for GenX chemicals to set a safe limit for air. RIVM's assessment focused on the precursor 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid (FRD-903) (a synonym for HFPO dimer acid), the processing agent ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate (FRD-902) (a synonym for HFPO dimer acid ammonium salt), and the transformation product heptafluoropropyl 1,2,2,2-tetrafluoroethyl ether (E1). Overall, RIVM concluded that there is no health risk expected for people living near plants from emissions of FRD-902 and FRD-903 at a limit of 73 nanograms per cubic meter (insufficient data are available to determine the toxicity of E1) (Beekman et al., 2016). RIVM used the oral carcinogenicity study in rats as the critical study (DuPont-18405-1238, 2013) and concluded that the study NOAEL was 0.1 mg/kg/day, based on increased albumin and the albumin-to-globulin (A/G) ratio observed at 12 months in males dosed with 1 mg/kg/day, an effect that indicates the potential for immunotoxicity. Using route-to-route extrapolation, RIVM converted this NOAEL to an air concentration to be used as the POD. UFs to account for intraspecies differences (10) and interspecies differences (1.8), and an additional factor to account for uncertainty in the human elimination of GenX chemicals (66) were applied to the POD to determine the chronic inhalation limit.

2.0 Nature of the Stressor

2.1 Chemical/Physical Properties

HFPO dimer acid and its ammonium salt are fluorinated organic compounds (Figure 1).



HFPO dimer acid
CASRN 13252-13-6

HFPO dimer acid ammonium salt
CASRN 62037-80-3

Figure 1. Structure of HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt

HFPO dimer acid is a liquid whereas its ammonium salt is a solid at room temperature. Both are highly soluble in water. Except in very acidic solvents (pH less than 3), the acid will dissolve and be present as the acid anion with a -1 charge. The associated cation ion will be a hydronium ion (H_3O^+) in water if other hydrogen ion acceptors are absent. Both compounds can volatilize from water to air, where they will dissolve in aerosolized water droplets or bind to suspended particulate matter. In soils, they will migrate with the aqueous phase or bind to the soil particle surfaces with areas of positive charge. The organic portions of HFPO dimer acid and its ammonium salt are stable to environmental degradation. Table 1 presents the chemical and physical properties of HFPO dimer acid and its ammonium salt.

Table 1. Chemical and Physical Properties of HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
CASRN	13252-13-6	62037-80-3	Chemical Abstracts Service.
CAS Index Name	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)	Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-ammonium salt (1:1)	Chemical Abstracts Service.
IUPAC Name	2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid	azanium;2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate	PubChem.
Synonyms	GenX Acid FRD 903 H-28307 C3 dimer acid 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid	GenX salt308 FRD 902 FDR 90208 H-21216 H-27529 H-28072 H-28397 H-28308 H-28548 HFPO dimer ammonium salt C3 dimer salt Ammonium, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate Ammonium perfluoro(2-methyl-3-oxahexanoate) PMOH	DuPont-24637, 2008; DuPont- 24698, 2008.
Chemical Formula	$C_6HF_{11}O_3$	$C_6H_4F_{11}NO_3$	
Molecular Weight	330.06 g/mol	347.08 g/mol	

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
Color/Physical State	Clear, colorless liquid (20 ˚C, 101.3 kPa)	Solid	DuPont-24637, 2008; DuPont-24698, 2008.
Boiling Point	129 ˚C	108 ˚C (as 86% salt solution in water) No measurement available for salt form	DuPont-24637, 2008; DuPont-24698, 2008.
Melting Point	< –40.0 ˚C	-21.0 ˚C (as 86% salt solution in water) No measurement available for salt form	DuPont-24637, 2008; DuPont-24698, 2008.
Vapor Pressure	306 Pa (2.7 mm Hg) (20 ˚C)	No measurement available	DuPont-24128, 2008; DuPont-24129, 2008.
Henry's Law Constant	< 2.5 x 10^{-4} atm-m^3/mol	No measurement available	Calculated from measured vapor pressure and highest measured solubility. Water solubility is reported to be "infinite" (DuPont-24128, 2008; DuPont-24129, 2008), so the actual K_h is expected to be much lower. These values should not be used to estimate partitioning between water and air.
Pk_a	2.84 (20 ˚C)	3.82	DuPont-26349, 2008.
Pk_b	8.1	8.1	DuPont-24198, 2008 (HFPO dimer acid ammonium salt).
K_{oc}		Soil–12 L/Kg (log 1.10) Sludge–12.6 L/Kg (log = 1.08)	DuPont-17568-1675, 2008.
K_{ow}	Not applicable[a]	Not applicable[a]	
Solubility in Water @ 20 ˚C	>751 g/L	>739 g/L	Highest tested values. Actual solubility not determined but described as "infinite" (DuPont-24128, 2008; DuPont-24129, 2008).

Property	HFPO dimer acid	HFPO dimer acid ammonium salt	Source
Half-life ($T_{1/2}$) in Water (25 ˚C)	Stable	Stable	Measured hydrolysis values for salt. No degradation in 5 days at 50 ˚C and pH 4, 7, and 9 (DuPont-24199, 2008).
Half-life ($T_{1/2}$) in Air	Stable	Stable	Ultraviolet-visible and visible spectrophotometry spectra for acid showed little absorption above 240 nm (DuPont-26349, 2008). EPA concurs with DuPont's assessment that the salt is assumed to be similar. Measured OH· reaction rate for E1 reaction product indicates T½ > 37 years.
Biodegradation	Biodegradation was not observed in ready biodegradation and inherent biodegradation tests	Biodegradation was not observed in ready biodegradation and inherent biodegradation tests	DuPont-A080558, 2009; DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010.
Bioconcentration (Fish BCF)	< 30 (log < 1)	< 30 (log < 1)	Measured BCF[b] < 30 at 0.02 mg/L and < 3 at 0.2 mg/L in Medaka 28 days (DuPont-A080560, 2009).
Bioaccumulation (Field BAF)	< 10	< 10	Pan et al., 2017.[c]

Notes: ºC = degrees Celsius; atm-m^3/mol = atmosphere cubic meters per mole; BAF = bioaccumulation factor; BCF = bioconcentration factor; g/L = grams per liter; g/mol = grams per mole; International Union of Pure and Applied Chemistry (IUPAC); K_{OC} = soil-water partition coefficient for organic compounds; K_{OW} = octanol-water partition coefficient; kPa = kilopascals; L/kg = liters per kilogram; mg/L = milligrams per liter; mm Hg = millimeters of mercury; nm = nanometer; Pk_a = acid dissociation constant; Pk_b = base dissociation constant; $T_{1/2}$ = half-life.

[a] Surfactants are surface acting agents that lower the interfacial tension between two liquids. Their amphiphilic nature (i.e., they contain both a hydrophilic part and a hydrophobic part) causes them to accumulate at interfaces such as the water-air interface, the water-food interface, and glass walls, which hampers the determination of their aqueous concentration. These surfactant properties present difficulties in applying existing methods for the experimental determination of log K_{ow} and produce unreliable results.

[b] The concentration of the propionate ion was not quantified in the BCF study, so the values are limits based on the limit of quantification for the analytical technique employed in the study.

[c] Pan et al. (2017) quantified the propionate ion and found that the concentrations were low in the tissues expected to most likely accumulate perfluorinated compounds (e.g., muscle, blood, and so forth). The tissue values indicate a BAF less than 10. Lipid tissue concentrations are not the basis for this BAF as is common for "traditional" organic compounds.

2.2 Environmental Fate

HFPO dimer acid and its ammonium salt are stable to photolysis, hydrolysis, and biodegradation. The degradation data suggest that the substances will be persistent (i.e., have a half-life ($T_{1/2}$) longer than 6 months) in air, water, soil, and sediments. Based on measured physical-chemical and sorption data, they are expected to run off into surface water and to rapidly leach to ground water from soil and landfills. As seen with PFOA and chemicals with similar properties, HFPO dimer acid and its ammonium salt might undergo long-range atmospheric transport in the vapor phase and associated with particulates. They are not expected to be removed during conventional wastewater treatment or conventional drinking water treatment.

When released to the freshwater environment, HFPO dimer acid will dissociate to the HFPO carboxylate anion and H_3O^+. The ammonium salt will dissolve to the HFPO carboxylate anion and the ammonium cation ($NH4^+$). It is expected that other salts of the HFPO dimer acid (e.g., potassium and sodium salts) will behave similarly. Both have high solubilities in water and are expected to remain in water with low sorption to sediment or soil. Given the vapor pressure, the acid can partition to air as well as to water. The salt can also be transported in air, although the mechanism of vapor phase transport is not understood (DuPont CCAS, 2009). In the vapor phase, the acid and salt are expected to be stable to direct photolysis and will undergo hydroxyl radical catalyzed indirect photolysis very slowly.

2.2.1 Water

Measured data for HFPO dimer acid and/or ammonium salt show that they are highly soluble in water (Table 1). The measured base dissociation constants (pK_b) indicate that the chemicals will exist primarily as the propionate ion at most environmental pH levels.

The chemicals are stable to hydrolysis. A hydrolysis study on the ammonium salt found no degradation at pH 4, 7, and 9 at 50 degrees Celsius (ºC) in 5 days, indicating a hydrolysis $T_{1/2}$ of more than 1 year at 20 ºC (DuPont-24199, 2008). Calculated Henry's Law constants (Table 1) suggest that partitioning from water to air might occur. Experimental data on the transfer of the acid and salt from water to air indicate that partitioning from surface water to the vapor phase might occur and some transfer from surface water to air is expected (DuPont CCAS, 2009). Water-air transport of these chemicals, however, is not well understood. Their surfactant properties, equilibrium between chemical forms as a function of pH, and interaction with dissolved cations make it difficult to accurately predict how the chemicals will behave in the aquatic environment.

2.2.2 Air

The acid was described as having "a significant vapor pressure" (DuPont CCAS, 2009). As observed with PFOA and other perfluorochemicals, these chemicals could be transported in the vapor phase or could associate with particulate material and be transported with the solids when released or partitioned into air.

When released to air or volatilized from water, the chemicals are stable and short- and long-range transport has occurred (D'Ambro et al., 2021; Galloway et al., 2020). For example, D'Ambro et al. (2021) demonstrated that just 2.5% of the total GenX concentrations (defined as the HFPO dimer acid and HFPO dimer acid fluoride) emitted from a fluoropolymer manufacturing facility in North Carolina were deposited within 150 kilometers of the facility. Removal from air is expected to occur through scavenging by water droplets and attachment to particulates followed by precipitation and settling. No studies of long-range transport or air removal rates are available.

2.2.3 Sediments and Soils

Organic carbon normalized sorption coefficients were measured by high-performance liquid chromatography (HPLC) (following OECD, 2001a). The sorption of the HFPO dimer acid ammonium salt to soil and sludge were 12.0 liters per kilogram (L/kg) (log = 1.10) and 12.6 L/kg (log = 1.08), respectively (DuPont-17568-1675, 2008; OECD, 2001a). Their high water solubility and low sorption potential indicate that the chemicals will tend to remain largely in water with little partitioning to soil or sediment. If applied or deposited to soil, they will run off or leach to ground water and, as indicated by the vapor pressure, could volatilize to air.

2.2.4 Biodegradation

GenX chemicals are resistant to biodegradation; no degradation was observed in standardized internationally recognized test methods for biodegradability. The aerobic aquatic biodegradation $T_{1/2}$ is on the order of years based on no measured inherent biodegradation of the acid or ammonium salt in OECD 302C, modified Ministry of International Trade and Industry studies (DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010; OECD, 2008b).[2] The HFPO dimer acid ammonium salt showed no inhibition of activated sludge respiration (OECD TG 209) (OECD, 2010a) at up to 1,000 milligrams per liter (mg/L) (DuPont-25938 RV1, 2008).

2.2.5 Incineration

A preliminary study submitted to EPA by DuPont/Chemours indicates that thermal degradation occurs (DuPont-PMN Attachment 119, 2008) and the potential for significant removal during incineration exists. Thermal degradation was reported to be rapid for HFPO dimer acid and/or its ammonium salt. The acid $T_{1/2}$ was reported to be about 2,500 seconds (about 42 minutes) at 150 ºC and about 1,900 seconds (about 32 minutes) at 200 ºC. The salt $T_{1/2}$ was 500 seconds (8.3 minutes) at 150 ºC and 200 seconds (3.3 minutes) at 200 ºC (DuPont-PMN Attachment 119, 2008).

2.2.6 Bioaccumulation

Measured steady-state fish BCFs in medaka (*Oryzias latipes*) exposed to the acid at 0.2 mg/L and 0.02 mg/L in a flow-through system for 28 days were less than 3 and less than 30, respectively (DuPont-A080560, 2009). These BCF results were observed—BCFs of less than 3

[2] HFPO dimer acid aerobic aquatic biodegradation $T_{1/2}$ = 0% by biochemical oxygen demand (BOD) and 1.5% by high-performance liquid chromatography-tandem mass spectrometry (HPLC/MS/MS); HFPO dimer acid ammonium salt aerobic aquatic biodegradation $T_{1/2}$ = < 1% by BOD and 0% by HPLC/MS/MS in 28 days (DuPont-1388231-R2009NC031(a)-02, 2010; DuPont-1388231-R2009NC031(s)-02, 2010).

and less than 30 when exposures were 0.2 mg/L and 0.02 mg/L of the acid, respectively—under the same conditions in common carp (*Cyprinus carpio*) (Hoke et al., 2016). A field-derived BAF was determined from a water body impacted by industrial perfluoroether releases. The log BAFs for specific tissues in the carp were 0.86 for blood, 0.50 for liver, and 0.61 for muscle. The tissue values indicate a BAF of less than 10 (Pan et al., 2017).

In a 4-day trout hepatocyte bioaccumulation screening test (non-TG) with the HFPO dimer acid ammonium salt, no metabolism was observed, suggesting that *in vivo* metabolism does not significantly affect potential bioaccumulation (DuPont-23459, 2007).

2.3 Toxicokinetics

In rats and mice, HFPO dimer acid and its ammonium salt are both absorbed from the gastrointestinal tract at levels that are proportional to dose following acute oral exposures. Transfer from plasma/serum to the liver, but not adipose tissue, was demonstrated in the few studies that conducted tissue analysis. The potential for maternal transfer to the fetus (Conley et al., 2019; Blake et al., 2020) during development and to the neonate during lactation (DuPont-18405-1037, 2010) was noted. Urine is the primary pathway for excretion. Based on data from studies of acute, single-dose, gavage, and intravenous exposures, $T_{1/2}$s in the beta (elimination) phase are longer in male rats and mice than in females. The male rats' $T_{1/2}$s in the beta (elimination) phase are relatively comparable to those for the male and female monkeys, whereas the female rats' $T_{1/2}$s are shorter.

HFPO dimer acid is a strong acid (acid dissociation constant (pK_a) = 2.84) and will be predominantly ionized in aqueous solutions with pH values higher than 4 and in both plasma and serum (DuPont-26349, 2008). Once in solution, the cation that counterbalances the HFPO dimer anion will vary with the salt used or the mineral ion composition of the solvent, plasma, serum, intercellular, and intracellular fluids. Based on the physical and chemical properties of HFPO dimer acid and its ammonium salt, once these chemicals enter physiologic compartments with pH values higher than 4 (e.g., most ambient water, serum, or blood), they will either dissociate (acid) or dissolve (ammonium salt) to yield the carboxylate anion. Thus, what is being measured in the studies outlined in this section is the HFPO dimer acid anion concentration regardless of whether animals are dosed with HFPO dimer acid or its ammonium salt.

2.3.1 Absorption

Oral. Sprague Dawley (Crl:CD(SD)) rats (five of each sex (5/sex)) were administered (via gavage) a single oral dose of 30 milligrams per kilogram (mg/kg) HFPO dimer acid ammonium salt in aqueous solution (purity 84%) in a study conducted according to EPA TG OPPTS 870.7485. Two animals of each sex served as controls. Urine and feces were collected at 0–6 hours, 6–12 hours, 12–24 hours, and every 24 hours until 168 hours post-dose. The 0–12-hour urine collections accounted for a mean of 95% to 97% of the dose, supporting a conclusion that these GenX chemicals are rapidly absorbed from the GI tract by male and female rats (DuPont-18405-1017 RV1, 2011).

In a similar guideline study with Crl/CD-1(ICR) mice (5/sex) (OPPTS 870.7485), the animals were administered a single oral dose of 3 mg/kg HFPO dimer acid ammonium salt (purity 84%) by gavage in aqueous solution (DuPont-18647-1017 RV1, 2011). Two animals of each sex served as controls. Urine and feces were collected at 0–6 hours, 6–12 hours, 12–24 hours, and

every 24 hours until 168 hours post-dose. In the 0–12-hour urine collections, 31% (mean) of the substance was found for the males and 39% (mean) for the females. By 168 hours post-dosing, the total accumulated urine values accounted for 90% and 92% of the total dose for male and female mice, respectively, indicating that both rats and mice extensively absorb the HFPO dimer acid anion. This study additionally shows mice either incompletely absorb HFPO dimer acid anions or eliminate it in urine at a slower rate than was seen in the rats (DuPont-18647-1017 RV1, 2011).

A 28-day gavage study by Rushing et al. (2017) indicates a potentially more complex toxicokinetic profile for HFPO dimer acid when dosing occurs over multiple days. Groups of six male and six female C57BL/6 mice were given doses of 1, 10, or 100 mg/kg/day of HFPO dimer acid daily for 28 days. Serum concentrations were measured at intervals of 1, 5, 14, and 28 days, and urine concentrations were measured on days 1, 2, 3, 5, 10, and 14. At each time point, serum levels reflected the magnitude of the dose, but not the exposure duration. The peak serum concentration occurred at day 5 for all but the high-dose males, where it occurred at day 14. Serum measurements for the 1- and 10-mg/kg/day doses were lower on days 14 and 28 than on day 5. The differences in serum concentration between days 5, 14, and 28 are not explained by the study authors, but could possibly indicate changes in absorption, tissue storage, or elimination after repeated dosing. The males exposed to 10 and 100 mg/kg/day had higher serum and urine concentrations than the females, as described in section 2.3.5 (Excretion). Based on the higher serum and urine concentrations, there appeared to be greater absorption in males than in females.

In a repeated-dose study following OECD TG 408 (OECD, 1998) guidelines, HFPO dimer acid ammonium salt (purity 84%) was administered to Crl:CD1(ICR) mice for 95 (males) or 96 (females) consecutive days via gavage at doses of 0, 0.1, 0.5, and 5 mg/kg/day (DuPont-18405-1307, 2010). Ten animals per sex per group (animals/sex/group) were included for toxicity evaluation, and an additional 15/sex/group were included for quantitation of the test substance plasma concentration 2 hours after dosing on day 0 (the first day of dosing) (5/sex/dose), providing a measure of post-dosing absorption (Table 2). Overall, plasma concentrations increased with increasing dose, indicating that absorption was not saturated, and displayed broad standard deviations indicative of considerable inter-animal variability in the absorption. The doses evaluated differ from those used by Rushing et al. (2017), limiting comparisons of the postexposure serum and plasma data. The sex difference seen by Rushing et al. (2017) (i.e., where male uptake to serum for the 1 and 10 mg/kg/day doses at the end of day 1 was greater than female uptake) is not as apparent at 2 hours post-dosing in this dataset.

Table 2. Plasma Concentration in Crl:CD1(ICR) Mice at 2 Hours after the First Gavage Exposure to HFPO Dimer Acid Ammonium Salt

Dose mg/kg/day	Males		Females	
	µg/mL	SD	µg/mL	SD
0	Not detected[a]	N/A	Not detected	N/A
0.1	0.736	0.099	0.824	0.072

Dose mg/kg/day	Males		Females	
0.5	3.806	1.197	3.606	1.308
5	42.58	5.214	35.34	9.262

Source: Dupont-18405-1307, 2010.
Notes: N/A = not applicable; µg/mL = micrograms per milliliter; SD = standard deviation.
[a] Detection limit of the method was 0.005 µg/mL in plasma.

Inhalation. There are no studies investigating HFPO dimer acid or its ammonium salt's uptake following inhalation exposures of aerosols. In a study conducted by Dupont (17751-723, 2009), one group of 5 male and 5 female Crl:CD(SD) rats were exposed to 5,200 milligrams per cubic meter (mg/m^3) and two groups of male and female Crl:CD(SD) rats (3/sex/group) were exposed to aerosols containing 0, 13, and 100 mg/m^3 of HFPO dimer acid ammonium salt (84% purity) for a single 4 hour period. One male and one female rat exposed to air only were used as the control. The rats in the 0, 13, and 100 mg/m^3 groups had a 2-day recovery period. The rats in the 5,200 mg/m^3 group recovered for 14-days. There were no measurements of the chemical in serum or plasma, however, to support an estimate of absorption by way of the respiratory tract.

Dermal. Absorption of HFPO dimer acid ammonium salt through the skin was determined *in vitro* with rat and human skin specimens (DuPont-25292, 2008). HFPO dimer acid ammonium salt (86% purity) was diluted with water to a concentration of 124 milligrams per milliliter (mg/mL). Serial receptor fluid samples were collected at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, and 24 hours and analyzed for cumulative HFPO anion concentration.

Steady-state penetration rates were 70.3 ± 5.3 and 6.2 ± 5.3 micrograms per square centimeter per hour for rat and human skin, respectively, which yielded dermal permeability coefficients of 5.71E-4 ± 4.3E-5 centimeters per hour (cm/hr) for rats and 5.02E-5 ± 4.3E-5 cm/hr for humans. These dermal kinetic parameters demonstrate dermal absorption occurs, but at a relatively slower rate than chemicals that are well absorbed dermally.

2.3.2 Distribution

Crl:CD(SD) rats (3/sex/dose) were administered a single oral dose of 10 or 30 mg/kg by gavage in aqueous solution of either HFPO dimer acid ammonium salt (purity 84.5%) or HFPO dimer acid (purity 98%) (DuPont-24281, 2008; DuPont-24286, 2008). Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours, as described in section 2.3.6 (Clearance and Half-life Data). Liver and fat samples were presumed to be collected for analysis after the 168-hour plasma sample collection. In male rats dosed with HFPO dimer acid ammonium salt, the mean concentration in plasma at 168 hours post-dose was 0.036 ± 0.011 micrograms per milliliter (µg/mL) (36 ± 11 nanograms per milliliter (ng/mL)) for the low dose (10 mg/kg) and 0.057 ± 0.036 µg/mL (57 ± 36 ng/mL) for the high dose (30 mg/kg). In male rats dosed with HFPO dimer acid, the mean concentration in plasma at 168 hours post-dose was 0.041 ± 0.01 µg/mL (41 ± 10 ng/mL) for the low dose (10 mg/kg) and 0.128 ± 0.023 µg/mL (128 ± 23 ng/mL) for the high dose (30 mg/kg). In female rats, plasma concentrations of HFPO dimer acid anion were not above the limit of quantification (LOQ) in any sample at 168 hours post-dosing. In males dosed with HFPO dimer acid ammonium salt, the mean concentration of HFPO dimer acid anion in the liver 168 hours post-dose was 73 ± 25 ng/g for the low dose (10 mg/kg)

and 38 ± 15 ng/g for the high dose (30 mg/kg). In males dosed with HFPO dimer acid, the mean concentration of HFPO dimer acid anion in the liver 168 hours post-dose was 24 ± 6 ng/g for the low dose (10 mg/kg) and 89 ± 4 ng/g for the high dose (30 mg/kg). The mean liver tissue-to-plasma concentration ratio was higher in males for the ammonium salt (2.19) than for the acid (0.64) at the low dose (10 mg/kg). At the high 30 mg/kg dose, the liver tissue-plasma concentration ratio values in male rats were similar: 0.78 for the ammonium salt and 0.71 for the acid. Females at both doses, however, had a lower accumulation of HFPO dimer acid and its ammonium salt in the liver than in the male did. Overall, 10 out of 12 female rats dosed with HFPO dimer acid or its ammonium salt had undetectable concentrations of HFPO dimer acid anion in the liver (LOQ = 20 ng/g). Two females dosed with HFPO dimer acid ammonium salt at the low dose (10 mg/kg) had liver HFPO dimer acid anion concentrations above the LOQ, containing 20.6 and 54.1 ng/g of HFPO dimer acid anion. Females dosed with HFPO dimer acid did not have liver anion concentrations above the LOQ (20 ng/g). HFPO dimer acid anion concentrations in the fat tissue samples were below the LOQ of 20 ng/g in all the rats given HFPO dimer acid or HFPO dimer acid ammonium salt (DuPont-24281, 2008; DuPont-24286, 2008).

Crl:CD1(ICR) mice (3/sex/dose) were administered a single oral dose of 10 or 30 mg/kg by gavage in aqueous solution of HFPO dimer acid ammonium salt (purity 86%) (DuPont-25300, 2008). Unlike the rat studies, HFPO dimer acid was not evaluated in the mice. Plasma samples were collected at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours, as described in section 2.3.6 (Clearance and Half-Life Data). Liver and fat samples were presumed to be collected for analysis after the 168 hour plasma sample collection. In males, the mean concentration of HFPO dimer acid anion in the liver was 384 ± 472 ng/g for the low dose (10 mg/kg) and 457 ± 337 ng/g for the high dose (30 mg/kg). The mean concentration in fat tissue was 31.6 ng/g in males for the high dose (30 mg/kg) and less than the LOQ (20 ng/g) for the low dose (10 mg/kg) and for both doses in females.

In male mice, the mean concentration in plasma at 168 hours post-dose was 0.759 ± 0.946 µg/mL (759 ± 946 ng/mL) for the low dose (10 mg/kg) and 0.83 ± 0.618 µg/mL (830 ± 618 ng/mL) for the high dose (30 mg/kg). In females, only one of three mice in each dose group had a plasma concentration above the LOQ at 168 hours post-dose, which was 0.0232 µg/mL (23.2 ng/mL) for the high dose (30 mg/kg) and 29.2 ng/g for the low dose (10 mg/kg). Based on the plasma and liver concentrations reported in the study, a liver-to-plasma ratio was calculated for males, but not for females because the females did not have liver concentrations above the LOQ. At the low dose (10 mg/kg), the average male liver-to-plasma ratio was 0.52, and at the high dose (30 mg/kg), it was 0.58.

Because the perfluorinated portion of the HFPO dimer acid ether is similar to that of the perfluorinated alkane acids (e.g., PFOA), HFPO dimer acid and its ammonium salt are anticipated to be transported in serum either freely dissolved or bound to serum protein (Gomis et al., 2018). Additionally, studies have demonstrated that 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) demonstrate that bovine serum albumin binds

HFPO dimer acid, and that the albumin/water partition coefficient is in the same range as other PFAS (e.g., perfluorobutanoic acid (PFBA) and perfluorohexane sulfonic acid (PFHxS)).

A study by Sheng et al. (2018) reported that the HFPO dimer acid anion also binds to fatty acid-binding protein (FABP). FABPs are intracellular lipid carrier proteins for long-chain fatty acids, phospholipids, and a variety of chemicals that induce peroxisome proliferation (Erol et al., 2003). They constitute 2%–5% of the cytosolic protein in the liver. FABPs can be synthesized in the gastrointestinal tract and act as a systemic carrier of long-chain fatty acids in plasma and serum (Storch and McDermott, 2009). Thus, FABPs likely play a role in the systemic distribution of HFPO dimer acid in both its neutral and ionized forms.

2.3.3 Distribution during Gestation and Lactation

HFPO dimer acid ammonium salt can be transferred (distributed) from a pregnant animal to the fetus, as demonstrated in multiple studies. In an OECD TG 421 (OECD, 2016a) reproduction/developmental toxicity study (DuPont-18405-1037, 2010), pregnant Crl:CD1(ICR) mice (25/sex/group) were administered, by gavage, 0, 0.1, 0.5, or 5 mg/kg/day HFPO dimer acid ammonium salt from premating day 14 to lactation day (LD) 20/21. Blood was collected from the dams 2 hours after dosing on LD/postnatal day (PND) 21 (scheduled termination) and pooled. The litters were normalized on PND4 to 8 pups per litter (4/sex). Blood was collected and pooled from the pups not randomly selected on PND4. The HFPO dimer acid anion was present in the pooled plasma of PND4 pups at concentrations approximately two to four times lower than the concentrations in the dams on LD21. These results indicate that there is transfer of HFPO dimer acid anion from maternal serum. The PND/LD21 plasma levels in both male and female pups, however, were forty- to sixtyfold lower than the maternal LD21 plasma concentrations, indicating that the majority of fetal transfer occurred during gestation (DuPont-18405-1037, 2010).

Blake et al. (2020) demonstrated that HFPO dimer acid can be transferred from the pregnant dam to the embryo during gestation. Pregnant CD-1 mouse dams were dosed from embryonic day (E) 1.5 to E11.5 or E17.5 with either deionized water (vehicle control), 1 or 5 mg/kg/day of PFOA, or 2 or 10 mg/kg/day of HFPO dimer acid. At E11.5 and E17.5, serum and a portion of the hepatic left lateral lobe were collected from pregnant dams after the final dose. Amniotic fluid was collected by needle aspiration from litters euthanized on E11.5 and whole embryos were collected on E11.5 and E17.5 to determine the concentration of HFPO dimer acid. HFPO dimer acid was detected in both the amniotic fluid and the whole embryo at 2 and 10 mg/kg/day and at both time points, demonstrating transfer of HFPO dimer acid from the pregnant dam to the fetus during gestation (Table 3).

Table 3. Concentrations of HFPO Dimer Acid in CD1 Pregnant Mice and Their Embryos at Embryonic Day 11.5 or 17.5[a]

Measurement [b]	Embryonic day	HFPO dimer acid	
		2 mg/kg/day	10 mg/kg/day
Maternal Serum (µg/mL)	11.5	33.5 ± 15.7	118.1 ± 10.4
	17.5 [c]	22.9 ± 17.1	58.5 ± 34.5

Measurement [b]	Embryonic day	HFPO dimer acid	
		2 mg/kg/day	10 mg/kg/day
Amniotic Fluid (µg/mL)	11.5	3.6 ± 2.2	9.3 ± 2.0
	17.5	NQ	NQ
Maternal Liver (µg/g)	11.5	5.45 ± 3.43	19.9 ± 4.2
	17.5	4.56 ± 2.80	14.2 ± 7.6
Whole Embryo (µg/g)	11.5	0.91 ± 0.22	3.21 ± 0.51
	17.5	3.23 ± 1.28	7.69 ± 2.92

Source: Blake et al., 2020.
Notes: µg/mL = micrograms per milliliter; µg/g = micrograms per gram embryo weight; SD = standard deviation; NQ = not quantified due to limited volume.
[a] For each reported measurement in this table, N = 6–8 per group.
[b] Limit of detection was 0.010 µg/mL; note all vehicle control samples were below the limit of detection.
[c] HFPO dimer acid was detected in the serum of vehicle control mice in the E17.5 group (0.211 ± 0.55 µg/mL).

HFPO dimer acid concentrations increased with increasing dose in all samples. The concentration of HFPO dimer acid in the whole embryo increased from E11.5 to E17.5 in both dose groups, indicating bioaccumulation in the embryo over the gestational period. Conversely, the concentration of HFPO dimer acid in the maternal serum decreases from E11.5 to E17.5 in both dose groups. The authors note that the decrease in maternal serum HFPO dimer acid could be the result of increased transfer to embryos over time or to dilution effect from blood volume expansion over the course of gestation.

In the DuPont-18405-1037 (2010) study, generally, the standard deviations were large in all dose groups, especially as compared to PND21. The male pups tended to have slightly higher plasma concentrations of HFPO dimer acid anion than the female pups at PND40. For example, at the 0.1 mg/kg/day-dose group, the concentration of HFPO dimer acid anion was 1.352 and 0.946 µg/mL (1,352 and 946 ng/mL) in male and female pups, respectively. Similarly, at the 0.5 mg/kg/day-dose group, the concentration of HFPO dimer acid anion was 6.282 and 4.074 µg/mL (6,282 and 4,074 ng/mL) in male and female pups, respectively, and it was 51.34 µg/mL (51,340 ng/mL) in male pups and 43.34 µg/mL (43,340 ng/mL) in female pups at 5 mg/kg/day (DuPont-18405-1037, 2010).

Transfer of HFPO dimer acid anion to the fetus was also demonstrated in groups of five Crl:CD(SD) rats exposed to doses of 0, 5, 10, 100, or 1,000 mg/kg/day from gestation day (GD)6 to GD20 (Dupont-18405-849 RV1, 2011). On GD20, blood was collected from individual dams 2 hours after dosing and trunk blood was collected from the fetuses and pooled by litter for analysis. The plasma concentration in the blood samples from the dams was three times higher than the plasma concentration in the pooled blood of their fetuses. The detection of HFPO dimer acid anion in the pooled fetus plasma demonstrates gestational transfer from dam to fetus.

Similarly, Conley et al. (2019) demonstrated transfer of HFPO dimer acid anion to the fetus by measuring serum concentrations of pregnant Crl:CD(SD) rats exposed to 0, 1, 3, 10, 30, 62.5, 125, 250, and 500 mg/kg/day HFPO dimer acid ammonium salt from GD14 through GD18.

Serum was collected from the dams in all dose groups and plasma was collected from the fetuses in the 0, 1, 3, 10 and 30 mg/kg/day groups. On GD18, trunk blood was collected from individual dams 2 hours after dosing and blood was collected from the fetuses' jugular vein and pooled per litter for analysis. HFPO dimer acid anion was detected in the pooled fetal plasma at all doses and the concentration increased with increasing maternal dose (Table 4). The study authors noted that, while the increases in maternal serum and fetal plasma were linear in the lower dose range (0–30 mg/kg/day), the maternal slope was significantly greater than the fetal slope. The maternal serum concentration of HFPO dimer acid anion increased 0.46 µg/mL (460 ng/mL) per 1 mg/kg increase in maternal dose while the fetal plasma concentration increased 0.12 µg/mL (120 ng/mL) per 1 mg/kg increase in maternal dose. Additionally, the study authors modeled uptake over the full maternal dose range (1–500 mg/kg) (Table 4) using exponential one-phase association and determined that a plateau was reached at 112 ± 15 µg/mL (112,000 ± 15,000 ng/mL), indicative of uptake saturation (Conley et al., 2019).

Table 4. Maternal Serum and Fetal Plasma Concentrations on GD18 in Crl:CD(SD) Rats Exposed to HFPO Dimer Acid Ammonium Salt from GD14-18

Oral dose mg/kg/day	Pregnant dam serum		Fetal plasma	
	µg/mL	SE	µg/mL	SE
0	0.027	0.008	0.018	0.01
1	0.68	0.08	0.13	0.06
3	1.2	0.3	0.49	0.04
10	4.6	1.1	1.9	0.2
30	13.9	3.1	3.5	0.4
62.5	30.7	2.9	N/A	N/A
125	46.0	10.3	N/A	N/A
250	81.8	21.6	N/A	N/A
500	100.7	26.4	N/A	N/A

Source: Conley et al., 2019, Table S10.
Notes: µg/mL = micrograms per milliliter; N/A = not applicable because no sample collected at that dose; SE = standard error.

Conley et al. (2021) also demonstrated transfer of HFPO dimer acid anion to the fetus and pup by measuring serum concentrations of pregnant Crl:CD(SD) rats exposed to 0, 1, 3, 10, 30, 62.5, or 125 mg/kg/day HFPO dimer acid ammonium salt from GD16 through GD20 or to 0, 10, 30, 62.5, 125, or 250 mg/kg/day from GD8 through PND2. Serum was collected from the dams and fetuses in all dose groups on GD20 in the GD16-20 experiment and from the dams on PND2 in the neonatal experiment. In the GD16–20 experiment, trunk blood and liver samples were collected from both dams and fetuses 2 to 4 hours after the final oral dose on GD20. Fetal serum was pooled per litter for analysis. On PND2 in the neonatal experiment, trunk blood and liver samples were collected from the dams 2 to 5.5 hours after the final oral dose and liver samples were collected from the pups. Maternal serum and liver HFPO dimer acid anion concentrations

increased as a function of dose during both experiments (Table 5). The study authors noted that there was no statistically significant difference in serum or liver concentration within a given dose group between the two experiments indicating that bioaccumulation did not occur after longer exposure. HFPO dimer acid anion was detected in the pooled fetal serum at all doses and the concentration generally increased with increasing maternal dose. Regression analyses showed that fetal and maternal serum concentrations increased log-linearly as a function of maternal oral dose, and maternal serum concentrations were approximately 2- to 3-fold greater than fetal serum concentrations. Liver concentrations of HFPO dimer acid anion in dams, fetuses, and pups also increased log-linearly. The fetal and maternal liver concentrations on GD20 were nearly identical for the 30–125 mg/kg/day dose levels. On PND2, male pup liver concentrations were significantly greater than female pup liver concentrations, which was most prominent at the 125 mg/kg/day dose level. PND2 liver concentrations for both sexes were approximately 10-fold lower than concentrations observed in GD20 fetal livers.

Table 5. Maternal and Offspring HFPO Dimer Acid Anion Concentrations in Serum and Liver Samples Collected on GD20 or PND2 from Crl:CD(SD) Rats Orally Exposed to HFPO Dimer Acid Ammonium Salt from GD16-20 or GD8-PND2

Oral dose mg/kg/day	Maternal serum GD20 (µg/mL)	Fetal serum GD20 (µg/mL)	Maternal serum PND2 (µg/mL)	Maternal liver GD20 (µg/g)	Fetal liver GD20 (µg/g)	Maternal liver PND 2 (µg/g)	Female pup liver PND 2 (µg/g)	Male pup liver PND 2 (µg/g)
0	0.016 ± 0.014	0.014 ± 0.008	<LOQ	0.29 ± 0.12	0.07 ± 0.04	0.14 ± 0.05	0.07 ± 0.021	<LOQ
1	0.54 ± 0.10	0.33 ± 0.03	NA	2.11 ± 0.78	0.23 ± 0.09	NA	NA	NA
3	1.15 ± 0.28	1.56 ± 0.84	NA	3.18 ± 1.01	0.46 ± 0.05	NA	NA	NA
10	3.05 ± 0.90	3.14 ± 0.71	1.76 ± 0.60	3.70 ± 0.92	2.07 ± 0.18	2.90 ± 0.91	0.21 ± 0.05	0.22 ± 0.02
30	7.46 ± 2.59	2.74 ± 1.88	4.22 ± 0.83	8.36 ± 2.35	9.09 ± 0.96	4.42 ± 1.21	0.64 ± 0.14	1.10 ± 0.26
62.5	13.81 ± 3.76	7.63 ± 1.16	16.09 ± 5.88	21.65 ± 3.81	22.30 ± 4.96	22.93 ± 7.23	1.64 ± 0.11	2.37 ± 0.60
125	31.96 ± 6.67	11.68 ± 2.77	28.39 ± 9.63	42.82 ± 9.05	44.08 ± 10.54	43.99 ± 15.57	1.83 ± 0.83	4.96 ± 1.36
250	NA	NA	41.57 ± 12.91	NA	NA	45.88 ± 14.43	NA	6.48 (n = 1)

Source: Conley et al., 2021, Table S11.
Notes: Values reported as mean ± standard error of the mean (SEM); sample size n = 2–6 except where noted; LOQ = limit of quantitation (0.005 µg/mL for serum, 0.1 µg/g for liver); µg/mL = micrograms per milliliter; µg/g = micrograms per gram; N/A = not applicable because no sample collected at that dose.

In the studies of rats dosed during pregnancy in which plasma concentrations in both the dams and fetuses were measured at GD20 (Dupont-18405-849 RV1, 2011) or GD18 (Conley et al., 2019), the HFPO dimer acid anion plasma concentration ratio for dams to fetuses is approximately two to four. In the study of mice dosed during pregnancy (Dupont 18405-1037, 2010), plasma concentrations were measured in dams on LD21 and in pups on PND4, PND21, and PND40. If the plasma concentrations in dams on LD21 are assumed to be representative of those on LD4, the comparison to pup plasma concentrations on PND4 indicate a dam-to-pup plasma concentration ratio of two to four. Together these data indicate the efficiency of transfer in rats and mice is of a similar magnitude.

2.3.4 Metabolism

In two *in vitro* studies, hepatocytes (1 x 10^6 cells/mL for clearance incubations and 5 x 10^6 cells/mL for biotransformation incubations) prepared from male and female Crl:CD(SD) rats were incubated with 2 micromolar (µM) (clearance) or 200 µM (biotransformation) solutions of HFPO dimer acid ammonium salt for a total of 120 minutes (DuPont-23460, 2007). Samples were analyzed for HFPO dimer acid ammonium salt and potential metabolites at 5, 15, 30, 45, 60, 90, and 120 minutes. Heat-inactivated hepatocytes were used as negative controls and 4-nonylphenol in live hepatocytes were used as a positive control. There was no difference in the concentration of HFPO dimer acid between the viable and heat-inactivated hepatocytes, indicating that HFPO dimer acid ammonium salt is not metabolized by rat hepatocytes. Additionally, no metabolites were detected in the biotransformation incubation samples (DuPont-23460, 2007). Similar *in vitro* studies were conducted in rat hepatocytes in Gannon et al. (2016). Hepatocytes (1 x 10^6 cells/mL for clearance incubations and 5 x 10^6 cells/mL for biotransformation incubations) prepared from male and female Crl:CD(SD)rats were incubated with 5 µM (clearance) or 50 µM (biotransformation) solutions of HFPO dimer acid ammonium salt for a total of 120 minutes. Heat-inactivated hepatocytes were used as negative controls and samples were collected at 0, 30, 45, 60, 90, and 120 minutes. Gannon et al. (2016) concluded that the test substance was not metabolized by rat hepatocytes because there was no difference in clearance rate between live and heat-inactivated hepatocytes and no metabolites were identified.

In the single oral (gavage) study of rats described in section 2.3.1 (Absorption), the total accumulated amount of HFPO dimer acid ammonium salt at 168 hours post-dosing in the collected urine accounted for 103% $\pm$ 2.73% and 99.8% $\pm$ 6.41% of the administered dose for male and female rats, respectively, and there was no detection of metabolites (DuPont-18405-1017 RV1, 2011).

Similarly, in the single oral (gavage) study of mice described in section 2.3.1 (Absorption), the total accumulated amount of HFPO dimer acid ammonium salt accounted for 89.5% ± 6.91% and 91.5% ± 6.04% of the total dose for male and female mice, respectively, and there was no detection of metabolites in the urine (DuPont-18647-1017 RV1, 2011).

2.3.5 Excretion

Urine. Studies in rats, mice, and monkeys indicate that urine is the primary excretory pathway for GenX chemicals. In the DuPont-18405-1017 RV1 (2011) study, Crl:CD(SD)rats (5/sex) administered a single oral (gavage) dose of 30 mg/kg HFPO dimer acid ammonium salt excreted 95% to 97% of the dose in urine within 12 hours. The pooled urine collections accounted for virtually all the substance administered with no evidence of metabolic alteration. Study authors calculated the elimination $T_{1/2}$ in the urine for male and female rats to be 3 hours and 8 hours, respectively. In a companion study, Crl/CD1(ICR) mice (5/sex) were administered a single oral (gavage) dose of 3 mg/kg HFPO dimer acid ammonium salt (purity 84%) (DuPont-18647-1017 RV1, 2011). Urinary elimination in mice appeared to be less efficient than in the rats given that only 31% (mean) and 39% (mean) of the dose material was found in the 12-hour pooled urine for the male and female mice, respectively. At 168 hours post-dosing, the mean values for the pooled urine samples accounted for 90% and 92% of the total dose for the male and female mice, respectively (DuPont-18647-1017 RV1, 2011). Study authors calculated the elimination $T_{1/2}$ in the urine for male and female mice to be 21 hours and 18 hours, respectively. Based on the amounts in urine and the clearance from blood (see section 2.3.6), mice appear to have less of an

ability than rats to clear the HFPO dimer acid anion by transferring it to urine in the early postexposure period. The differences in the results of these studies might have been influenced by the different doses given to the rats (30 mg/kg) and the mice (3 mg/kg) (DuPont-18647-1017 RV1, 2011; DuPont-18405-1017 RV1, 2011).

The dynamic relationship across dose and exposure duration observed in serum measurements from the Rushing et al. (2017) study is also reflected in their data on urinary excretion. Urine concentrations were monitored on exposure days 1, 2, 3, 5, 10, and 14. For the 1- and 10-mg/kg/day doses, urinary concentration peaked on day 3 and, thereafter, declined monotonically. Males had higher urine concentrations than females at each time point, consistent with their higher serum concentrations. For the 100-mg/kg/day-dose group, the concentrations in urine peaked at day 2 and again at day 14 in males while in females they appeared to peak at 5 days followed by a decline at 10 and 14 days.

Feces. Fecal elimination of HFPO dimer acid appears to be minor in rats and mice in the available single-dose studies (DuPont-18405-1017 RV1, 2011; DuPont-18647-1017 RV1, 2011). Specifically, feces + cage wash (dried fecal matter) from male and female rats had 2% and 6% of recovered compound, respectively, while feces + cage wash from male and female mice had 12% and 8% of recovered compound, respectively. The data for combined fecal matter and cage wash suggest that mice might lose slightly more HFPO dimer acid through fecal matter than rats. Low fecal excretion could reflect low levels of hepatic loss via biliary excretion.

2.3.6 Clearance and Half-Life Data

Clearance time. In multiple study reports, the study authors did not calculate pharmacokinetic parameters such as $T_{½}$ or area under the curve and instead defined the metric "clearance time" as the time when 98.4% of the anion from the HFPO dimer acid ammonium salt was cleared from the plasma.

A total of 12 Crl:CD(SD) rats, 3/sex/dose, received a single oral dose of 10 or 30 mg/kg/day HFPO dimer acid ammonium salt (84.5% purity) by gavage (Dupont-24281, 2008). Plasma samples were collected from animals serially at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours. In males, plasma levels peaked within the first 1–2 hours after dosing for the low dose, and within the first 30 minutes to 1 hour for the high dose. By days 4 to 5, plasma concentrations were less than 1% of the peak level, although still above the LOQ (0.02 µg/mL (20 ng/mL)). In females, the plasma levels peaked at 1 hour for the low dose and had usually declined to the LOQ (0.02 µg/mL (20 ng/mL)) by 24 hours. At the 30-mg/kg dose, the plasma levels of female rats peaked at 30 minutes to 1-hour post-dosing and declined to the LOQ (0.02 µg/mL (20 ng/mL)) by 24 or 48 hours. In male rats, the authors identified 12 hours as the clearance time at the low dose and 22 hours at the high dose (Table 6). In female rats, the clearance values were 4 hours and 8 hours for the low dose and high dose, respectively.

Table 6. Clearance Times in Plasma for Male and Female Rats and Mice Following a Single Oral Dose[a]

Chemical	Male rat	Male mouse	Female rat	Female mouse
10 mg/kg				
HFPO dimer acid ammonium salt	12 hr	143 hr	4 hr	57 hr
HFPO dimer acid	28 hr	ND	8 hr	no data
30 mg/kg				
HFPO dimer acid ammonium salt	22 hr	139 hr	8 hr	62 hr
HFPO dimer acid	22 hr	ND	4 hr	no data

Sources: Dupont-24281, 2008; Dupont-24286, 2008; Dupont-25300, 2008.
Notes: hr = hour
[a] "Clearance time" is defined as the time when 98.4% of the HFPO dimer acid ammonium salt was cleared from the plasma.

The same protocol was followed using HFPO dimer acid (98% purity) (Dupont-24286, 2008). At the low dose, plasma concentrations peaked within 1 hour in both male and female rats, while at the high dose, the peak plasma concentrations occurred in males at 1 or 2 hours and in females at 15 minutes. The clearance times in males were 28 hours and 22 hours for the low dose and high dose, respectively. The clearance times in females were 8 hours and 4 hours for the low dose and high dose, respectively (Table 6).

The protocol outlined in this section was also followed for mice with a total of 12 Crl:CD(ICR) mice, 3/sex/dose, receiving a single oral dose of 10 or 30 mg/kg/day HFPO dimer acid ammonium salt (86% purity) by gavage (Dupont-25300, 2008). Plasma samples were collected from animals serially at 0, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours post-dosing. Peak plasma HFPO dimer acid anion concentrations were reached within 8 hours for the males and 4 hours for the females at the 10-mg/kg dose. At the 30-mg/kg dose, the peak HFPO dimer acid anion concentrations were reached within 2 hours for both males and females. The mean clearance time was slower in the males (143 hours and 139 hours at the low dose and high dose, respectively) than in the females (57 hours and 62 hours at the low dose and high dose, respectively) (Table 6).

In the oral toxicokinetic studies, the clearance times were shorter in rats than in mice and were shortest in female rats compared to male rats for both anions from HFPO dimer acid and its ammonium salt. In rats at the 10-mg/kg dose, HFPO dimer acid took longer to clear than its ammonium salt in both male and female rats. At the 30-mg/kg dose, however, both HFPO dimer acid and its ammonium salt had the same clearance times in male rats, but the HFPO dimer acid ammonium salt took longer to clear in female rats.

In a cross-species pharmacokinetic study, Crl:CD(SD) rats (3/sex) were administered a single intravenous bolus of 10 or 50 mg/kg of HFPO dimer acid ammonium salt and Cynomolgus monkeys (3/sex) were administered a single intravenous bolus of the HFPO dimer acid ammonium salt (10 mg/kg) (DuPont-17751-1579 RV1, 2009). Plasma samples were collected at intervals over the first 24 hours post-dosing and once per day for the subsequent 7 days in the

rats and 21 days in the monkeys. In the rats, the plasma concentrations were consistently higher for the males than for females by approximately one to two orders of magnitude, consistent with the indication that female rats have more rapid elimination. The clearance times for male rats were 22 hours and 17 hours in the 10- and 50-mg/kg dose groups, respectively. The clearance times for female rats were 3 hours and 4 hours in the 10- and 50-mg/kg dose groups, respectively. Notably, the calculated clearance time in the male rats was longer for the 10-mg/kg dose group (22 hours) than the clearance time calculated in Dupont-24281 (2008) for male rats in the 10-mg/kg dose group (12 hours). Female rats had similar clearance times. Additionally, the standard deviations on each serum mean were broad for the rats in the 50-mg/kg dose group, indicative of wide differences between the three males and three females evaluated at that dose. In the monkeys, the standard deviations on each serum mean were broad, especially for the female monkeys over the first 2 hours, which is indicative of wide differences between the three males and three females evaluated. The plasma levels were generally higher in females over the first 2 hours, were nearly identical at 4 hours, and were slightly higher in the males from 4 to 336 hours. The levels of the anion from HFPO dimer acid ammonium salt were very low at 168 hours in male (0.004 µg/mL (4 ng/mL)) and female (0.001 µg/mL (1 ng/mL)) monkeys. For 408 hours and beyond, concentrations were below the LOQ of 0.001 µg/mL (1 ng/mL). The clearance times calculated for the male and female monkeys were 11 hours and 10 hours, respectively.

Half-lives. In Gannon et al. (2016), the goodness of fit was calculated for the plasma concentrations after oral and intravenous dosing (DuPont studies outlined above) using one- and two-compartment models, and the two-compartment model had a better fit. Pharmacokinetic parameters identified by Gannon et al. (2016) are presented for the intravenous studies in Table 7 and for the oral studies in Table 8. The alpha phase $T_{1/2}$ represents the plasma concentration in the early post-injection period and is considered to reflect the plasma distribution phase (Klaassen, 1996). The beta phase $T_{1/2}$ represents the period during which the chemical in the plasma has established an equilibrium with the levels in the body tissues and represents the elimination phase. The two-compartment model is a refinement of the prior pharmacokinetic analysis in which the clearance time was calculated. The two-compartment model better fits the data and separates distribution and elimination phases; therefore, generally for comparisons across the datasets, the $T_{1/2}$s are preferred.

Table 7. $T_{1/2}$ Estimates from Intravenous Injection in Sprague Dawley Rats and Cynomolgus Monkeys

$T_{1/2}$	Intravenous Exposures (in hours)			
	Male rat	Male monkey	Female rat	Female monkey
Alpha (Plasma Distribution) Phase	3.6	2.3	0.4	1.9
Beta (Plasma Elimination) Phase	89.1	64.1	22.6	79.6

Source: Gannon et al., 2016.

In the intravenous injection studies, the $T_{1/2}$ of the alpha phase of distribution is similar (about 2 hours) for male and female monkeys, but the $T_{1/2}$ of the beta (elimination) phase is longer in female monkeys. The $T_{1/2}$ of the beta (elimination) phase in female monkeys is longer than it is in the female rats, which could be a result of female monkeys having higher tissue stores than

female rats or clearance of HFPO dimer acid anion from their tissues might be slower. There are no studies, however, to distinguish these explanations such as a study of tissue concentrations over time. In rats, both the alpha and beta phases are shorter in females than in males; the beta phase $T_{1/2}$ is about four times longer in males, suggesting higher levels in tissues of males or slower clearance of HFPO dimer acid anion from their tissues (Gannon et al., 2016).

Gannon et al. (2016) also used the data from the single oral dose studies in rats and mice to derive estimates of alpha and beta phase $T_{1/2}$s to represent the distribution and elimination phases. The oral exposure data are not ideal for this calculation because the chemical is not directly injected into the blood. However, because intestinal uptake of HFPO dimer acid anion from the ammonium salt is believed to be rapid and there appears to be no metabolism, the estimates are reasonable for a two-compartment model.

In rats, following oral exposure, the alpha (distribution) $T_{1/2}$ phase is shorter in females than in males and the beta (elimination) phase $T_{1/2}$ is comparable for both sexes (Table 8). In mice, the $T_{1/2}$ estimates for the alpha phase are similar for both sexes and the $T_{1/2}$ estimates for the beta phase are shorter for females than for males (Table 8). The $T_{1/2}$ estimated for the beta phase in female rats is shorter from the intravenous data (22.6 hours) than from the oral gavage data (67.4 hours), while the other estimates of $T_{1/2}$ from the intravenous and oral gavage data for males and females are similar.

Table 8. $T_{1/2}$ Estimates from Single Oral Dose in Sprague Dawley Rats and Crl/CD1(ICR) Mice

$T_{1/2}$	Oral Exposures (in hours)			
	Male rat	Female rat	Male mouse	Female mouse
Alpha (Plasma Distribution) Phase	2.8	0.2	5.8	4.6
Beta (Plasma Elimination) Phase	72.2	67.4	36.9	24.2

Source: Gannon et al., 2016.

The time it takes to achieve a balance between gastrointestinal uptake and excretion (i.e., steady state) following daily gavage exposures to the HFPO dimer acid anion is dependent on the $T_{1/2}$s of the alpha and beta phases. When the data are well described by a multicompartmental model, the steady state is a function of the multiple $T_{1/2}$s for the intercompartmental distribution (alpha phase) and elimination (beta phase); however, at later times, the elimination $T_{1/2}$ is expected to dominate the time to steady state and to be reached approximately within four $T_{1/2}$s, or 6.15 days, for male mice (Ito, 2011). This was calculated by multiplying the oral gavage beta phase $T_{1/2}$ (36.9 hours) for male mice by 4 and dividing that product by 24 hours. The data from Rushing et al. (2017) for male mice clearly demonstrate a lack of serum steady state for male mice after receiving doses of 1, 10, and 100 mg/kg/day for 28 days because the serum concentrations do not remain constant after the expected 6 days. In fact, HFPO dimer acid concentrations continue to change between 5 and 14 days and 14 and 28 days. These continual changes in plasma concentration after 6 days indicate dynamics over multiple days that are not represented by typical multicompartment models and, therefore, are not appropriate for modeling the complexity of the pharmacokinetics of HFPO dimer acid and its ammonium salt.

Repeated-dose study. In a repeated-dose study with Crl:CD1(ICR) mice dosed with 0, 0.1, 0.5, or 5 mg/kg/day for at least 90 days, plasma measurements were determined 2 hours post-dosing on days 0, 28, and 95 (Dupont 18405-1307, 2010). Plasma concentrations increased less than twofold between the 2 hour and the 28-day measurements for both the males and females in all dose groups (Table 9). Unfortunately, the study provides no measurements between the 2-hour and 28-day time points to allow for a determination regarding steady state. As mentioned above, however, the Rushing et al. (2017) study in mice provides measurements in serum at 1, 5, 14, and 28 days following daily gavage dosing of C57BL/6 mice that clearly establish the lack of steady-state conditions, which supports development of a more complex model to represent these data.

Table 9. Mean Plasma Concentrations with Standard Deviations of Dosing Crl:CD1(ICR) Mice with HFPO Dimer Acid Ammonium Salt for at Least 90 Days

Dose mg/kg/day	Day 0			Day 28			Day 95		
	µg/mL	SD	COV	µg/mL	SD	COV	µg/mL	SD	COV
Males									
0	ND[a]	N/A	N/A	ND	N/A	N/A	ND	N/A	N/A
0.1	0.736	0.099	13%	1.124	0.238	21%	1.276	0.309	24%
0.5	3.806	1.197	31%	7.182	3.055	43%	7.068	2.398	34%
5	42.58	5.214	12%	52.240	16.725	32%	67.98	13.717	20%
Females									
0	ND	N/A	N/A	N/D	N/A	N/A	ND	N/A	N/A
0.1	0.824	0.072	9%	0.704	0.35	50%	0.74	0.282	38%
0.5	3.606	1.308	36%	4.198	1.239	30%	5.438	1.696	31%
5	35.34	9.262	26%	46.58	16.842	36%	45.58	5.741	13%

Source: Dupont 18405-1307, 2010.
Notes: COV = coefficient of variation (SD / mean); µg/mL = micrograms per milliliter; N/A = not applicable; ND = not detected; SD = standard deviation.
[a] Limit of detection = 0.005 µg/mL

Plasma concentrations remained relatively constant between 28 days and 95 days for male and female mice administered the 0.1-mg/kg/day dose in the Dupont 18405-1307 (2010) study (Table 9). At the 0.5-mg/kg/day dose, plasma concentrations were relatively constant from day 28 to 95 days for the males, but the females' plasma concentrations increased from 4.198 to 5.438 µg/mL (4,198 ng/mL to 5,438 ng/mL) (a 30% increase). This indicates that the HFPO dimer acid anion does not appear to accumulate at 0.1 mg/kg/day; however, it might have accumulation potential at 0.5 mg/kg/day. Interestingly, this increase in female plasma concentrations from 28 days to 95 days is equal to the coefficient of variation (COV) in the 28-day measurement, thus the difference between days 28 and 95 could be the result of inter-animal

differences in response to the same dose. Also interesting is that, at the 5-mg/kg/day dose, female plasma levels returned to approximately the same levels at 28 and 95 days (46.58 and 45.58 µg/mL (46,580 and 45,580 ng/mL), respectively) (Table 9). In the males, the plasma levels at 28 days increased from 52.24 to 67.98 µg/mL (52,240 ng/mL to 67,980 ng/mL) at 95 days (a 30% increase), again equaling the COV in the 28-day measurement. Thus, the difference between days 28 and 95 could be the result of variability in these measurements as a result of inter-animal differences and might not necessarily reflect accumulation of HFPO dimer acid anion.

3.0 Problem Formulation

3.1 Conceptual Model

The conceptual model provides useful, publicly available information to characterize and communicate the potential health hazards related to oral exposure to HFPO dimer acid and its ammonium salt. Figure 2 depicts in a conceptual diagram the sources of these GenX chemicals, the routes of exposure to biological receptors of concern (e.g., human activities related to ingested tap water such as drinking, food preparation, and consumption), the potential assessment endpoints (e.g., effects such as liver toxicity), and populations at risk of exposure to HFPO dimer acid and its ammonium salt. As outlined in the legend for Figure 2, the green boxes indicate where there are limited data available for these GenX chemicals. This includes quantitative data for oral exposure to HFPO dimer acid and its ammonium salt, as well as the limited data available for some of the potential sources of exposure to these chemicals. The quantitative data for oral exposure to HFPO dimer acid and its ammonium salt includes animal toxicity and toxicokinetic studies; no epidemiological studies on health effects in humans are available. The white boxes indicate that no data are publicly available to allow for determining if GenX chemicals are found in certain sources and that no human toxicity data exist.

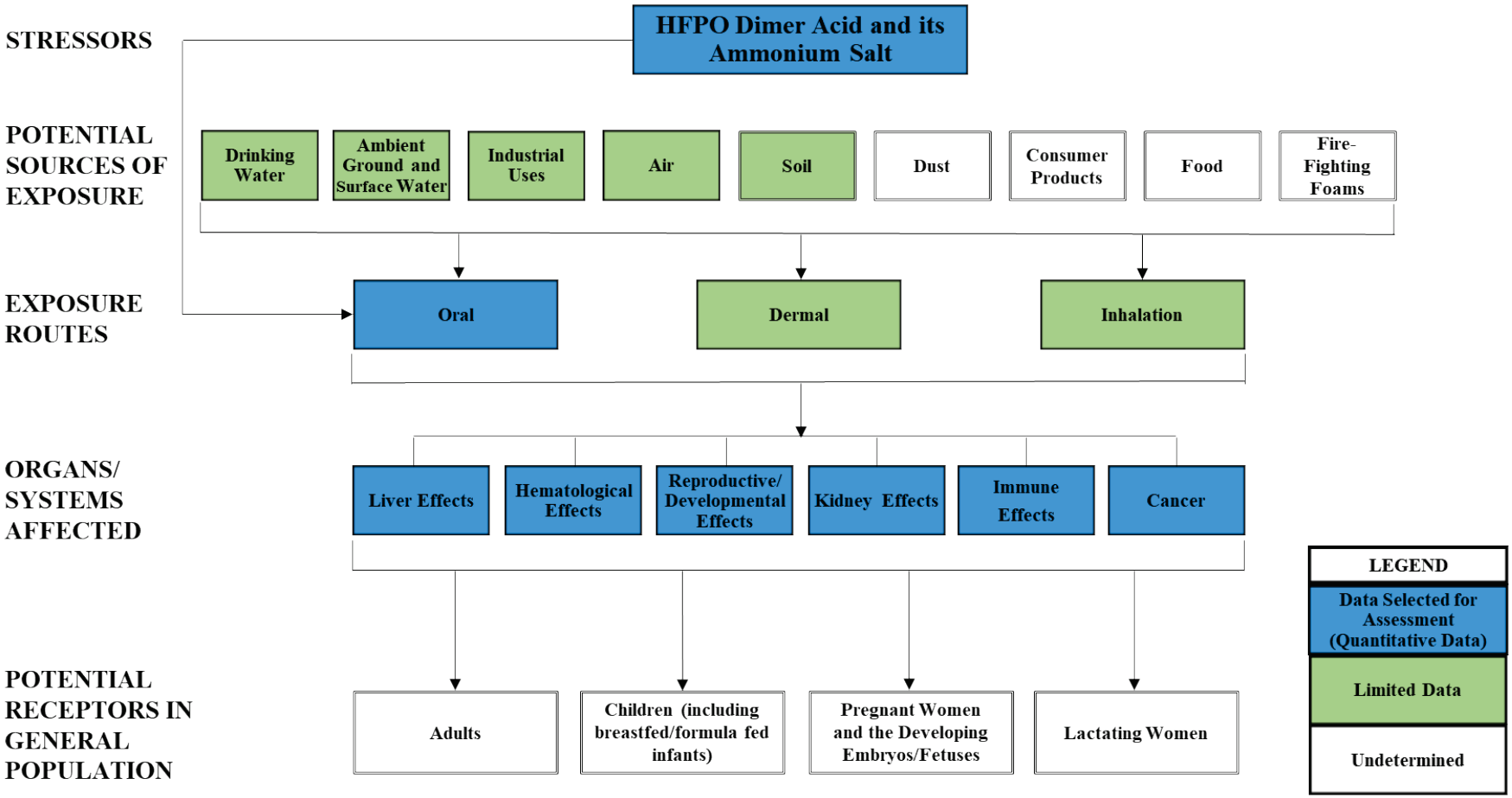


Figure 2. Conceptual Model for HFPO Dimer Acid and Its Ammonium Salt

3.2 Overall Scientific Objectives

This document provides the health effects basis for the development of oral RfDs for subchronic and chronic durations for GenX chemicals, including the science-based decisions providing the basis for estimating the POD. This section discusses the factors EPA considers in the process of developing a POD (depicted in Figure 2).

Stressors: This assessment addresses only HFPO dimer acid and its ammonium salt. It does not address any other chemicals used in the GenX processing technology or any other precursors, metabolites, or degradate of HFPO dimer acid and its ammonium salt. Uses of GenX chemicals include as intermediates and as polymerization aids in the production of fluoropolymers. These chemicals are two of several replacements for PFOA and its ammonium salt and could have many applications in consumer products (e.g., stain- and water-repellant textiles) and industrial processes (e.g., pharmaceutical and semiconductor manufacturing). Information on specific products containing GenX chemicals is not available, however, GenX chemicals may be used in the manufacture of the same or similar commercial fluoropolymer end products that formerly used PFOA. GenX chemicals may also be generated as a byproduct of fluoromonomer production. Publicly available data, although limited, indicate that sources of exposure to GenX chemicals include both ground and surface waters used for drinking. Many other potentially important sources of exposure to GenX chemicals exist given their use as a replacement for PFOA, including foods; indoor dust in a home or work environment; indoor and outdoor air; soil; biosolids; and consumer products within the home, workplace, children's schools, and daycare centers. Very little quantitative information on these sources of exposure, however, is available.

Routes of Exposure: Nonoccupational exposure to GenX chemicals in water can occur through oral exposure (i.e., drinking water, cooking with water, and incidental ingestion from showering) and is expected to occur by dermal exposure (i.e., contact of exposed parts of the body with water containing GenX chemicals during bathing or showering, and dishwashing) and inhalation exposure (e.g., volatilization of the GenX chemicals from the water during bathing or showering, or while using a humidifier or vaporizer). There is limited information identifying health effects from inhalation or dermal exposures to GenX chemicals in animals. Specifically, two acute dermal toxicity tests (one in rats and one in rabbits), one dermal irritation study in rabbits, and one acute inhalation toxicity test in rats (see section 4.1) have been conducted. Repeated-dose toxicity data are available for oral exposure, but not for inhalation and dermal exposures. Since the only quantitative data available for HFPO dimer acid and its ammonium salt are for oral exposure, this assessment applies only to that route.

Receptors: The receptors are those in the general population who could be exposed to GenX chemicals in tap water through ingestion (i.e., adults, the elderly, women of childbearing age, pregnant women, fetuses, infants, and children). In the conceptual model in Figure 2, the box for adults includes sensitive life stages (e.g., women of childbearing age and the elderly). In this toxicity assessment, 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 are addressed. This toxicity assessment summarizes potential health effects associated with exposure to GenX chemicals and identifies levels at which those health effects might occur. Potential exposure to receptors is not determined. Toxicity values from this assessment can be combined with specific exposure information (Step 3. Exposure Assessment) to help characterize the

potential public health risks associated with exposure to these chemicals (Step 4. Risk Characterization) to the receptors outlined here.

Endpoints: No human epidemiological studies for GenX chemicals are available. Oral exposure studies of acute, subchronic, and chronic duration are available in rodent species, including rats and mice. The recommended definitions of study duration were applied as outlined in *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002). Using this approach, the employed study durations are as follows:

- **Acute:** Exposure by the oral, dermal, or inhalation route for 24 hours or less.
- **Short-term:** Repeated exposure by the oral, dermal, or inhalation route for more than 24 hours, up to 30 days.
- **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).

Adverse effects observed following exposure to HFPO dimer acid and/or its ammonium salt include liver toxicity (e.g., hypertrophy, single-cell necrosis, focal necrosis and apoptosis), hematological effects (e.g., decreased red blood cell (RBC) count, hemoglobin, and hematocrit), kidney toxicity (e.g., increased kidney weight, necrosis, and hyperplasia), reproductive and developmental effects (e.g., placental lesions, changes in maternal gestational weight gain (GWG), and BW changes), immune effects (e.g., T cell-dependent antibody response (TDAR) suppression and lymphocyte increases), and *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans (e.g., liver and pancreatic acinar cell tumors).

In most of the available animal studies, hepatocellular hypertrophy and necrosis of the liver appear to be the most sensitive effects observed. The increases in relative liver weight, hepatocellular hypertrophy, and peroxisome activity (e.g., peroxisomal beta-oxidation induction) can be associated with activation of cellular peroxisome proliferator-activated receptor alpha (PPARα) receptors, making it difficult to determine if this change is a reflection of PPARα activation or an indication of GenX chemical toxicity. This is important because the PPARα response could be more relevant to rodents than humans. EPA evaluated liver effects resulting from exposure to GenX chemicals in the context of the Hall criteria (Hall et al., 2012), through which changes in liver weight or hepatocellular hypertrophy can be considered adverse when they are accompanied by histologic or clinical pathology indicative of liver toxicity such as necrosis, inflammation, and/or fibrosis. In this assessment, EPA listed hepatocellular hypertrophy or changes in serum liver enzymes as adverse only when they were accompanied by histologic pathology indicative of liver toxicity such as necrosis, inflammation, and/or fibrosis. The observance of liver necrosis indicates that cytotoxicity also could be a mode of action (MOA) for liver damage.

No physiologically based pharmacokinetic (PBPK) models are available that address the relationship between external exposure and internal dose for GenX chemicals; however, allometric scaling methodology is available to calculate a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans (EPA, 2011b). The use

of allometric scaling addresses some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes.

The toxicity values for this assessment include a chronic oral RfD (chronic RfD) and a subchronic oral RfD (subchronic RfD) for HFPO dimer acid and its ammonium salt. An RfD is an estimate of the concentration or dose of a substance (with uncertainty spanning perhaps an order of magnitude) to which a human population (including sensitive subgroups) can be exposed that is likely to be without an appreciable risk of deleterious effects during a lifetime. In addition to chronic RfDs, other durations of exposure can be considered, including subchronic exposures. RfDs are derived for noncarcinogenic toxicological endpoints of concern.

3.3 Methods

3.3.1 Literature Search Strategy and Results

EPA assembled and evaluated available information on toxicokinetics; acute, short-term, subchronic, and chronic toxicity; developmental and reproductive toxicity; neurotoxicity; immunotoxicity; genotoxicity; and cancer in animals. Most of the available data for HFPO dimer acid and its ammonium salt were submitted with PMNs to EPA by DuPont/Chemours, the manufacturer of GenX chemicals, under TSCA, 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 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) and TSCA (EPA, 2021b).

All available studies were considered for inclusion. Most of the studies considered for dose-response analysis in this assessment adhered to the principles of GLP, and full study reports were submitted for Agency review. As noted by OECD,[3] the OECD TGs are accepted internationally as standard methods for safety testing and:

> ...are covered by the Mutual Acceptance of Data, implying that data generated in the testing of chemicals in an OECD member country, or a partner country having adhered to the Decision, in accordance with OECD Test Guidelines and Principles of GLP, be accepted in other OECD countries and partner countries having adhered to the Decision, for the purposes of assessment and other uses relating to the protection of human health and the environment.

To identify public literature available for HFPO dimer acid and its ammonium salt, literature searches were conducted of four databases (PubMed, Toxline, Web of Science (WOS), and Toxic Substances Control Act Test Submissions (TSCATS)) using CASRN, synonyms, and additional relevant search strings (see Table A-2 in appendix A for a full list). Because the results of this core search were so limited, additional databases were searched for

[3] http://www.oecd.org/chemicalsafety/testing/oecdguidelinesforthetestingofchemicals.htm.

physicochemical property information, health effects, toxicokinetics, and mechanistic information. A list of the additional databases searched is provided in Table A-3 and Table A-4 in appendix A. The initial searches of these databases specific to HFPO dimer acid were conducted in July 2017 and specific to the HFPO dimer acid ammonium salt in January and February 2018. They returned 27 studies for HFPO dimer acid and its ammonium salt, after accounting for duplicates. Additional updates to the literature search were completed in February 2019, October 2019, and March 3, 2020 using the same search strategy as described in appendix A. These searches returned an additional 48 studies.

The submitted studies from DuPont/Chemours and the literature identified by the search of publicly available sources are available through EPA's Health & Environmental Research Online website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

3.3.2 Study Screening and Evaluation

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, 2020). These studies were subjected to title and abstract screening to determine relevancy according to the PECO criteria statement/inclusion and exclusion criteria outlined in Table A-6 in appendix A. The title and abstract of each study were independently screened by two screeners using Distiller SR[4]. 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. These supplemental categories are outlined in Table A-7 in appendix A. 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 twelve studies providing dose-response information were then evaluated for study quality using an approach consistent with the draft ORD Handbook for developing IRIS assessments (DuPont-24447, 2008; DuPont-24459, 2008; DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1037, 2010; DuPont-18405-841, 2010; DuPont-18405-1238, 2013; Rushing et al., 2017, Conley et al., 2019, 2021; Thompson et al., 2019; Blake et al., 2020; EPA, 2020). 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[5]. A third reviewer made the final decision on the quality ratings based on the primary ratings. The results of the study quality evaluation are provided in Figure 3 and an interactive version of the heatmap can be found here:

[4] Distiller SR is a fee-based, multi-user, web-based platform that manages, tracks, and streamlines the screening of literature reviews.

[5] HAWC is a free and open-source web-based software application that enables multiple users to synthesize multiple data sources into an overall human health assessment of chemicals.

https://hawcprd.epa.gov/summary/visual/assessment/100500273/GenX-SQE-Heatmap/. All twelve studies were rated as medium or high-quality studies and were summarized in section 4 and considered for dose response in section 7.

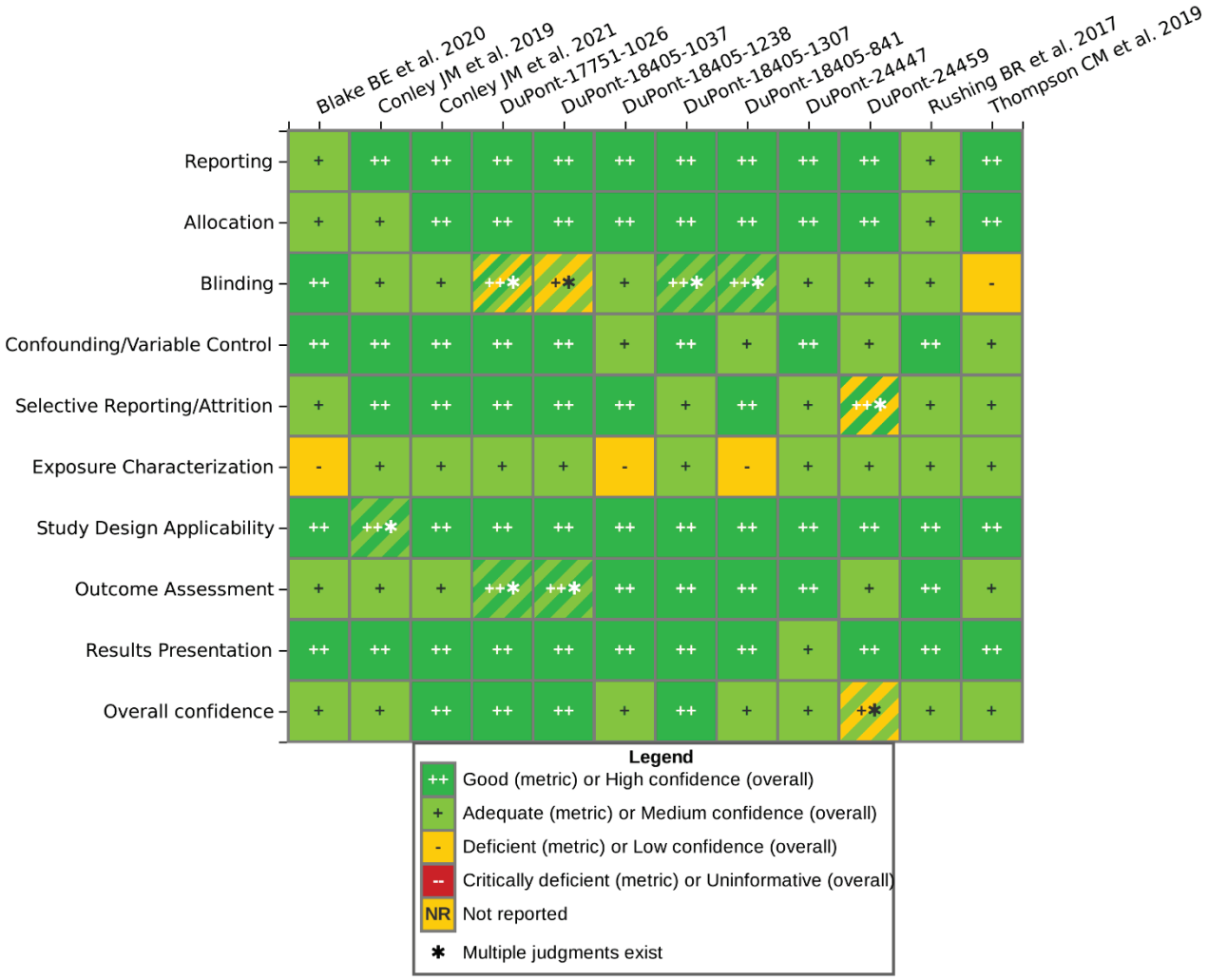


Figure 3. Evaluation Results for Animal Studies Assessing Effects of GenX Chemicals Exposure (Click to see interactive data graphic for rating rationales)

Additionally, all studies tagged as supplemental that provided toxicokinetic or mechanistic information were summarized and incorporated into the assessment in sections 2.3 and 4.6, respectively. Study summaries were also provided for all acute toxicity studies in section 4.1. Finally, two mechanistic studies were included in this assessment that were published after the final literature search (Gaballah et al., 2020; Cannon et al., 2020).

3.4 Approach to Deriving Reference Values

Development of the hazard identification and dose-response assessment for HFPO dimer acid and its ammonium salt has followed the general guidelines for risk assessment published by the National Research Council (1983) and EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (EPA, 2014a). Additional EPA guidelines and other Agency reports used in developing this assessment include the following:

- *Guidelines for Developmental Toxicity Risk Assessment* (EPA, 1991)
- *Guidelines for Reproductive Toxicity Risk Assessment* (EPA, 1996)
- *Guidelines for Neurotoxicity Risk Assessment* (EPA, 1998)
- *A Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002)
- *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a)
- *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (EPA, 2005b)
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (EPA, 2006a)
- *Exposure Factors Handbook* (EPA, 2011a)
- *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b)
- *Benchmark Dose Technical Guidance Document* (EPA, 2012)
- *Child-Specific Exposure Scenarios Examples* (EPA, 2014b)
- *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (EPA, 2014c)

EPA's *A Review of the Reference Dose and Reference Concentration Processes* describes a multistep approach to dose-response assessment, including analysis in the range of observation followed by extrapolation to lower levels (EPA, 2002). EPA conducted a dose-response assessment to define a POD and extrapolated from the POD to an RfD. For HFPO dimer acid and its ammonium salt, EPA used benchmark dose (BMD) modeling to refine the critical effect POD in deriving the RfD.

The steps for deriving an RfD are summarized below.

Step 1: Evaluate the data to identify and characterize endpoints related to exposure to GenX chemicals. This step involves determining the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data are collected, evaluated for study quality, and characterized for adverse outcomes, the risk assessor selects endpoints judged to be relevant and the most sensitive (typically defined by the NOAEL value). Considerations that might influence selection of endpoints include data with dose response, percent change from controls, adversity of effect, and consistency across studies.

Step 2: Conduct BMD Modeling. Using EPA's *Benchmark Dose Technical Guidance Document* (EPA, 2012), a benchmark response (BMR) is selected and BMD modeling is applied to the endpoints selected as most relevant. The BMR is a predetermined change in the response rate of an adverse effect. It serves as the basis for obtaining the benchmark dose lower limit (BMDL), which is the 95% lower bound of the BMD. A family of BMD models are fit to the

dose-response data that describe the dataset of the identified adverse effect. From the family of models, either a best fitting model with the corresponding BMD and BMDL is derived or, if no adequate models are found, the NOAEL or lowest-observed-adverse-effect level (LOAEL) identified in step 1 is used as the POD.

Step 3: Convert the POD to a human equivalent dose (HED) or point of departure human equivalent dose (POD_{HED}). The POD (either a BMDL, NOAEL, or LOAEL) is then converted to an HED following the method described in EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b).

Step 4: Provide rationale for selecting UFs. UFs are selected in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the critical study compared to the lifetime of the species studied, and the completeness of the toxicology database.

Step 5: Calculate the chronic and subchronic RfDs. The RfDs are calculated by dividing POD_{HED} by the selected UF.

$$RfD = \frac{POD_{HED}}{\text{Total UF}}$$

where:

- POD_{HED} = calculated from the BMDL or NOAEL/LOAEL using a $BW^{3/4}$ allometric scaling approach consistent with EPA guidance (EPA, 2011b).
- UF = Total UF established in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, duration of exposure in the critical study compared to the lifetime of the species studied, and completeness of the toxicology database.

3.5 Measures of Effect

The available dataset regarding the toxicity of these GenX chemicals includes *in vivo* and *in vitro* studies. The *in vivo* studies were considered in the dose-response assessment for HFPO dimer acid and its ammonium salt. The available data indicate that the liver, kidney, RBCs, immunological responses, and reproductive and developmental effects (BW and fetal development) are adversely impacted by exposure to GenX chemicals. Tumors were also observed following oral exposure to GenX chemicals (DuPont-18405-1238, 2013). In this analysis, all reported changes in relative organ weights were presented as relative to BW (data relative to brain weight were not included). The endpoints presented in this assessment represent potentially adverse effects that were statistically significantly different ($p < 0.05$ or 0.01) from control unless otherwise noted. Additionally, statistically significant changes from the control are presented as the percent change from control, unless otherwise noted.

The animal studies demonstrated dose-related effects on the liver in rodent species (rats and mice) following exposure to HFPO dimer acid and/or its ammonium salt for durations of 28 days to 104 weeks. The studies and endpoints reviewed as possible critical studies and effects for determination of the POD were evaluated for experimental design, data quality, and dose response identified through the range of experimental NOAELs/LOAELs. A route-to-route

extrapolation of oral toxicity data from which to derive an inhalation reference concentration was not conducted because of data limitations. For example, no toxicokinetic data are available characterizing the uptake of GenX chemicals through the lung for systemic distribution, and only one acute inhalation toxicity study is available (DuPont-17751-723, 2009). This study identifies the portal of entry effects, albeit at a high dose.

4.0 Study Summaries

4.1 Acute Toxicity Studies

There are over 10 studies available detailing the acute toxicity and irritation effects of HFPO dimer acid and its ammonium salt. This section summarizes the available acute oral, dermal, and inhalation toxicity studies as well as dermal and eye irritation studies for HFPO dimer acid and its ammonium salt. Appendix B provides additional details on each of the studies.

Oral Toxicity. Several studies have evaluated oral toxicity in rats and mice from single doses of the HFPO dimer acid ammonium salt at doses ranging from 1.5 mg/kg to 17,000 mg/kg (DuPont-22932, 2007; DuPont-24126, 2007; DuPont-25438 RV1, 2008; DuPont-2-63, 1963; DuPont-770-95, 1996). Also, male and female rats were evaluated with doses of 175–5,000 mg/kg HFPO dimer acid (DuPont-25875, 2008). The rats and mice in these studies received a single dose of the compound and were observed for clinical effects of toxicity for 14 days.

Four studies were conducted according to OECD TG 425 (OPPTS 870.1100) (OECD, 2008c) using the Up-and-Down Procedure (DuPont-22932, 2007; DuPont-25438 RV1, 2008; DuPont-25875, 2008; DuPont-24126, 2007). Two studies that estimated approximate lethal doses (ALDs) did not have identified TGs (DuPont-2-63, 1963; DuPont-770-95, 1996). For HFPO dimer acid, the oral median lethal doses (LD_{50}s) were 1,730 mg/kg and 1,750 mg/kg in male rats and female rats, respectively (DuPont-25875, 2008). For the HFPO dimer acid ammonium salt, the LD_{50} was 3,129 mg/kg for female rats (DuPont-22932, 2007); 1,030 mg/kg for female mice (DuPont-24126, 2007); and 1,750 mg/kg for male rats (DuPont-25438 RV1, 2008). The estimated ALD for male rats for the ammonium salt ranged from 5,000 mg/kg to 7,500 mg/kg (DuPont-2-63, 1963; DuPont-770-95, 1996).

The more common clinical signs observed across studies included wet fur, fur/skin stain or discoloration, altered posture, and lethargy; changes in BW were also seen (DuPont-770-95, 1996; DuPont-22932, 2007; DuPont-24126, 2007; Dupont-25438 RV1, 2008; DuPont-25875, 2008). Effects in mice were observed after exposure to HFPO dimer acid ammonium salt (86% purity) doses at 550 mg/kg and higher. Effects in rats were observed after exposure to either HFPO dimer acid (98% purity) or its ammonium salt (82.6% to 99% purity) at doses of 175 mg/kg and higher (DuPont-22932, 2007; DuPont-25875, 2008).

Gross evidence of organ or tissue damage included discoloration of lungs, stomach, skin, lymph nodes, liver, and/or esophagus (DuPont-22932, 2007; DuPont-25438 RV1, 2008; DuPont-25875, 2008). Enlarged livers and enlarged hepatocytes were observed in young male rats following single doses of 2,250, 3,400, or 5,000 mg/kg for HFPO dimer acid ammonium salt (DuPont-2-63, 1963).

Dermal Toxicity. Two studies reported acute dermal toxicity of HFPO dimer acid ammonium salt in rats or rabbits following acute dermal exposure (DuPont-24113, 2007; DuPont-839-95, 1996). In an OECD TG 402 (OPPTS 870.1200) (OECD, 2017) study, 5,000 mg/kg HFPO dimer acid ammonium salt (86% purity) was applied to shaved, intact skin of male and female rats under a semi-occlusive dressing for 24 hours. The dermal LD_{50} was more than 5,000 mg/kg (both sexes). Erythema was observed only in females, whereas hyperkeratosis and ulceration were observed in rats of both sexes. All dermal effects cleared by 13 days posttreatment (DuPont-24113, 2007). In another study (in which no guideline is cited), HFPO dimer acid ammonium salt (99% purity) was applied to shaved, intact skin of New Zealand white rabbits for 24 hours. The ALD was determined to be more than 5,000 mg/kg. In this study, erythema persisted for 13 days post application and was accompanied by scaling and sloughing of skin. One of the rabbits also exhibited necrosis for 2–6 days post application (DuPont-839-95, 1996).

Inhalation Toxicity. One study (conducted using the GLP Compliance Statement in compliance with Title 40 of the Code of Federal Regulations (CFR) part 792) evaluated acute inhalation toxicity of HFPO dimer acid ammonium salt (84% purity) in male and female rats following a single 4-hour nose-only exposure to aerosol concentrations of 0, 13, 100, and 5,200 mg/m^3. The median lethal concentration (LC_{50}) was more than 5,200 mg/m^3. Red discharge from the nose, eyes, and mouth was observed in rats at doses of 100 and 5,200 mg/m^3 for up to 2 days postexposure. No gross lesions were observed. Microscopic evaluation of respiratory tract tissue (lung, larynx/pharynx, trachea, and nose) from rats exposed to concentrations of 0, 13, and 100 mg/m^3 detected no substance-related effects (DuPont-17751-723, 2009).

Dermal Irritation. In an OECD TG 404 (OPPTS 870.2500) (OECD, 2002) dermal irritation study, very slight-to-well-defined erythema was observed in three male New Zealand white rabbits following a single application of a 0.5-mL aliquot of HFPO dimer acid ammonium salt (86% purity) in an area of shaved skin for a period of 4 hours on the day of application. Erythema cleared by 24 hours postexposure (DuPont-24030, 2007).

Eye Irritation. New Zealand white rabbits were administered a single application of a 0.1 mL aliquot of HFPO dimer acid ammonium salt (86% purity) to the lower conjunctival sac in an eye irritation study conducted according to OECD TG 405 (OPPTS 870.2400) (OECD, 2020a). At 28 hours after instillation of the compound, necrosis, corneal opacity, iritis, conjunctival chemosis (swelling), discharge, and corneal injury were observed (DuPont-24114, 2007).

4.2 Short-Term Toxicity Studies

Seven-Day Toxicity Studies. Hepatic effects were observed in 6-week-old mice and rats of both sexes in four 7-day studies (in which no TG is cited) evaluating repeated-dose oral toxicity of HFPO dimer acid and its ammonium salt (DuPont-24010, 2008; DuPont-25281, 2008; DuPont-24116, 2008; DuPont-24009, 2008). Water was used as the vehicle control in all studies. Two 7-day studies evaluated the toxicity of HFPO dimer acid ammonium salt (86.6% purity) and HFPO dimer acid (99% purity) at doses of 30 mg/kg/day in male mice and rats, respectively. In both studies, a twofold increase in liver weight relative to control, cell necrosis of hepatocytes, and hepatocellular hypertrophy were observed in all exposed animals (DuPont-24010, 2008; DuPont-25281, 2008). A third 7-day study evaluating toxicity of HFPO dimer acid (99% purity) also detected increased liver weight in male rats (at 30, 100, and 300 mg/kg/day) and in female rats (at 300 mg/kg/day). Hepatocellular hypertrophy was present in both sexes at all doses (DuPont-

24116, 2008). Hypertrophy and increased liver weight were observed in another similar 7-day gavage study evaluating effects of HFPO dimer acid ammonium salt (86.6% purity). Males appeared to be more sensitive to hepatic effects because increases in liver weight were observed at 30, 300, and 1,000 mg/kg/day, whereas increased liver weight was observed in females only at 1,000 mg/kg/day. These effects were accompanied by increases in β-oxidation and increases in cytochrome P450 enzyme activity, biomarkers for activation of PPARα nuclear receptors. Mild-to-minimal hepatocellular hypertrophy was observed in both sexes at 1,000 mg/kg/day (DuPont-24009, 2008).

Twenty-Eight-Day Toxicity Studies. Two 28-day studies evaluating systemic toxicity in rats and mice are available for HFPO dimer acid ammonium salt.

DuPont-24447 (2008)

In a study with 7-week-old Crl:CD(SD) rats (10/sex/group) conducted according to OECD TG 407 (OECD, 2008a), HFPO dimer acid ammonium salt (purity 88%) was administered on 28 consecutive days via gavage (vehicle was deionized water) (OECD, 2008a; DuPont-24447, 2008). Male rats received doses of 0, 0.3, 3, or 30 mg/kg/day while females received 0, 3, 30, or 300 mg/kg/day. In this study, there were no mortalities and clinical signs were confined to high-dose females (e.g., urogenital staining).

Hematological evaluation revealed statistically significantly decreased RBC count, hemoglobin, and hematocrit at greater than or equal to 3 mg/kg/day in males. The maximum decreases compared to control at 4 weeks were observed at the highest dose (30 mg/kg/day) and were 6%, 7%, and 8% for RBC count, hemoglobin, and hematocrit, respectively. Increases in absolute reticulocyte counts were also observed in males at all dose levels, but this increase was only statistically significant from control at the highest dose (27%) at 4 weeks. No statistically significant hematological effects were observed in the females (DuPont-24447, 2008).

Alterations in serum clinical chemistry parameters were seen in both sexes, but most of the significant effects were observed in the male rats. Decreases in total globulin and increases in the A/G ratio were observed in males and females. In males, total serum albumin increased (15% at 30 mg/kg/day) while total globulin decreased 13% and 22% compared to control at 3 mg/kg/day and 30 mg/kg/day, respectively. This resulted in an increase in the A/G ratio to 16% and 41% in the 3 mg/kg/day and 30 mg/kg/day males, respectively, most likely the result of underproduction of globulin. Females exhibited a 9% decrease in total globulin and a 20% increase in the A/G ratio compared to control at 300 mg/kg/day. Males also showed statistically significant decreases in serum cholesterol at all doses, with the largest decrease compared to control (28%) in the 30-mg/kg/day group. Triglyceride levels were decreased at all doses but were significantly decreased (22%) only at 3 mg/kg/day. Males also exhibited increases in blood urea nitrogen (BUN) (24%) and glucose (15%) at 30 mg/kg/day when compared to controls (DuPont-24447, 2008).

In males, relative kidney weight was significantly increased (15% compared to control) only at the highest dose tested. Minimal mineralization of the kidneys was also observed in 1/10 male rats in the high-dose group. There were no statistically significant changes in kidney weight in the females; however, there was minimal basophilic staining of cells in the tubules for 3/10 female mice in the 300-mg/kg/day group, while none were observed in the control group. Dose response could not be determined for basophilic tubules because no rats were examined in the 3-

mg/kg/day-dose group and only one rat was examined in the 30-mg/kg/day-dose group. No statistical analyses were completed on these microscopic observations.

Relative liver weights were statistically increased in a dose-response manner in males, 19% and 56% compared to control at 3 mg/kg/day and 30 mg/kg/day, respectively. These increases were accompanied by decreases compared to control in sorbitol dehydrogenase (SDH) at 0.3 mg/kg/day (-36%) and 30 mg/kg/day (-21%) in males. In females, the only statistically significant change in liver weight was a 12% increase compared to control at the highest dose (300 mg/kg/day). Microscopically, 4/10 and 7/10 male rats exhibited hepatocellular hypertrophy at 3-mg/kg/day and 30-mg/kg/day doses, respectively. In female rats, hepatocellular hypertrophy was observed in 4/10 rats in the high-dose group. Hepatocellular necrosis (3/10) and single-cell necrosis (1/10) were observed in males at 30 mg/kg/day. No statistical analyses were completed on these histological observations. The authors note that hepatic peroxisomal β-oxidation activity was induced in both sexes at the middle and high doses. Specifically, β-oxidation activity was determined using [14C] palmitoyl-coenzyme A (CoA) as the substrate and total cytochrome P450 content as markers of peroxisome proliferation. In the males, β-oxidation activity was significantly increased compared to control at dosages of 0.3 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day by 42%, 274%, and 772%, respectively, and total cytochrome P450 content was significantly increased by 23% at 30 mg/kg/day (DuPont-24447, 2008). In female rats dosed with 30 mg/kg/day and 300 mg/kg/day, β-oxidation activity was significantly increased compared to control by 49% and 198%, respectively, while total cytochrome P450 content remained unaltered (DuPont-24447, 2008). EPA identified the NOAEL to be 0.3 mg/kg/day and the LOAEL to be 3 mg/kg/day based on hematological (decreased hemoglobin, RBC count, and hematocrit) and immune (decreased globulin levels) findings in males (DuPont-24447, 2008). These findings were also accompanied by liver effects, including an increase in relative liver weight and hepatocellular hypertrophy; however, necrosis was observed only at the high dose (30 mg/kg/day).

DuPont-24459 (2008)

In another repeated-dose study conducted according to OECD TG 407 (OECD, 2008a), 7-week-old Crl:CD-1 mice (10/sex/group) were administered 0, 0.1, 3, or 30 mg/kg/day HFPO dimer acid ammonium salt (purity 88%) for 28 consecutive days via gavage (vehicle was deionized water) (DuPont-24459, 2008). Increases in mean BW gain were observed at 30 mg/kg/day in both males and females. In males, increases in mean cumulative BWs were reported as statistically different from the control group in the 30-mg/kg/day group during study weeks 1, 2, 3, and 4. In females, mean cumulative BW gains were significantly increased in the 30-mg/kg/day group during study weeks 2, 3, and 4.

Similar to the findings observed in the 28-day toxicity study in Crl:CD(SD) rats (DuPont-24447, 2008), decreases of 5.0% in hemoglobin and hematocrit were reported at greater than or equal to 3 mg/kg/day, and RBC count was significantly decreased by 7.6% in the Crl:CD-1 male mice at 30 mg/kg/day. In both males and females, the A/G ratio was statistically increased compared to control at greater than or equal to 3 mg/kg/day. Albumin alone was significantly increased by 31.3% compared to controls in males at 30 mg/kg/day, and globulin alone was decreased in females at greater than or equal to 3 mg/kg/day by 15.8% and 21.1% at 3 mg/kg/day and 30 mg/kg/day, respectively. Finally, in males, the serum liver enzymes aspartate aminotransferase (AST) (478%), alanine aminotransferase (ALT) (1,254%), alkaline

phosphatase (ALP) (1,222%), and SDH (1,800%) were significantly increased from control at the 30-mg/kg/day dose. Note that the hematology measures in female mice were inexplicably underpowered. Though a sample size of 9-10 mice per dose group was expected, only 2, 6, 3, and 5 female mice had hematology measurements in the 0, 0.1, 3 and 30 mg/kg/day dose groups, respectively.

In male mice, no statistically significant effect was observed on kidney weight. Female kidney weight findings were equivocal with the mean relative kidney weight showing statistically significant increases compared to control only at the low dose (8%) and high dose (17%). Minimal increases in basophilic tubular cells and tubular dilatation were observed in females at 30 mg/kg/day (3 of 10 animals for both effects) (DuPont-24459, 2008).

Macroscopic and microscopic tissue pathology evaluations were conducted for all dose groups. The inspection of male adrenal cortex at the highest dose found minimal hypertrophy in 8 of 10 tissue samples examined, while females showed mild or minimal adrenal cortex congestion at only the highest dose (DuPont-24459, 2008). No statistical analyses were completed on these microscopic observations.

Liver effects were also reported in both males and females in this study. In males, relative liver weights were significantly increased compared to control at 3 mg/kg/day and 30 mg/kg/day by 78% and 163%, respectively. In females, relative liver weights were increased at 3 mg/kg/day and 30 mg/kg/day by 32% and 103%, respectively, compared to controls. Absolute liver weights also increased at these doses in both sexes and to similar extents. Increases in liver weight correlated with microscopic liver findings (including single-cell necrosis, increased mitosis, and hepatocellular hypertrophy). Single-cell necrosis was observed in 40% (4/10) and 100% (10/10) of the male mice at 3 mg/kg/day and 30 mg/kg/day, respectively, while no liver necrosis was observed in the control mice. As noted above, serum liver enzymes were significantly increased from control at the 30 mg/kg/day dose: AST (478%), ALT (1,254%), ALP (1,222%), and SDH (1,800%). Single-cell necrosis was also detected in 40% (4/10) of female mice at 30 mg/kg/day compared to zero in the control. This was associated with an increase in serum SDH (186%) at 30 mg/kg/day. Hepatic peroxisomal β-oxidation activity was induced in both sexes. Specifically, β-oxidation activity was determined using [14C] palmitoyl-CoA as the substrate and total cytochrome P450 content as markers of peroxisome proliferation. In the male mice, β-oxidation activity significantly increased compared to control at doses of 0.1 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day HFPO dimer acid ammonium salt by 57%, 744%, and 648%, respectively, yet total cytochrome P450 content significantly decreased at 3 mg/kg/day and 30 mg/kg/day by 26% and 53%, respectively (DuPont-24459, 2008). β-oxidation activity significantly increased relative to control in female mice at 3 mg/kg/day and 30 mg/kg/day by 495% and 823%, respectively, with no alterations in total cytochrome P450 content. EPA identified the NOAEL for this study as 0.1 mg/kg/day and the LOAEL as 3 mg/kg/day based on increase in single-cell necrosis in males, which was accompanied by increased relative liver weight and hepatocellular hypertrophy, hematological, and immune effects.

4.3 Subchronic Toxicity Studies

DuPont-17751-1026 (2009)

In a repeated-dose study with rats, HFPO dimer acid ammonium salt (purity 84%) was administered to 8-week-old Crl:CD(SD) rats (10–20/sex/dose) on 90 consecutive days via oral

gavage (vehicle was deionized water) in accordance with OECD TG 408 (DuPont-17751-1026, 2009; OECD, 1998). Male rats were administered the test substance at doses of 0, 0.1, 10, or 100 mg/kg/day while females received 0, 10, 100, or 1,000 mg/kg/day. In this study, three high-dose females died before dosing was complete (two deaths considered as treatment-related; one death of undetermined cause).

Hematological evaluations revealed decreased hemoglobin, erythrocyte counts, and hematocrit in males administered greater than or equal to 10 mg/kg/day. The decreases in all three parameters for males were significantly different from control at 10 and 100 mg/kg/day and decreased in a dose-dependent manner at 90 days (study week 13). The maximum decreases from control in males were observed at the highest dose and were 11%, 13%, and 12% for RBC count, hemoglobin, and hematocrit, respectively. Likewise, female rats exhibited significant and dose-dependent decreases in RBC count (28%), hemoglobin (21%), and hematocrit (18%), but only at the 1,000 mg/kg/day dose. In males, absolute (52%) and percent (67%) reticulocytes and platelet count (17%) were significantly increased from control at the highest dose and exhibited a dose response. Additionally, both the absolute and percent of basophils (a type of white blood cell) were significantly decreased relative to control at 10 mg/kg/day (25%) and 100 mg/kg/day (50%) in males. Finally, female rats saw significant increases from control in mean corpuscular volume (15%), mean corpuscular hemoglobin (11%), mean corpuscular hemoglobin concentration (4%), platelet count (30%), and absolute (212%) and percent (392%) reticulocytes and a decrease relative to control in the percent of basophils (33%) at the high dose (1,000 mg/kg/day) (DuPont-17751-1026, 2009).

There were alterations in the clinical chemistry values in both sexes. Males exhibited a dose-dependent increase in total albumin and the A/G ratio and a decrease in total globulin compared to control. These changes were statistically significant at 10 mg/kg/day and 100 mg/kg/day. The maximum increases compared to control observed at the highest dose in total albumin, total globulin, and A/G ratio were 12%, 15%, and 35%, respectively. As in the 28-day study, females exhibited a dose-dependent decrease in globulin (33%) and an increase in A/G ratio (58%) that was significantly different from control for both effects at the highest dose only. Males and females also showed dose-dependent decreases in serum cholesterol that were statistically significantly different from control at 100 mg/kg/day (31%) in males and at both 100 mg/kg/day (20%) and 1,000 mg/kg/day (31%) in females. BUN was significantly increased relative to control in males at 100 mg/kg/day (38%). The trend for BUN was dose-related and positive in both sexes. ALP levels were significantly increased from control in a dose-dependent manner at 10 mg/kg/day (48%) and 100 mg/kg/day (106%) in the males and at 1,000 mg/kg/day (66%) in the females. Serum phosphorus levels increased dose-dependently in males and females and were significantly different from control at 10 mg/kg/day (10%) and 100 mg/kg/day (11%) in males and at 1,000 mg/kg/day (18%) in females. Total bilirubin was significantly decreased from control in a dose-dependent manner at the mid-dose (25%) and high dose (50%) only in females. Total protein and γ-glutamyl transferase decreased 10% and 69%, respectively, at the high dose in females. Finally, a slight but significant and dose-dependent decrease compared to controls in urine pH (8%) and a large increase in total urine volume (252%) were observed in female rats at 1,000 mg/kg/day (DuPont-17751-1026, 2009).

Kidney weight relative to BW was significantly and dose-dependently increased from control at 10 mg/kg/day (13%) and 100 mg/kg/day (16%) in male rats. Likewise, kidney weight relative to

BW was significantly increased at all dose levels in females and reached a maximum increase of 23% from control; however, microscopic damage of the kidney (tubular and papillary necrosis) was observed in only one of the rats at the highest dose. Additionally, one of the females that died prior to study termination exhibited tubular and papillary necrosis of the kidney. Transitional cell hyperplasia and mild acute inflammation were observed in the kidney of 1/10 male rats at the 100-mg/kg/day dose. Statistical analyses were not completed for the microscopic renal findings.

Liver weight relative to BW was significantly and dose-dependently increased from control at 10 mg/kg/day (31%) and 100 mg/kg/day (67%) in male rats. Females exhibited an 85% increase from control in liver weight at the high dose (1,000 mg/kg/day). Hepatocellular hypertrophy was observed in 3/10 and 10/10 males at the 10-mg/kg/day dose and 100-mg/kg/day dose, respectively, and in 10/10 females at the 1,000-mg/kg/day dose. Statistical analyses were not conducted for hepatocellular hypertrophy. Furthermore, it is not documented in the data tables whether other histological effects such as liver necrosis were detected in the 90-day study, although the pathology report states that the hypertrophy was not associated with microscopic changes indicative of liver injury such as necrosis (DuPont-17751-1026, 2009). EPA has determined the study NOAEL to be 0.1 mg/kg/day and the LOAEL to be 10 mg/kg/day based on blood effects (i.e., decreased RBC count, hemoglobin, and hematocrit) in males.

DuPont-18405-1307 (2010)

DuPont-18405-1307 (2010) was submitted to EPA under a TSCA Consent Order (see section 1.1 for more detail). Subsequently, in comments submitted to regulations.gov (Docket EPA-HQ-OW-2018-0614) by ToxStrategies LLC (2019a,b) a reevaluation of the study results for DuPont-18405-1307 (2010) and DuPont-18405-1037 (2010) was submitted. The reevaluation of DuPont-18405-1037 (2010) was published as Thompson et al. (2019) (discussed in section 4.5); however, the results of the reevaluation of DuPont-18405-1307 (2010) were not included in this publication. In response to these comments and the publication, EPA requested an independent review of DuPont-18405-1307 (2010) by the National Toxicology Program (NTP, 2019) Pathology Working Group (PWG) (appendix D). The results of the DuPont-18405-1307 (2010) and the NTP PWG review are described next.

In a repeated-dose, subchronic study with 7-week-old Crl:CD1(ICR) mice, the HFPO dimer acid ammonium salt (purity 84%) was administered to 10/sex/group for 95 days (males) or 96 days (females) via gavage (vehicle was deionized water) at doses of 0, 0.1, 0.5, and 5 mg/kg/day in accordance with OECD TG 408 (DuPont-18405-1307, 2010; OECD, 1998). A statistically significant increase in male BW and overall BW gain was observed at the high dose only. Mean daily food consumption was statistically increased in males between days 0 and 91 in a dose-related manner. The study authors reported that there were no treatment-related deaths. Two female mice (one at 0.5 mg/kg/day on day 6 and one at 5 mg/kg/day on day 20) died during the study. The authors reported that these animals displayed signs indicative of injury from gavage misdosing. The mice that died prematurely were included in the study results presented in the report.

A small decrease compared to control in mean corpuscular hemoglobin concentration (3%) in males and increased bilirubin (14%) in females was reported at 5 mg/kg/day. Clinical chemistry changes were more evident among male mice than female mice. Specifically, AST, ALT, and

ALP were statistically increased from control 106%, 420%, and 1,134%, respectively, at the 5-mg/kg/day dose in males. Comparatively, female mice saw significant increases relative to control in ALT (42%) and ALP (143%). SDH levels significantly increase compared to control in both males (308%) and females (32%) at 5 mg/kg/day. Albumin levels were increased relative to control in the 5-mg/kg/day-dose group in both males (14%) and females (4%), but total serum protein was significantly increased (14%) only in males at this dose (DuPont-18405-1307, 2010).

Macroscopic and microscopic tissue pathology evaluations were conducted for all dose groups. Male mice exhibited kidney tubular epithelial hypertrophy (9/10 treated mice compared to 0 in control) while females exhibited dilated kidney tubules (4/10 in treated compared to 2/10 in control) in the 5-mg/kg/day-dose group. Both effects were classified as minimal by the study authors. Female mice exhibited a decrease in relative spleen weight (10%, 21%, and 18% at 0.1 mg/kg/day, 0.5 mg/kg/day, and 5 mg/kg/day, respectively). No effects on the spleen were observed in male mice in any dose group. The study authors reported that changes in female spleen weight did not occur in a dose-related manner and were not associated with changes in absolute spleen weights or histological abnormalities in the spleen (DuPont-18405-1307, 2010).

Increased relative liver weights compared to control in both male mice (130%) and female mice (69%) were accompanied by minimal-to-mild hepatocellular hypertrophy at 5 mg/kg/day in all dosed mice. Minimal hepatocellular hypertrophy was also observed at the 0.5-mg/kg/day dose as well in males (8/10 mice). No hepatocellular hypertrophy was observed in the control group. Large and discolored livers were observed at doses greater than or equal to 0.5 mg/kg/day in males, but only in the 5-mg/kg/day-dose group in females. Key treatment-related findings considered as adverse at 5 mg/kg/day included increased enzymes indicative of liver injury (i.e., AST, ALT, ALP, and SDH) and increased total bile acids that co-occurred with histopathological findings in the liver. Histopathological findings in male mice included an increase in the incidence of single-cell necrosis (10/10 treated mice versus 0 in control), Kupffer cell pigments (10/10 treated mice versus 0 in control), and mitotic figures (9/10 treated mice versus 0 in control). Females also exhibited histopathological liver findings, but to a lesser degree. For example, 3/10 female mice exhibited focal necrosis and only 1/10 mice presented single-cell necrosis at 5 mg/kg/day (DuPont-18405-1307, 2010).

EPA concluded that the NOAEL in this study is 0.5 mg/kg/day and the LOAEL is 5 mg/kg/day based on the histological findings for the liver (i.e., necrosis and mitotic figures) accompanied by the clinical chemistry changes (i.e., AST, ALT, ALP, and SDH).

Reanalysis of DuPont 18405-1307 (2010) by National Toxicology Program Pathology Working Group (NTP, 2019)

The National Institute of Environmental Health Sciences (NIEHS), NTP in Research Triangle Park, NC convened a pathology working group (PWG) to provide an independent review of slides from the 90-day mouse study (DuPont-18405-1307, 2010) and the reproductive/developmental study (DuPont-18405-1037, 2010). All pathology slides provided by DuPont/Chemours were reviewed by the NTP PWG, including those of animals that died on study. The data and slides were reviewed per NTP standards (Sills et al., 2019).

As part of this PWG, one pathologist reviewed slides from the two studies and classified liver effects according to the International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) Organ Working Group's diagnostic criteria which describes how pathologists can

distinguish between apoptosis and single-cell necrosis in standard hematoxylin and eosin- (H&E) stained tissue sections (Elmore et al., 2016). The PWG coordinator then confirmed the classifications and selected example slides representative of the observed liver effects for review by the full, eight-member PWG. 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. The PWG's description of cytoplasmic alteration indicates that this endpoint includes hepatocellular hypertrophy occurrence along with eosinophilic change to the hepatocytes. There was a majority consensus for 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 of this revised assessment).

The PWG's classification of liver lesions included, but was not limited to, the following: apoptosis, single-cell necrosis, cytoplasmic alteration, and focal necrosis. Single-cell necrosis was observed in the high-dose group for male and female mice (DuPont-18405-1307, 2010). The PWG agreed that the observed single-cell necrosis was often accompanied with inflammation. Findings of apoptosis were also observed in the high-dose groups in both sexes.

Additionally, the PWG offered general observations about the histopathology reported in the original study. The NTP pathologists identified hepatocellular hypertrophy, including morphological changes such as eosinophilic stippling. The pathologists agreed that hypertrophy was present, but often less severe than reported in the original study. In addition, the pathologists recommended adding the diagnosis of cytoplasmic alteration to account for the eosinophilic, granular appearance of the cytoplasm of the hepatocytes. The pathologists recommended using this term to account for hypertrophy and eosinophilic changes as they are considered part of the same process. Cytoplasmic alteration was noted in the mid- and high-dose groups in males.

The PWG majority consensus opinion for each slide was recorded in review worksheets in a final report to EPA (see appendix D). Overall, the PWG review confirmed the results of the original study. Specifically, the PWG confirmed that single-cell necrosis was observed and is a treatment-related, adverse effect. The PWG concluded that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion individually, represents adversity within these studies (appendix D). EPA interpreted the NTP PWG's definition that the constellation of liver lesions is adverse applies to the dose group level instead of the individual animal level since the histopathological evaluation represents a snapshot in time of a biological process within one portion of the liver that can vary across animals. Table 10 presents a comparison of the incidence data for the 90-day mouse study (DuPont-18405-1307, 2010) and the NTP (2019) PWG reevaluation. Because the PWG analysis reflects more recent histopathological criteria for the grading of liver lesions, the incidence data as reported by NTP (see appendix D) were considered the more appropriate measure of response in the liver from the 90-day mouse study (DuPont-18405-1307, 2010). The NTP PWG reported that 10 out of 10 male mice exhibited cytoplasmic alteration, compared to 0 in control at the 0.5-mg/kg/day dose in this study. Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions considered adverse, no other liver lesions indicative of liver damage (i.e., single-cell or focal necrosis or apoptosis) were observed at the 0.5-mg/kg/day dose level in males. Consistent with the Hall criteria, EPA did not consider the cytoplasmic alteration findings

alone as an adverse effect in the 0.5 mg/kg/day dose group but considered the constellation of liver lesions observed across the male mice in the high-dose group as adverse. Additionally, the female mice in this study did not exhibit a dose response for the constellation of liver lesions. Based on EPA's interpretation of the NTP PWG results, EPA derived the study NOAEL for DuPont-18405-1307 (2010) of 0.5 mg/kg/day and the LOAEL is 5 mg/kg/day based on the histological findings for the liver (i.e., cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) in male and female mice.

Table 10. Comparison of Results from 90-Day Mouse Study (DuPont-18405-1307, 2010) and NTP PWG Reevaluation (NTP, 2019)

Reference	Results				
DuPont-18405-1307 (2010)	**Doses mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	9/10 [90]
	Hepatocellular hypertrophy [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	8/10 [80]	9/10 [90]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Mitotic figures [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	9/10 [90]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	0/10 [0]
	Pigment increased, Kupffer cells [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/10 [0]	0/10 [0]	2/10 [20]
NTP (2019) PWG Reevaluation of DuPont-18405-1307 (2010)	**Doses mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	0/10 [0]	9/10 [90]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	3/9[b] [33]
	Cytoplasmic alteration [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	10/10 [100]	10/10 [100]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	9/9[b] [100]
	Focal necrosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	1/10 [10]

Reference	Results				
	Female	1/10 [10]	0/9[a] [0]	2/9[b] [22]	3/9[b] [33]
	Apoptosis [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/9[a] [0]	0/9[b] [0]	3/9[b] [33]
	Combined Necrosis (single cell and focal necrosis) [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	0/10 [0]	9/10 [90]
	Female	1/10 [10]	0/9[a] [0]	2/9[b] [22]	4/9[b] [44]
	Constellation of lesions (cytoplasmic alteration, focal necrosis, single-cell necrosis, apoptosis) [incidence (%)]				
	Male	0/10 [0]	1/10 [10]	10/10 [100]	10/10 [100]
	Female	1/10 [10]	0/9[b] [0]	2/9[b] [22]	9/9[b] [100]
	Mitotic figures increased [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	7/10 [70]
	Female	0/10 [0]	0/9[b] [0]	0/9[b] [0]	0/9[b] [0]
	Pigment increased [incidence (%)]				
	Male	0/10 [0]	0/10 [0]	0/10 [0]	10/10 [100]
	Female	0/10 [0]	0/9[b] [0]	0/9[b] [0]	4/9[b] [44]

Notes:
[a] Slides for animal number 251 were not provided for analysis.
[b] EPA did not include animals that died due to gavage misdosing in the presentation of incidence data from the NTP PWG.

4.4 Chronic Toxicity and Carcinogenicity Studies

DuPont-18405-1238 (2013)

In a combined chronic toxicity/carcinogenicity study in 7-week-old Crl:CD(SD) rats, HFPO dimer acid ammonium salt (purity 84%) was administered by oral gavage (vehicle was deionized water) for up to 104 weeks (80/sex/group, of which 10/sex/group were designated for a 12-month interim necropsy in accordance with OECD TG 453) (DuPont-18405-1238, 2013; OECD, 2009, 2018b; Caverly Rae et al., 2015). Dose levels administered were 0, 0.1, 1, and 50 mg/kg/day for males and 0, 1, 50, and 500 mg/kg/day for females. Numerous animals in all dose groups (both male and female) were found dead or euthanized *in extremis* over the course of the study. Across all dosing groups in both male and female rats, 25.4% of the test animals survived to their planned terminal necropsy while 74.6% of the animals experienced unscheduled death/ moribundity prior to the scheduled study termination at 104 weeks. The authors state that mean survival in males and females was unaffected by treatment; however, all females were sacrificed before study termination at 101 weeks because of decreased survival across all groups, including the control. There were no statistically significant differences in survival across the female

dosing groups and female survival was comparable across all dosing groups. Among the animals that experienced unscheduled death/moribundity on study due to effects determined to be unrelated to treatment, DuPont stated the males most commonly died from pituitary tumors and undetermined causes while the females most commonly died from pituitary tumors and mammary tumors.

The females in the high-dose group were observed to have papillary necrosis and inflammation of the kidneys deemed by the authors to be related to treatment. BW and BW gain were unaffected in males but reduced compared to control (13% and 20%, respectively) in high-dose females at 52 weeks. The incidence of alopecia and hypotrichosis (abnormal patterns of hair growth) was statistically significantly increased in females at 500 mg/kg/day.

Statistically significant hematological effects were observed in this study, primarily in female rats. Blood samples were taken at 3, 6, and 12 months. At 3 months, RBC count, hemoglobin, and hematocrit were significantly decreased at the highest dose in males and females, although these decreases did not occur in a dose-dependent manner. Similarly, at 6 months, hemoglobin and hematocrit were significantly decreased at the highest dose in males, yet these decreases did not occur in a dose-dependent manner. There were no significant differences in any of these parameters in male rats at the 12-month time point. At 6 and 12 months, female rats exhibited a significant decrease in RBC count, hemoglobin, and hematocrit at 500 mg/kg/day and in a dose-dependent manner. The RBC count was also significantly decreased at 50 mg/kg/day in females at the 12-month time point; however, hemoglobin and hematocrit were not. The largest decreases compared to control in RBC count, hemoglobin, and hematocrit in female rats were 28%, 24%, and 20%, respectively, which were observed at 12 months. Additionally, the percent change from control of these effects increased over time (i.e., 3 months < 6 months < 12 months). At 12 months, serum albumin levels increased in males at 1 mg/kg/day and 50 mg/kg/day by 8% and 16% from control, respectively, which led to a concomitant increase in the A/G ratio by 16% and 28%, respectively.

Statistically significant changes from control were observed in the kidneys of females, but only at the highest dose (500 mg/kg/day). For example, there were increased incidences of tubular dilatation (increased by 34% compared to control), edema of the renal papilla (increased by 56% compared to control), transitional cell hyperplasia (increased by 39% compared to control), tubular and pelvic mineralization (increased by 15% and 24% compared to control, respectively), renal papillary necrosis (increased by 23% compared to control), and chronic progressive nephropathy (increased by 36% compared to control), all statistically significant from control. These microscopic indications of kidney damage were also associated with a 15% increase in relative kidney weight compared to control in females administered 500 mg/kg/day of HFPO dimer acid ammonium salt.

Liver enzyme levels also were affected by exposure to HFPO dimer acid ammonium salt at 12 months in the chronic study. In males, statistically significant increases in ALP (180%), ALT (228%), and SDH (141%) were observed at 50 mg/kg/day. These enzyme changes were correlated with microscopic findings in the liver, including focal necrosis. Relative liver weights were increased in high-dose males (16% compared to controls) and females (69% compared to controls) at the 12-month sacrifice. The change in liver weight in females corresponded to centrilobular hypertrophy in the high-dose females at the interim sacrifice. Females exposed to 500 mg/kg/day of HFPO dimer acid ammonium salt for 2 years also had significantly increased

relative liver weights (43% compared to control) at terminal sacrifice. There was no difference in organ weights in males at any dose at terminal sacrifice despite the changes observed at 12 months. Male and female rats exposed to 50 mg/kg/day and 500 mg/kg/day, respectively, had statistically significantly increased centrilobular hepatocellular hypertrophy compared to control rats (7/70 in treated males compared to 0/70 in control; 65/70 in treated females compared to 0/70 in control) and centrilobular hepatocellular necrosis (5/70 in treated males compared to 1/70 in control; 7/70 in treated females compared to 1/70 in control). Male rats also saw a decrease in incidence from control of 16% and 10% in focal and periportal vacuolization, respectively, at 50 mg/kg/day, and female rats had a 4% decrease from control in centrilobular vacuolation at 500 mg/kg/day. Finally, in females, panlobular hepatocellular hypertrophy (increase in incidence compared to control of 4%), individual cell hepatocellular necrosis (increase in incidence compared to control of 4%), and angiectasis (i.e., dilation of a blood or lymphatic vessel) (increase in incidence compared to control of 6%) were reported at the high dose.

Nonneoplastic effects also were observed in the stomach and tongue of females exposed to the high dose. Specifically, there were increased incidences of hyperplasia of the limiting ridge of the nonglandular stomach (increased by 13% compared to control; incidence was 9/70 for treated females and 0/70 in control) and of the squamous cell in the tongue (increased 16% from control; incidence was 13/70 in treated females and 2/70 in control). The tongue also exhibited an increased incidence of inflammation (increased 14% from control; incidence was 13/70 in treated and 3/70 in control). EPA concluded that the NOAEL for chronic toxicity in this study was 1 mg/kg/day and the LOAEL was 50 mg/kg/day for the liver effects in males.

Statistically significant increases in the incidence of liver tumors in females at 500 mg/kg/day and pancreatic acinar cell tumors in males at 50 mg/kg/day were reported. An increase in testicular interstitial (Leydig) cell tumors was noted at the high dose but was not statistically significant. Because of the observed early deaths in both control and treated male rats, EPA recommended that the submitter (a) reexamine their test data, (b) identify the animals that died without Leydig cell tumor within the first year, (c) exclude the animals identified in the previous step (i.e., those that died within the first year and had no tumors) from consideration for cancer data analysis, (d) recalculate tumor incidences, and (e) perform statistical analyses. Because the initial results indicated that the increased incidences of liver tumors in female rats (500 mg/kg/d) and combined pancreatic acinar tumors in male rats (50 mg/kg/d) were significantly increased from control despite the inclusion of early deaths, EPA decided to limit the reanalysis to testicular hyperplasia and tumors in male rats only. Additional discussion of tumor findings for the liver, pancreas, and testes is presented below.

Females. There were increases in the incidence of liver tumors at the high dose only (500 mg/kg/day), where degenerative and necrotic changes were also observed. The tumor incidences were 0/70 (0%), 0/70 (0%), 0/70 (0%), and 11/70 (15.7%) for hepatocellular adenomas and 0/70 (0%), 0/70 (0%), 0/70 (0%), and 4/70 (5.7%) for hepatocellular carcinomas at the doses of 0, 1, 50, and 500 mg/kg/day, respectively. The increased incidences of hepatocellular adenomas were statistically significant by the Cochran-Armitage trend test, the Peto test, and the pairwise Fisher Exact test and the increased incidences of hepatocellular carcinomas were statistically significant by the Cochran-Armitage trend test and the Peto test. The incidences of adenomas and carcinomas observed at 500 mg/kg/day also exceeded the test laboratory historical control ranges of 0%–5% and 0%–1.7%, respectively.

Males: A statistically significant increase was reported in the incidence of pancreatic acinar cell adenomas/carcinomas combined (but not adenomas or carcinomas alone) at 50 mg/kg/day. Incidences of pancreatic acinar cell adenomas were 0/70 (0%), 1/70 (1.4%), 0/70 (0%), and 3/70 (4.3%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively. The increased incidence at the high dose was not statistically significant and was within the test laboratory historical control range (0%–5%). The incidence of pancreatic acinar cell carcinomas was 0/70 (0%) in all groups other than the high-dose group, in which 2/70 (2.9%) were observed. The incidence of carcinomas at 50 mg/kg/day was not statistically significant but was slightly higher than the upper end of the laboratory's historical control range (0%–1.7%). When these two types of tumor were combined, the incidences of adenoma/carcinoma were 0/70 (0%), 1/70 (1.4%), 0/70 (0%), and 5/70 (7.1%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively, with the increased incidence at the high dose significant by the Cochran-Armitage trend test and the Peto test. For reference, the incidences of pancreatic acinar cell hyperplasia were 16/70 (22.9%), 18/70 (25.7%), 7/70 (10%), and 21/70 (30%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively, indicating a lack of dose-response relationship for this finding. Furthermore, the increased incidence of hyperplasia at the high dose was not statistically significant (compared to control).

In the testes, the incidences of interstitial cell adenomas were 4/70 (5.7%), 4/70 (5.7%), 1/70 (1.4%), and 8/70 (11.4%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively at 2 years. An interstitial cell adenoma was also present in 1/10 high-dose males at the interim sacrifice (12 months). The increased adenoma incidence at 50 mg/kg/day (11.4%) was not statistically significant but was slightly higher than the upper end of the testing laboratory's historical control range (0%–8.3%). For reference, the incidences of interstitial cell hyperplasia were 7/70 (10%), 7/70 (10%), 3/70 (4.3%), and 15/70 (21.4%) at 0 mg/kg/day, 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day, respectively. The increased incidence of hyperplasia at the high dose was not statistically significant (compared to control), although the incidence of hyperplasia at 50 mg/kg/day exceeded the historical control range (0%–8.3%). The observed incidences in the control and low-dose groups (both 10%) were also slightly above the upper end of historical controls. DuPont's reanalysis of these findings in the testes indicated that the number of male rats that died before 1 year was 4, 9, 8, and 3 in the 0 mg/kg/day (control), 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day groups, respectively. The causes of death were generally dosing injury or undetermined causes, and there were no testicular lesions or tumors in the testicular tissues of these animals. Excluding these early deaths, the incidences of testicular interstitial cell hyperplasia were 7/66 (10.6%), 7/61 (11.5%), 3/62 (4.8%), and 15/67 (22.4%) in the 0 mg/kg/day (control), 0.1 mg/kg/day, 1 mg/kg/day, and 50 mg/kg/day groups, respectively. The corresponding incidences of testicular interstitial cell adenomas were 4/66 (6.0%), 4/61 (6.6%), 1/62 (1.6%), and 8/67 (11.9%). Thus, there were no statistically significant differences for either hyperplasia or adenoma, consistent with results from the original report in which all early deaths were included. 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/day group cannot be ruled out" while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015).

Based upon EPA's review of the study, the increased incidence of liver tumors in females at 500 mg/kg/day and combined pancreatic acinar adenomas and carcinomas in males at

50 mg/kg/day are treatment related. The increased incidence of testicular interstitial cell adenoma was not statistically significant, and EPA accepted the results of the reanalysis that excluded the early deaths. EPA concluded that the NOAEL is 1 mg/kg/day and the LOAEL is 50 mg/kg/day based on the reported liver effects (i.e., centrilobular necrosis in both sexes; increased ALP, ALT, and SDH in males; and increased centrilobular hepatocellular hypertrophy and cystic focal degeneration in males).

4.5 Reproductive and Developmental Toxicity Studies

DuPont-18405-1037 (2010)

DuPont-18405-1037 (2010) was submitted to EPA under a TSCA Consent Order (see section 1.1 for more detail). Subsequently, Thompson et al. (2019), a contractor to Chemours (previously DuPont), performed a reevaluation of the study results for DuPont-18405-1037 (2010). In response to this publication, EPA requested an independent review of DuPont-18405-1037 (2010) by the NTP PWG (appendix D). The results of the original DuPont study, and these two reanalyses are described next.

In a combined oral gavage reproductive/developmental toxicity study in mice with HFPO dimer acid ammonium salt, the test compound (purity 84%) was administered by oral gavage (vehicle was deionized water) to Crl:CD1(ICR) mice (25/sex/group) at doses of 0, 0.1, 0.5, or 5 mg/kg/day, according to a modified OECD TG 421 (DuPont-18405-1037, 2010; OECD, 2016a). The male mice were approximately 6 weeks old and the female mice were approximately 10 weeks old. Parental (F_0) males were dosed 70 days prior to mating and throughout mating through 1 day prior to scheduled termination, for a total of 84 to 85 total doses. Parental F_0 females were dosed for 2 weeks prior to pairing and were dosed through LD20 for a total of 53 to 65 doses (exceptions include females with no evidence of mating or those that failed to deliver yet were administered a total of 37 to 50 doses). F_1 animals (offspring) were dosed daily beginning on PND21 through PND40.

In this study, increases in BWs and food consumption were observed at 5 mg/kg/day in F_0 animals. In F_0 males, increased mean BW gains were reported in the 5-mg/kg/day group during study days 0–49; differences from the control group achieved significance during study days 0–7, 14–21, and 21–28. Significantly higher mean BW gains were observed in this high-dose male group when the overall premating period (study days 0–69) and treatment period (study days 0–84) were evaluated. Mean BW gains were statistically significantly increased in females during both the premating period and throughout gestation at 0.5 and 5 mg/kg/day. Specifically, during the pre-mating period, BW gain increased by 100% and 70% in the 0.5- and 5-mg/kg/day-dose groups, respectively. Mean maternal GWG, calculated from individual differences, also significantly increased over the gestational period (0–18 days) by 18% and 22% in the 0.5- and 5-mg/kg/day-dose groups, respectively. At the high dose, mean BW gains were increased (5.1%–14.0%) compared to controls throughout lactation; the differences were significant on LD1, LD4, and LD21. BWs were unaffected at 0.1 and 0.5 mg/kg/day during lactation. Overall, final BW was significantly increased from control by 9% and 14% in males and females, respectively, administered 5 mg/kg/day.

The authors reported no treatment-related deaths in the F_0 mice. However, three males (one in each of the dose groups) and six females (one in the control, three in the low-dose group, and one each in the mid- and high-dose group) did not survive until scheduled sacrifice. The cause of

death was undetermined in all cases except the male in the mid-dose group, which appeared to have ulcerative dermatitis. Due to the lack of dose response, the study authors concluded that these deaths were not related to treatment. The study authors did not include the mice with premature deaths in the study results (e.g., histopathological incidence counts).

An increase in relative kidney weight compared to control by 6.5% was observed only in F_0 females at the 5-mg/kg/day dose. Mild increases in tubular cell hypertrophy were observed in the kidneys of males at greater than or equal to 0.5 mg/kg/day–6/24 mice or 25% and 18/24 mice or 75% of male mice at 0.5 mg/kg/day and 5 mg/kg/day, respectively, compared to 1/25 mice or 4% in the control. Chronic progressive nephropathy was also noted in males at 0.5 mg/kg/day (4/24 mice or 17%) and 5 mg/kg/day (5/24 mice or 21%). This effect was not associated with any evidence of tubular cell degeneration.

Liver effects also were reported in both males and females in this study. In males, mean absolute liver weights were increased 26% and 142% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, as compared to control values. Mean relative liver weights were increased by 26% and 121%, respectively, at the 0.5-mg/kg/day and 5-mg/kg/day doses. In females, mean absolute liver weights were increased by 26% and 101% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, as compared to control values. Mean relative (% BW) liver weights were increased by 17% and 80%, respectively. Microscopic findings observed in the liver of F_0 males and females administered 0.5–5 mg/kg/day included increases in hepatocellular hypertrophy, single-cell necrosis, mitotic figures, and lipofuscin pigment. F_0 females exhibited an increase in the incidence of gross white areas in the liver at 5 mg/kg/day, which correlated with microscopic focal and single-cell necrosis. At doses greater than or equal to 0.5 mg/kg/day, minimal-to-moderate hepatocellular hypertrophy was observed in both sexes, along with the corresponding increases in relative liver weight outlined above. Specifically, male mice exhibited a 50% and 100% increase in the incidence of hepatocellular hypertrophy compared to control at 0.5 mg/kg/day and 5 mg/kg/day, respectively, and similar increases in incidence was also observed in female mice (58% and 100% at 0.5 mg/kg/day and 5 mg/kg/day, respectively, compared to control). At greater than or equal to 0.5 mg/kg/day, single-cell necrosis of hepatocytes was observed in males. Specifically, single-cell necrosis was observed in 5/24 mice at 0.5 mg/kg/day and 24/24 mice at 5 mg/kg/day compared to 1/25 mice in the control. Female mice exhibited an increase compared to control in both focal/multifocal necrosis and single-cell necrosis at 5mg/kg/day. Specifically, 5/24 mice had focal/multifocal necrosis compared to 1/24 in the control and 21/24 mice had single-cell necrosis compared to 1/24 mice in the control. Finally, the incidence of mitotic figures increased in males and females administered 5 mg/kg/day by 75% and 21% compared to control, respectively, while the incidence of lipofuscin pigment increased by 88% and 21% compared to control, respectively.

No treatment-related effects were identified for reproductive parameters (mating, fertility, and copulation indices; mean days between pairing and coitus), although male epididymal weight relative to final BW was statistically decreased at 5 mg/kg/day in both the left and right testes (12% decrease relative to control). No treatment-related effects were observed for mean gestation length, mean numbers of implantation sites, mean numbers of pups born, live litter size, percentage of males at birth, postnatal survival, or general condition of pups. At 5 mg/kg/day, however, F_1 male and female pups exhibited lower mean BWs at PND4, PND7, PND14, PND21, and PND28. F_1 male pups continued to exhibit lower mean BWs at PND35 and PND40.

Although values for the attainment of balanopreputial separation and vaginal patency (markers of pubertal onset) were within the range of historical control values, the pups showed statistically significant delays in these endpoints at 5 mg/kg/day (a finding that might be related to the observed effects on BW during the preweaning period). Additionally, the day for attainment of vaginal patency did not exhibit a dose response. The NOAEL (F_0) is 0.1 mg/kg/day, and the LOAEL is 0.5 mg/kg/day based on liver effects (single-cell necrosis in males). The NOAEL (F_1) is 0.5 mg/kg/day based on decreased pup BW and delays in attainment of balanopreputial separation and vaginal patency at the high dose.

Reanalysis of DuPont 18405-1037 (2010) published by Thompson et al. (2019)

In a publication presenting alternative approaches to deriving toxicity values and subsequent drinking water concentrations for GenX chemicals, Thompson et al. (2019) present a reevaluation of slides of liver sections in the reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010). Thompson et al. (2019) presents the reevaluation of the liver sections from the reproductive/developmental toxicity study in mice in the supplemental file, Table S3.

Thompson et al. (2019) reevaluated these slides using more current diagnostic criteria (Elmore et al., 2016) than those used in the original study (DuPont-18405-1037, 2010) to distinguish between apoptosis and single-cell necrosis in standard H&E-stained tissue sections. Cell death was classified as apoptosis and necrosis based on the proposed nomenclature from the Terminology Recommendations from the INHAND Apoptosis/Necrosis Working Group described by Elmore et al. (2016). The INHAND Nomenclature for Non-neoplastic Findings of the Rodent Liver was also consulted for final diagnostic nomenclature (Thoolen et al., 2010). The samples were specifically evaluated for the presence and type of individual hepatocyte necrosis. The veterinary pathologist who reviewed the slides concluded that apoptosis was the primary adverse effect of note at 5 mg/kg/day. Thompson et al. (2019) also reported increased mitosis at doses with apparent increased apoptosis; the study authors concluded that it is well established that peroxisome proliferator-activated receptor (PPAR) activators can increase mitosis and apoptosis *in vivo*. Therefore, the authors conclude that this effect is likely a part of PPARα signaling pathways that are specific to rodents. EPA identified the NOAEL for this study as 0.5 mg/kg/day and the LOAEL as 5 mg/kg/day based on increased apoptosis in male mice.

Reanalysis of DuPont 18405-1037 (2010) by National Toxicology Program Pathology Working Group (2019)

As described in section 4.3, slides from the 90-day mouse study (DuPont-18405-1307, 2010) and the reproductive/developmental study (DuPont-18405-1037, 2010) were reevaluated by an NTP PWG (see appendix D). The same protocol was used by the PWG in their analysis of each of these studies (see section 4.3 for protocol details). The NTP PWG consensus opinion for each slide was recorded on a review worksheet. Worksheets for the slides were provided as appendices A and B in the final PWG report to EPA (see the full report provided in appendix D of this assessment). Similar to the Thompson et al. (2019) publication, the NTP used the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al., 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al., 2016). For the reproductive/developmental study (DuPont-18405-1037, 2010), the PWG confirmed single-cell necrosis and focal necrosis in the mid- and high-dose groups of both sexes. Single-cell

necrosis alone and single-cell and focal necrosis combined exhibited a dose-response relationship in both sexes. The PWG agreed that the observed single-cell necrosis was often accompanied with inflammation in this study. Findings of apoptosis were observed but were limited to the highest dose groups in both sexes. Additionally, cytoplasmic alteration (which includes hepatocellular hypertrophy occurrence along with eosinophilic change to the hepatocytes) was noted in the mid- and high-dose groups in both males and females.

The PWG review confirmed the results of the original DuPont study and did not agree with the conclusion of the reanalysis published by Thompson et al. (2019). Specifically, the PWG concluded that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of the study. Table 11 presents a comparison the incidence data for the reproductive/developmental toxicity study (DuPont-18405-1037, 2010), Thompson et al. (2019), and the NTP PWG reevaluation (NTP, 2019) of DuPont-18405-1037 (2010). The incidence data as reported by NTP (see appendix D) were considered the more appropriate measure of response in the liver from the reproductive/developmental study (DuPont-18405-1037, 2010) because the PWG analysis reflects the more recent scientific histopathological criteria developed for the grading of liver lesions and the PWG results were the consensus of eight pathologists. The NTP PWG confirmed that the study NOAEL for DuPont-18405-1037 (2010) is 0.1 mg/kg/day and the LOAEL is 0.5 mg/kg/day based on the constellation of liver effects (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) in male and female mice.

Table 11. Comparison of Study Results from DuPont-18405-1037 (2010), Thompson et al. (2019), and NTP PWG Reevaluation of DuPont-18405-1037 (NTP, 2019)

Reference	Results				
DuPont-18405-1037 (2010)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	1/25 [4]	1/24 [4]	5/24 [21]	24/24 [100]
	Female	1/24 [4]	3/22 [14]	2/24 [8]	21/24 [88]
	Focal /multifocal necrosis [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	1/24 [4]	1/24 [4]
	Female	1/24 [4]	0/22 [0]	0/24 [0]	5/24 [21]
	Hepatocellular hypertrophy [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	12/24 [50]	24/24 [100]
	Female	0/24 [0]	0/22 [0]	14/24 [58]	24/24 [100]
	Mitotic figures [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	0/24 [0]	18/24 [75]

Reference	Results				
	Female	0/24 [0]	0/22 [0]	0/24 [0]	5/24 [21]
	Pigment increased [incidence (%)]				
	Male	0/25 [0]	0/24 [0]	0/24 [0]	21/24
	Female	0/24 [0]	0/22 [0]	0/24 [0]	5/24
Thompson et al. (2019)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Apoptosis [incidence (%)]				
	Male	2/25 [8]	1/25 [0]	0/25 [0]	23/25 [92]
	Female	N/A	N/A	N/A	N/A
	Necrosis[a] [incidence (%)]				
	Male	2/25 [8]	0/25 [0]	1/25 [4]	1/25 [4]
	Female	N/A	N/A	N/A	N/A
	Mitosis [incidence (%)]				
	Male	0/25 [0]	0/25 [0]	0/25 [0]	15/25 [60]
	Female	N/A	N/A	N/A	N/A
NTP (2019) PWG Reevaluation of DuPont-18405-1037 (2010)	**Doses (mg/kg/day)**	**0**	**0.1**	**0.5**	**5**
	Single-cell necrosis [incidence (%)]				
	Male	1/25 [4]	1/24[b] [4]	2/24[b] [8]	23/24[b] [96]
	Female	0/24[b] [0]	2/22[b] [9]	3/24[b] [13]	19/24[b] [79]
	Cytoplasmic alteration [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	10/24[b] [42]	24/24[b] [100]
	Female	0/24[b] [0]	1/22[b] [5]	16/24[b] [67]	24/24[b] [100]
	Focal necrosis [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	4/24[b] [17]	3/24[b] [13]
	Female	2/24[b] 8]	1/22[b] [5]	4/24[b] [17]	5/24[b] [21]
	Apoptosis [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	21/24[b] [88]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	10/24[b] [42]
	Combined Necrosis (single cell and focal necrosis) [incidence (%)]				

Reference	Results				
	Male	1/25 [4]	1/24[b] [4]	6/24[b] [25]	24/24[b] [100]
	Female	2/24[b] [8]	3/22[b] [14]	6/24[b] [25]	20/24[b] [83]
	Constellation of lesions (cytoplasmic alteration, focal necrosis, single-cell necrosis, apoptosis) [incidence (%)]				
	Male	1/25 [4]	1/24[b] [4]	13/24[b] [54]	24/24[b] [100]
	Female	2/24[b] [8]	3/22[b] [14]	17/24[b] [71]	24/24[b] [100]
	Mitotic figures increased [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	17/24[b] [71]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	2/24[b] [8]
	Pigment increased [incidence (%)]				
	Male	0/25 [0]	0/24[b] [0]	0/24[b] [0]	20/24[b] [83]
	Female	0/24[b] [0]	0/22[b] [0]	0/24[b] [0]	2/23[b] [9]

Notes: N/A = not applicable

[a] Thompson et al. (2019) stated that "Emphasis was placed on evaluating the samples for the presence and type of individual hepatocyte necrosis. The two terms recommended for hepatocyte death were apoptosis and necrosis based on the proposed nomenclature from the Terminology Recommendations from the INHAND Apoptosis/Necrosis Working Group."

[b] EPA did not include animals that died due to gavage misdoing in the presentation of incidence data from the NTP PWG.

DuPont-18405-841 (2010)

In a prenatal and developmental toxicity study in 12-week-old female Crl:CD(SD) rats, HFPO dimer acid ammonium salt (purity 84%) was administered via oral gavage (vehicle was deionized water) once daily from GD6 through GD20 at doses of 0, 10, 100, and 1,000 mg/kg/day (22 females/group), according to OECD TG 414 (DuPont-18405-841, 2010; OECD, 2001b). The parental males and females were not dosed prior to or during mating and dosing for the dams was not initiated until GD6. Lack of dosing for males and females prior to and during mating and failure to dose the dams during the GD0 to GD6 period are limitations when evaluating this study to fully reflect the ability of the HFPO dimer acid ammonium salt to cause reproductive/developmental toxicity.

The dams' BW decreased at all doses, but significantly decreased (-22% compared to control) at 1,000 mg/kg/day. This decrease in BW also resulted in a decrease (-25%) in maternal GWG compared to control at 1,000 mg/kg/day. Moreover, gravid uterine weight was significantly decreased by 10% and 25% compared to control at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Food consumption in the dams was significantly decreased by 9% over the dosing period (GD6–GD21) at the highest dose. Early delivery on GD21 was observed in 18% and 41% of the dams at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Importantly, the authors noted that, in the available historical controls data for early deliveries in this rat strain (17 datasets), no females showed early deliveries (i.e., before GD21).

Statistically significant increases relative to control in absolute liver weight (12% and 34%) were observed at 100 mg/kg/day and 1,000 mg/kg/day, respectively. Changes in liver weight relative to BW were not documented. This increase in liver weight was associated with hepatocellular hypertrophy at the high dose (19/22 rats, or 86%) and focal necrosis was observed in 9% and 23% of the dams dosed with 100 mg/kg/day and 1,000 mg/kg/day, respectively. Additionally, absolute kidney weight increased dose-dependently in the dams and was significantly increased compared to control (10%) at the highest dose. Changes in kidney weight relative to BW were not documented, and there were no notable microscopic changes in the kidney tissue of the dams. Of note is that a 1,000-mg/kg/day dam that died on GD20 had moderate multifocal/focal necrosis of the liver and disseminated intravascular coagulation in the kidney glomerular capillaries.

The pups experienced a 9% and 28% decrease compared to control in fetal weight at doses of 100 mg/kg/day and 1,000 mg/kg/day, respectively. The percentage of male (47%) and female (53%) pups born were significantly altered from control (55% male; 45% female) at 1,000 mg/kg/day. Additionally, a 14th rudimentary rib developed in 9% of the control fetuses, 10% of fetuses in the 10-mg/kg/day-dose group, 12% of fetuses in the 100-mg/kg/day-dose group, and 27% of the fetuses in the 1,000-mg/kg/day-dose group. Statistical analyses were not completed for the development of the 14th rudimentary rib in individual pups, but a statistically significant increase in the number of litters developing a 14th rudimentary rib was observed for those receiving the high dose.

The NOAEL for this prenatal and developmental toxicity study is 10 mg/kg/day based on an increase in early deliveries, decreases in gravid uterine weight, and decreased fetal weights for both sexes, all occurring at the LOAEL of 100 mg/kg/day.

Conley et al. (2019)

Conley et al. (2019) reported on two experiments evaluating the effects of oral gestational exposures to HFPO dimer acid ammonium salt. In the first experiment, pregnant Crl:CD(SD) rats were dosed from GD14 to GD18 with either water (control), or 1, 3, 10, 30, 62.5, 125, 250, or 500 mg/kg/day of HFPO dimer acid ammonium salt. HFPO dimer acid purity was 100% as determined by the supplier via perchloric acid titration. Dams were dosed during GD14 to GD18 because this window is identified as the critical period for masculinization of the male reproductive tract. The study authors stated that the experiment was completed in three separate "blocks" of animals (15 animals/block). There was a total of nine control animals (three control animals/block), three animals each for the 62.5-, 125-, 250-, and 500-mg/kg/day doses (first block) and six animals each for the 1-, 3-, 10-, or 30-mg/kg/day doses (second and third blocks). Across all three blocks, GWG, reproductive output (number of fetuses and absorptions), maternal sera, and maternal liver weight were measured. In the first two blocks, fetal testis gene expression and testosterone production, fetal BW, fetal and maternal liver gene expression, and maternal serum thyroid hormone and lipid concentrations were also evaluated. In the third block, fetal plasma was collected for determining HFPO dimer acid ammonium salt concentrations (see section 2.3.3 for detail).

A variety of effects were observed in the dams at doses greater than or equal to 30 mg/kg/day. Serum total triiodothyronine (T3) levels were decreased at doses greater than or equal to 30 mg/kg/day and total thyroxine (T4) levels decreased at doses greater than or equal to 125

mg/kg/day. Liver weight was increased on GD18 at doses greater than or equal to 62.5 mg/kg/day. Decreases in serum low-density lipoprotein (LDL) were observed at doses greater than or equal to 125 mg/kg/day and in serum high-density lipoprotein (HDL) and total cholesterol at doses greater than or equal to 250 mg/kg/day. Additionally, serum triglycerides were decreased at the highest dose tested. GWG was also decreased at doses greater than or equal to 250 mg/kg/day.

No significant effects from control were observed on the number of fetuses or resorptions.

In a second pilot experiment evaluating postnatal development, five Crl:CD(SD) dams were dosed from GD14 through GD18 with either water (control; *n*=2 pregnant dams) or 125 mg/kg/day of HFPO dimer acid ammonium salt (*n*=3 pregnant dams) (Conley et al., 2019). The single dose of 125 mg/kg/day was selected because it was the highest dose evaluated that did not cause a significant decrease in GWG during the study described above. Pup delivery began on GD22 and the following schedule was followed for postnatal monitoring:

- PND2: Pups were weighed and sexed and anogenital distance (AGD) was measured.
- PND13: Pups were weighed, sexed, and evaluated for retention of female-like nipples/areolae.
- PND27: Dams were euthanized, and uterine implantation sites scored. Pups were weaned to 2/sex/treatment group.
- PND31–PND37: F_1 female offspring were examined daily for vaginal opening (a marker of pubertal onset).
- PND41–PND45: F_1 male offspring were evaluated daily for balanopreputial separation (a marker of pubertal onset).
- PND128: F_1 females were weighed, euthanized, and examined for reproductive tract malformations. Tissue weights were recorded for the uterus, paired ovaries, liver, paired kidneys, and visceral adipose tissues.
- PND146: F_1 males were weighed, euthanized, and examined for reproductive track abnormalities. Tissue weights were collected for glans penis, ventral prostate, paired seminal vesicles, paired testes, paired epididymis, levator ani-bulbocavernosus, paired bulbourethral (Cowper's) glands, paired kidneys, and visceral and epididymal adipose tissues. Total sperm counts were measured in epididymal sections.

Viable pup number was not affected by treatment. The only significant effect in the treated F_1 generation was a decrease in right epididymis weight on a litter mean basis compared to control. However, multiple significant effects were observed on an individual pup basis. For example, F_1 female BW was significantly decreased compared to control on PND2, PND27, and at the time of vaginal opening (PND31–PND37). Additionally, AGD and liver weight were significantly decreased in F_1 female offspring on an individual pup basis. For F_1 males, paired testes, paired epididymides, right testis, right corpus/caput, right epididymis, left testis, and epididymal adipose tissue were significantly decreased compared to control on an individual pup basis.

Conley et al. (2019) conducted gene expression analyses to determine if HFPO dimer acid ammonium salt activates PPAR signaling pathways. Maternal and fetal livers and fetal testes were collected on GD18 for gene expression analyses. Gene expression was assessed using reverse transcriptase real-time polymerase chain reaction (PCR) of complementary

deoxyribonucleic acid (DNA). Maternal and fetal livers were assessed for 84 target genes relevant to PPARα, PPAR beta/delta (PPAR-β/δ), and -γ signaling pathways in the rat.

Maternal and fetal livers shared upregulation of 16 genes and most of these shared genes were associated with fatty acid metabolism. Enoyl-CoA, hydratase/3-hydroxyacyl-CoA dehydrogenase *(Ehhadh*) was the most highly upregulated gene in both the maternal (fifty-five-fold at 500 mg/kg/day) and fetal (321-fold at 500 mg/kg/day) livers. Other shared upregulated genes were associated with adipogenesis (e.g., *Ech1*), PPAR transcription factors (e.g., *Rxrg*), and PPAR ligand transporters (e.g., *Slc27a5*). Generally, the fetal liver tended to display a greater sensitivity to HFPO dimer acid ammonium salt exposure with respect to the number of genes upregulated and the magnitude of upregulation. For example, the fetal liver exhibited upregulation of 12 genes that were not affected in the maternal liver (e.g., *Pck1, Aqp7, Gk* (gluconeogenesis) and *Angptl4* (lipid transport)). Additionally, all but one of the upregulated genes shared by maternal and fetal livers (i.e., *Ech1*) was upregulated to a greater extent in the fetal liver. In maternal livers, the genes most sensitive to HFPO dimer acid ammonium salt exposure were *Ech1* and *Rxrg* and, in the fetal livers, *Cpt1b* (mitochondrial fatty acid metabolism), *Acox1* (fatty acid metabolism) and *Angptl4* were the most sensitive. These genes were significantly increased at 1 mg/kg/day of HFPO dimer acid ammonium salt.

Overall, Conley et al. (2019) concluded that HFPO dimer acid ammonium salt activated PPAR signaling pathways in maternal and fetal livers, but the effects observed in this study are not exclusive to PPARα or even general PPAR signaling.

Conley et al. (2019) also measured fetal testis testosterone production and gene expression to understand if HFPO dimer acid ammonium salt exposure produces effects similar to those of some phthalate ester metabolites. Fetal testes were collected from male pups on GD18, with a single testis from the first three male pups used for the *ex vivo* testosterone production assay and the remaining testes for gene expression analysis. Unlike some phthalate ester metabolites, there was no effect of HFPO dimer acid ammonium salt exposure on fetal testis testosterone production or on the expression of genes that are typically changed in the fetal testis by exposure to phthalates (e.g., steroidogenic enzymes).

HFPO dimer acid ammonium salt was also assessed for *in vitro* agonism and antagonism of transcriptional activation for estrogen (100 picomolar (pM) to 10 μM), androgen (100 pM to 100 μM), and glucocorticoid (100 pM to 100 μM) receptors (Conley et al., 2019). HFPO dimer acid ammonium salt displayed no agonism of any of the receptors. At 100 μM, the study authors classified HFPO dimer acid ammonium salt antagonism as slight for the glucocorticoid receptor (28% reduction in luciferase expression) and as moderate for the androgen receptor (AR) (42% reduction in luciferase expression). The study authors noted that the 100 μM dose was approaching the cytotoxic dose of 300 μM.

EPA concluded that the study NOAEL is 62.5 mg/kg/day and the LOAEL is 125 mg/kg/day based on the indications of reduced BW in F_1 females and tissue weights in F_1 animals, decreased maternal GWG, and decreased maternal serum total T4 levels. Although maternal serum total T3 levels were significantly decreased compared to control at 30 mg/kg/day, EPA selected the LOAEL at 125 mg/kg/day because the deiodination of free T4 results in the formation of T3 (Forhead and Fowden, 2014), and T4 is the thyroid hormone that preferentially crosses the placenta of humans and rodents during early gestation (Calvo et al., 2002).

Blake et al. (2020)

Blake et al. (2020) evaluated the effects of gestational PFOA and HFPO dimer acid exposure on maternal and embryonic endpoints in mice. Pregnant CD-1 dams were dosed from E1.5 to E11.5 or E17.5 with either deionized water (vehicle control), 1 or 5 mg/kg/day PFOA, or 2 or 10 mg/kg/day HFPO dimer acid. PFOA results, as they compare to HFPO dimer acid, are presented in section 2.3.3. These time points were selected because the placenta had not fully matured at E11.5 and this time point overlaps with critical periods of placental development, including vascularization with the uterine wall and chorioallantoic branching of vessels. The E17.5 time point was selected to capture treatment-related effects on embryo weight and because the placenta is fully mature at E17.5.

Blake et al. (2020) evaluated albumin, ALP, ALT, AST, BUN, total cholesterol, creatine, glucose, HDL, LDL, SDH, total bile acid, total protein, triglycerides, and urinary creatine in maternal serum at E11.5 and E17.5. Total cholesterol and HDL were significantly increased 66% and 56%, respectively, compared to vehicle control in the 2 mg/kg/day HFPO dimer acid-dose group at E11.5, but these effects did not reach statistical significance at E17.5. Additionally, serum triglyceride levels were significantly decreased at 2 mg/kg/day (-43%) and 10 mg/kg/day (-61%) of HFPO dimer acid at E11.5 and remained significantly decreased in the 10-mg/kg/day (-74%) dose group at E17.5. Finally, serum ALP was significantly increased (53%) compared to vehicle control at E17.5 in the 10-mg/kg/day HFPO dimer acid-dose group.

Absolute and relative maternal liver weights significantly increased compared to vehicle control at both time points and in both HFPO dimer acid dose groups. Specifically, absolute liver weight increased by 41% and 91% and relative liver weights increased 37% and 73% compared to vehicle control at 2 and 10 mg/kg/day, respectively, at E11.5. At E17.5, absolute liver weight increased by 30% and 70% and relative liver weights increased 31% and 69% compared to vehicle control at 2 and 10 mg/kg/day, respectively. A variety of hepatocellular lesions were observed to increase as compared to vehicle control, including cytoplasmic alteration, mitotic figures, cell death (included both apoptosis and single-cell necrosis), and vacuolation. At E11.5, all dosed livers presented with cytoplasmic alteration, which increased in severity at the 10 mg/kg/day HFPO dimer acid dose. Mitotic figures and cell death increased in both dose groups and vacuolation rated as minimal was observed in 100% of the 10 mg/kg/day-dose group livers. At E17.5, all dosed livers presented with more severe cytoplasmic alteration than at E11.5, and this cytoplasmic alteration was most severe in the 10-mg/kg/day HFPO dimer acid-dose group. Mitotic figures were no longer increased at E17.5 and increased cell death was only observed in the 10-mg/kg/day-dose group. Vacuolation rated as minimal and mild was observed in the 10-mg/kg/day-dose group. Additionally, a portion of E17.5 livers from all dose groups were processed for transmission electron microscopy (TEM). As compared to vehicle control, the livers from the 2- and 10-mg/kg/day HFPO dimer acid-dose groups exhibited "abnormal ultrastructure with enlarged hepatocytes containing more abundant cytoplasmic organelles consistent with mitochondria and peroxisomes and vacuolation" (Blake et al., 2020). Additionally, the livers in the 10-mg/kg/day HFPO dimer acid-dose group presented "vacuolation often with remnant membrane material as myelin figures, abundant rough endoplasmic reticulum with few ribosomes present, and unevenly dispersed glycogen appearing as clustered clumps" (Blake et al., 2020).

Absolute and relative kidney weights were unchanged at E11.5. Absolute (19%) and relative (16%) kidney weight was increased compared to vehicle control in the 10-mg/kg/day HFPO dimer acid-dose group at E17.5. No histopathologic changes in kidneys were noted in any dose groups.

GWG was significantly increased (30%) relative to vehicle control at E11.5 in the 10-mg/kg/day HFPO dimer acid-dose group. When controlling for litter size, GWG was significantly greater in the 10-mg/kg/day HFPO dimer acid-dose group than in vehicle control at both E11.5 (7.1%) and E17.5 (19.1%). Finally, GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at E17.5 using effect estimates from mixed effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day.

Implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than vehicle control in any dose group. Placental weight was significantly increased by ~15.5 milligrams (mg) and the embryo:placental weight ratio significantly decreased by 15% in the 10-mg/kg/day HFPO dimer acid-dose group relative to vehicle control at E17.5. Additionally, placentas from litters (an average of seven individual placentas per litter) per treatment group and sacrifice time point were evaluated for histopathology. There were no significant histopathological changes at E11.5 between vehicle control and the HFPO dimer acid dose groups, with nearly all the placentas evaluated within normal limits. However, 58% and 83% of placentas evaluated at E17.5 were classified as abnormal in the 2 and 10 mg/kg/day HFPO dimer acid dose groups, respectively, compared to 2% in the vehicle control group. The number of abnormal placentas in the 10 mg/kg/day HFPO dimer acid dose group was significantly different than vehicle control. The most frequent lesion detected was labyrinth atrophy, which was observed in 0/41 (0%), 15/31 (48%), and 16/35 (46%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Labyrinth congestion and early fibrin clots increased with increasing HFPO dimer acid dose. Specifically, labyrinth congestion was observed in 0/41 (0%), 1/31 (3%), and 8/35 (23%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively, and early fibrin clot was observed in 0/41 (0%), 1/31 (3%), and 4/35 (11%) placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Placental lesions were evaluated against the proportion of placentas within a litter within normal limits to account for litter effects, and the proportion of abnormal placentas was significantly higher at the 2- and 10-mg/kg/day HFPO dimer acid-dose groups relative to vehicle control. Finally, placental thyroid hormones (reverse triiodothyronine (rT3), T3, and T4) were quantified at E17.5 from 2-3 pooled placental tissues of same-sex embryos. Each pooled sample was considered as one biological replicate and three replicates were used for each sex and treatment group. There was no significant effect of sex or treatment on rT3, T3, T3:T4 ratio, or rT3:T4 ratio. A significant increase (60%) in T4 relative to vehicle control was reported for the 10-mg/kg/day HFPO dimer acid-dose group.

The authors noted that, in some HFPO dimer acid-exposed dams, gross anomalies were apparent, including excess abdominal fluid, edematous tissues, and clotted placentas.

EPA concluded that there is no NOAEL for this study because the study LOAEL is 2 mg/kg/day, which is the lowest dose tested. The LOAEL is based on increased incidence of placental lesions within a litter and increased GWG using effect estimates from mixed-effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day in maternal mice.

Conley et al. (2021)

In a follow-up to their 2019 study, Conley et al. (2021) reported on two experiments evaluating the effects of oral gestational exposures to HFPO dimer acid ammonium salt. In one experiment, pregnant Crl:CD(SD) rats were dosed once daily by gavage from GD16 to GD20 with either water (control) or 1, 3, 10, 30, 62.5, or 125 mg/kg/day of HFPO dimer acid ammonium salt. HFPO dimer acid purity was 100% as determined by the supplier via perchloric acid titration. The study authors stated that the experiment was completed in two separate "blocks" of animals (15 animals/block). There were three control animals/block (total of six control animals) and two animals/treatment group/block (total of four treated animals/group). In both blocks, dams and fetuses were euthanized on GD20 and maternal and fetal sera were collected for determining HFPO dimer acid concentrations (see section 2.3.3 for detail). Maternal serum was also analyzed for thyroid hormone concentrations (total T3 and total T4) and clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)). Maternal weight gain, reproductive output (number of fetuses and resorptions), and maternal liver weight were measured. Maternal liver samples were collected for determining HFPO dimer acid concentrations and gene expression analyses. In the first block, two male and two female fetuses were randomly selected from each litter for measurements of body and liver weight and HFPO dimer acid concentration in liver samples. The individual body weights of the remaining fetuses were recorded irrespective of sex. Because there was no indication of an effect of sex on fetal body weight in the first block, body weights in the second block were recorded for three randomly selected fetuses per litter (irrespective of sex); out of those fetuses, one was randomly selected per litter to determine liver weight and HFPO dimer acid concentration in the liver and for gene expression analyses.

There were no significant differences observed for fetal body weight or liver weight (broken out by sex or combined), maternal body weight gain, or maternal terminal body weight in any dose groups compared with controls. Maternal liver weight was increased at 62.5 and 125 mg/kg/day. Maternal serum T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day. Albumin was decreased at 3, 62.5, and 125 mg/kg/day. Triglycerides were decreased at doses ≥10 mg/kg/day and cholesterol was decreased at doses ≥30 mg/kg/day. There were no significant effects on the numbers of viable fetuses or resorptions in any dose groups compared with controls.

In another experiment reported in Conley et al. (2021), pregnant Crl:CD(SD) rats (five per group) were dosed once daily by gavage from GD8 to PND2 with either water (control) or 10, 30, 62.5, 125, or 250 mg/kg/day of HFPO dimer acid ammonium salt. Dams gave birth naturally and were checked for parturition beginning on GD22. Once delivery was complete, pups were counted and the litter weight was recorded. All pups were returned to the nest except for two randomly selected pups per litter that were sacrificed. Trunk blood was collected, and serum was analyzed for HFPO dimer acid concentration and clinical chemistry parameters. Livers were collected for histopathological examination and gene expression analyses. The carcasses of three deceased newborn pups (one each from 30, 125, and 250 mg/kg/day dose groups) were sent for histopathological examination. On PND2, dams received their final dose in the morning and were weighed and euthanized 2–5.5 hours later. Maternal trunk blood was collected, and serum was analyzed for thyroid hormone concentrations (total T3 and total T4), clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)), and HFPO dimer acid concentration. Maternal liver weight was recorded, and liver samples were collected for gene expression analyses and HFPO dimer acid determination. Uterine implantation

sites were scored. The pups were sexed and weighed, anogenital distance was measured, trunk blood was collected, liver weight was recorded (one male and one female per litter), and liver samples were analyzed for HFPO dimer acid concentration. PND2 pup serum was analyzed for clinical chemistry parameters (ALT, AST, triglycerides, cholesterol, albumin, and glucose (non-fasting)) and HFPO dimer acid concentration.

All pups were alive at birth with no remarkable gross external malformations. Dams displayed typical nesting behaviors; however, shortly after delivery, pups in the higher dose groups began displaying lethargy, morbidity, or were found dead. Pups continued to die or require euthanasia throughout PND0 and PND1. Pup survival was reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day. Many of the pups that died had visible milk bands indicating they had nursed. Pup survival scores on PND2 were 100 ± 0, 96 ± 2, 97 ± 2, 87 ± 5, 38 ± 13, and 5 ± 5% in the 0, 10, 30, 62.5, 125, 250 mg/kg/day groups, respectively. Pup survival scores were significantly decreased at doses ≥62.5 mg/kg/day Pup body weight gain (birth to PND2) and PND2 body weight in the surviving pups were both reduced at doses ≥30 mg/kg/day. Anogenital distance was not affected in male or female pups, but relative liver weight was increased in all dose groups. No remarkable histopathological lesions were observed in pup livers, but glycogen accumulation scores in the liver were significantly lower in all dose groups compared with control pups. Significant changes were observed in some pup serum clinical chemistry parameters. Glucose was decreased at doses ≥62.5 mg/kg/day in newborn pups and at doses ≥125 mg/kg/day in PND2 pups. Albumin was decreased at 62.5 and 250 mg/kg/day only in newborn pups. Cholesterol was increased at doses ≥125 mg/kg/day in newborn pups and at doses ≥62.5 mg/kg/day in PND2 pups. Triglycerides were increased at doses ≥125 mg/kg/day in newborn pups, and AST was increased at doses ≥30 mg/kg/day in PND2 pups.

Purple discoloration of the entire right hind limb was observed in one pup each from the 30, 125, and 250 mg/kg/day dose groups beginning on PND1 and those pups were examined for histopathology. All three had milk protein in the stomach lumen, vascular thrombi in various vessels, and small dense basophilic cells throughout liver lobes. The two from the higher dose groups also had moderate subcutaneous hemorrhage in the area of the umbilical artery and vein. Subcutaneous edema or vascular congestion of the lower limb was observed in the pups from the 30 and 250 mg/kg/day dose groups.

A variety of significant adverse effects were observed in the dams. Maternal body weight (on GD22 and PND2) and gestational weight gain were reduced at doses ≥125 mg/kg/day. At necropsy on PND2, maternal absolute liver weight was increased at doses ≥30 mg/kg/day, and relative liver weight was increased at all dose levels. There was no significant effect on the number of uterine implants. Maternal serum total T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day (with the exception of T3 at 250 mg/kg/day). Albumin was decreased at 250 mg/kg/day, and triglycerides were increased at 125 and 250 mg/kg/day. Serum AST was increased at all dose levels. The study authors noted that, even though maternal serum and levels of HFPO dimer acid ammonium salt did not increase when dosing was extended from 4 days in the fetal study to 16 days in the postnatal study (see section 2.3.3), maternal liver weight was more affected in the postnatal study and at lower dose levels.

Conley et al. (2021) also conducted gene expression analyses using liver samples from both experiments (GD16–20 and GD8–PND2) to determine if HFPO dimer acid ammonium salt activates PPAR signaling pathways or alters genes related to glucose and glycogen metabolism.

Gene expression was assessed using reverse transcriptase real-time polymerase chain reaction (PCR) of complementary deoxyribonucleic acid (DNA) synthesized from ribonucleic acid (RNA) extracted from sample homogenates. Maternal (GD20), fetal (GD20), and neonatal (PND0) livers were assessed for 84 target genes relevant to PPARα, PPARβ/δ, and PPAR-γ signaling pathways in the rat. Fetal and neonatal livers were also assessed for 84 genes involved in the regulation and enzymatic pathways of glucose and glycogen metabolism.

Expression of five genes related to glucose metabolism were affected in the GD20 fetal livers. Four genes (*Pck1, Pdk4, G6pc, Pdp2*) were significantly upregulated compared with controls and one (*Ugp2*, critical to glycogen synthesis) was significantly downregulated. All genes were significantly different at doses ≥10 mg/kg/day, except for *G6pc* (critical to gluconeogenesis) which was significantly different at doses ≥3 mg/kg/day. *Pck1* (critical to gluconeogenesis) was the most highly upregulated gene (37.5-fold compared with control at the highest dose). No genes were significantly affected at 1 mg/kg/day.

Conley et al. (2021) compared the gene alterations observed in PPAR signaling pathways for fetal livers exposed GD16 to 20 (this study) with those exposed GD14 to 18 (reported in Conley et al., 2019). All 28 genes involved in PPAR signaling that were significantly upregulated on GD18 were also upregulated on GD20, and 16 of these genes had a highly significant interval effect with greater upregulation on GD20 than on GD18. The remaining upregulated genes did not differ significantly between GD18 and GD20. There were no significantly downregulated PPAR signaling genes. Overall, Conley et al. (2021) concluded that greater gene expression effects were observed later in gestation on genes that code for proteins critical to mitochondrial (*Acaa2, Acadm, Cpt1a*) or peroxisomal (*Acox1, Ech1, Ehhadh*) fatty acid β-oxidation or both (*Mlycd*), gluconeogenesis (*Pck1*), glycerol metabolism (*Gk*), fatty acyl-CoA conversion (*Acsl1, Acsl3*), mediation of triglyceride clearance (*Angptl4*), triglyceride biosynthesis (*Dgat1*), fatty acid biosynthesis (*Fads2, Scd1*), and PPAR coactivation (*Rxrg*).

Analysis of maternal livers showed that the 19 PPAR signaling genes that were upregulated on GD18 (Conley et al., 2019) were also upregulated on GD20, and seven of those showed greater upregulation on GD20. The upregulated genes code for proteins critical to mitochondrial and peroxisomal fatty acid β-oxidation, ketogenesis, fatty acid transport, fatty acyl-CoA conversion, triglyceride turnover, carnitine transport, mitochondrial protein import, accumulation of reactive oxygen species, and transcriptional coactivation. Conley et al. (2021) concluded that the data from this study provide evidence for PPARα activation in the maternal, fetal, and neonatal livers following exposure to HFPO dimer acid ammonium salt.

Gene expression analyses of newborn pup livers showed that 13 glucose metabolism genes were upregulated and 15 were downregulated, 11 of which were significantly different from controls in all dose groups. *Pdk4* was upregulated and *Ugp2* was downregulated, similar to fetal livers, but *Pck1* and *G6pc* were unaffected. The most highly affected upregulated genes were *Fbp2* (gluconeogenesis) and *Ldha* (anaerobic glycolysis); the most highly affected downregulated genes included *Aldob* (glycolysis), *Agl* (glycogen degradation), *Ugp2* (glycogen synthesis), and *Gsk3a* (glycogen synthesis). There were 21 upregulated and 8 downregulated PPAR signaling pathway genes, 21 of which were significantly different from controls in all dose groups. Several gene expression changes were unique to PND0 livers including *Fabp2* (downregulated, a lipid sensor and high affinity long-chain fatty acid binding protein), *Slc27a5* (downregulated, activates very long-chain fatty acids and bile acids), *Apoc3* (downregulated, associated with metabolism of

triglyceride-rich lipoproteins), *Ppara* (downregulated, codes for the PPAR alpha nuclear receptor), and *Cd36* (upregulated, has pleiotropic effects associated with angiogenesis, inflammation, and fatty acid metabolism). Overall, Conley et al. (2021) concluded that many genes associated with carbohydrate and lipid metabolism were affected at multiple stages of development by HFPO dimer acid ammonium salt exposure.

Conley et al. (2021) observed multiple significant adverse effects when dams were dosed with HFPO dimer acid ammonium salt from GD8 to PND2. Significant pup mortality was observed at doses ≥62.5 mg/kg/day and growth rates were significantly lower at doses ≥30 mg/kg/day. Newborns displayed hypoglycemia at doses ≥62.5 mg/kg/day, elevated serum lipid levels at doses ≥125 mg/kg/day, and significantly lower glycogen accumulation scores in the PND2 livers of all dose groups. Maternal body weights were decreased at doses ≥125 mg/kg/day, and maternal relative liver weight was increased in all dose groups. Maternal serum total T3 and T4 levels were decreased at doses ≥62.5 mg/kg/day (with the exception of T3 at 250 mg/kg/day). The authors noted that disruption of carbohydrate and lipid metabolism across the maternal-placental-fetal unit (beginning in the 1 mg/kg/day dosing group) were likely key events in the observed adverse effects, including decreases in pup body weight and survival. EPA concluded that the study NOAEL is 10 mg/kg/day and the LOAEL is 30 mg/kg/day based on reduced BW in F_1 pups at PND0 and PND2.

4.6 Other Studies

4.6.1 Immunotoxicity Studies

Rushing et al. (2017)

Male and female C57BL/6 mice (6–12/sex/group) were administered HFPO dimer acid by gavage at doses of 0, 1, 10, or 100 mg/kg/day for 28 days (Rushing et al., 2017). The animals were immunized with sheep RBC antigen on day 24 and, 5 days later, were evaluated for TDARs and splenic lymphocyte subpopulations. Organs were collected 1 day after the final gavage exposure.

T lymphocyte numbers were significantly increased (the average increase of $CD8^+$, $CD4^+/CD8^+$, and $CD4^-/CD8^-$ T cells was 74%) in males at 100 mg/kg/day, yet suppression of TDAR was observed in female mice only at 100 mg/kg/day. TDAR suppression was measured through immunoglobulin M (IgM) antibody production, which decreased by 7.3% in females at the high dose. Liver weight relative to BW significantly increased (40%–160%) in both sexes at 10 mg/kg/day in a dose-dependent manner. Relative spleen weights significantly decreased by 11% in females treated with 100 mg/kg/day, and there were no significant changes in thymus weight.

Peroxisomal fatty acid oxidation was measured using hepatic acyl-CoA oxidase activity as a readout. In male mice, hepatic acyl-CoA oxidase activity increased 122% and 222% at 10 mg/kg/day and 100 mg/kg/day, respectively. Female mice had a 100% increase in acyl-CoA oxidase activity at the highest dose tested. The NOAEL for immune effects that include TDAR suppression in females and increased T cells in males is 10 mg/kg/day.

4.6.2 Mechanistic Studies

The studies in this section provide mechanistic insight into the effects of HFPO dimer acid and/or its ammonium salt. Available studies address biological mechanisms applicable to liver

effects, serum lipids and lipoproteins, thyroid hormones, and developmental effects. Of note, many of the studies outlined here report using dimethyl sulfoxide (DMSO) to prepare HFPO dimer acid or its ammonium salt. This is important because a 2020 publication (Gaballah et al., 2020) demonstrates that HFPO dimer acid is unstable in DMSO but is stable in deionized water. Where reported, EPA has listed the vehicle that the study authors used to dissolve these chemicals and the vehicle control.

Wang et al. (2017)

In one study investigating changes in gene expression, male ICR mice (*n*=12/group) were administered either (control) or 1 mg/kg/day HFPO dimer acid ammonium salt prepared in 0.5% Tween-20 via oral gavage for 28 days (Wang et al., 2017). Although the authors state that HFPO dimer acid was tested and its chemical structure is presented, the CASRN is listed as 62037-80-3, which is the HFPO dimer acid ammonium salt. Nevertheless, whether the chemical evaluated was the acid or the ammonium salt does not impact the form dissolved in serum or plasma. In both cases, the HFPO dimer anion is present in solution.

At the end of 28 days, blood samples were collected and analyzed. After sacrifice the liver was recovered for measurement of organ weight and histological examination. High-throughput ribonucleic acid (RNA)-sequencing was conducted to gain mechanistic insights into the observed liver effects. Liver tissue samples from three controls and three treated animals were frozen for RNA isolation, library preparation, and sequencing.

Statistically significant treatment-related findings reported include increased absolute liver weight (31%) and relative liver weight (28%), ALP (51%), LDL cholesterol (50%), decreased total bilirubin (-37%), and decreased direct bilirubin (-45%) when compared to control. Qualitative hepatic histopathological findings documented abnormalities from the treated animals, including lipid droplet accumulation, hepatocellular hypertrophy, mild steatosis, and karyolysis.

High-throughput RNA-sequencing of liver tissues resulted in the identification of 146 transcripts (101 upregulated and 45 downregulated) with altered differential gene expression due to treatment with the HFPO dimer. Pathway analyses (using the National Center for Biotechnology Information, Ensemble, gene ontology, and Kyoto Encyclopedia of Genes and Genomes databases) revealed four enriched pathways from these altered hepatic transcripts: the PPAR signaling pathway, arachidonic acid (an essential polyunsaturated fatty acid) metabolism, retinol metabolism, and fatty acid degradation. All four of these pathways are associated with lipid metabolism. Gene ontology analyses of the 146 altered transcripts identified several other enriched processes, cellular components, and molecular functions related to immune system function, lipid metabolism, membrane parameters, and others that were altered by HFPO dimer acid treatment.

Behr et al. (2018)

H295R, MDA-kb2, HEK293T, LNCaP, and MCF-7 cell lines were cultured and incubated with various individual PFAS in a variety of experiments to investigate effects on cytotoxicity, estrogen and AR activity, and steroidogenesis (Behr et al., 2018). The study authors do not report how the HFPO dimer acid ammonium salt was prepared but report 99% purity.

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was employed to assess cell viability in HEK293T, H295R, MCF-7, and LNCaP cell lines following exposure to various concentrations of each PFAS. HEK293T and LNCaP cells were exposed for 24 hours, H295R cells for 48 hours, and MCF-7 cells for 6 days. The WST-1 assay was used to determine viability of MDA-kb2 cells following 24 hours of exposure to each PFAS. Although the study authors do not report how the HFPO dimer acid ammonium salt was prepared, 0.1% DMSO was used as the vehicle control in this assy. HFPO dimer acid ammonium salt (referred to as "PMOH" (ammonium perfluoro(2-methyl-3-oxahexanoate)) in this study) was not cytotoxic in HEK293T, MDA-kb2, H295R, or LNCaP cell lines up to concentrations of 500 μM and caused cytotoxicity in the MCF-7 cell line at 500 μM.

HEK293T cells were assayed for agonistic and antagonistic estrogen receptor (ER) alpha and beta (ERα and ERβ) transactivation. None of the tested PFAS were found to affect either ERα or ERβ at the highest tested concentrations (500 μM for HFPO dimer acid ammonium salt). Estrogen co-exposure with 500 μM HFPO dimer acid ammonium salt was found to co-stimulate ERβ activity. Additionally, HFPO dimer acid ammonium salt was found to enhance estrogen-mediated ERβ activation.

The agonistic and antagonistic AR reporter gene assay was performed in MDA-kb2 cells. HFPO dimer acid ammonium salt was found to be negative for AR transactivation and inhibition up to concentrations of 100 μM. HFPO dimer acid ammonium salt enhanced dihydrotestosterone-stimulated AR activity in a dose-responsive fashion at concentrations above 50 μM.

A steroidogenesis assay was performed in which H295R cells were exposed for 48 hours and enzyme-linked immunosorbent assay (ELISA) kits were used to quantify estradiol (E2), estrone, testosterone, and progesterone levels. HFPO dimer acid ammonium salt significantly decreased testosterone at 100 μM.

An E-screen assay was used to evaluate proliferation of MCF-7 cells following 6 days of exposure to various PFAS. HFPO dimer acid ammonium salt did not significantly affect cell proliferation compared to estrogen. Exposure to high concentrations of HFPO dimer acid ammonium salt (100 μM) in combination with estrogen slightly diminished cell proliferation, but the effect was not statistically significant.

MCF-7 and LNCaP cells were cultured with HFPO dimer acid ammonium salt for 24 hours, and H295R cells were cultured for 48 hours prior to RNA extraction followed by quantitative reverse transcription PCR. HFPO dimer acid ammonium salt did not stimulate estrogenic responsive gene expression of *TFF1, GREB1, PGR, ESR1, ESR2,* or *CTSD* in MCF-7 cells, or *AR, PSA, NKX3-1, TMPRSS2,* or *CDKN1A* in LNCaP cells at concentrations up to 100 μM. Additionally, HFPO dimer acid ammonium salt did not affect expression of *CYP19A1, CYP17A1, CYP21A2, CYP11A1, STAR,* or *HSD3B* at concentrations up to 100 μM.

Sheng et al. (2018)

Sheng et al. (2018) used *in vitro* experiments to investigate perfluoroalkyl cytotoxicity and binding to proteins for HFPO dimer acid ammonium salt (referred to as "HFPO-DA" in this study), HFPO dimer acid trimer, HFPO dimer acid tetramer, PFOA, and perfluorooctane sulfonate (PFOS) in a human liver HL-7702 cell line. The study authors assessed cell viability to determine the cytotoxicity of the various perfluoroalkyl substances and used flow cytometry to

investigate effects on cell proliferation. The authors noted, however, that no effects of HFPO dimer acid ammonium salt on cytotoxicity and cell proliferation could be determined through these assays because of the chemical's low boiling point and high volatility. The study authors do not report how the HFPO dimer acid ammonium salt was prepared.

Data quantifying the HFPO dimer acid anion's ability to bind to human liver fatty acid-binding protein (hL-FABP) was also generated. Binding affinity was explored because other PFAS compounds have exhibited effective binding to hL-FABP and such binding might explain how PFAS can enter into hepatocytes, a potential target cell for HFPO dimer acid and/or its ammonium salt (Luebker et al., 2002; Sheng et al., 2016; Zhang et al., 2013). Binding affinity was measured in a fluorescence competitive binding assay and found that HFPO dimer acid anion exhibited a weaker binding affinity than PFOA or PFOS. However, the study found that the HFPO dimer acid anion fit well in the hL-FABP binding pocket with a docking energy in between PFOS and PFOA. This indicates direct interaction between the HFPO dimer acid anion and hL-FABP. Additionally, the HFPO dimer acid anion bound differently to hL-FABP than PFOA and PFOS (Sheng et al., 2018). These results were replicated using a predictive model of binding affinity to hL-FABP (Cheng and Ng, 2018).

Li et al. (2019)

Li et al. (2019) investigated the binding affinity of HFPO dimer acid (referred to as "HFPO-DA" in the paper), HFPO trimer acid (HFPO-TA), and PFOA to human and mouse PPAR gamma (PPARγ) ligand binding domains. PPARγ is a second member of the PPAR family of nuclear receptors. It functions as a regulator of cell proliferation and differentiation in addition to impacting lipid metabolism. The study authors report that HFPO dimer acid was dissolved in DMSO to make stock solutions and was reported as 97% pure. Binding affinity was measured in a fluorescence competitive binding assay. The study authors observed a higher affinity for the human PPARγ ligand binding domain for HFPO-TA and PFOA, while HFPO dimer acid bound with greater affinity for the mouse PPARγ ligand binding domain. Among the three PFAS tested, a binding potency order of HFPO-TA> PFOA>HFPO dimer acid was identified for both human and mouse ligand binding. Li et al. (2019) also assessed the activity of HFPO dimer acid, HFPO-TA, and PFOA using HEK293 cells transfected with a luciferin-tagged PPARγ vector. After exposure to HFPO dimer acid, HFPO-TA, and PFOA, the luciferase activity of the cells was quantified as an indicator of the PFAS's ability to impact PPARγ transcription. The authors conclude that HFPO dimer acid, HFPO-TA, and PFOA acted as transcriptional agonists, resulting in enhanced PPARγ transcriptional activity in a dose-dependent manner.

Because PPARγ activation is involved in the modulation of adipogenesis (Tontonoz et al., 1994), Li et al. (2019) also exposed cultured human (HPA-s) and mouse (3T3-L1) preadipocytes to the three compounds for ten days during a period of cellular differentiation into adipocytes. To quantify adipogenic activity, an Oil Red O staining assay was performed to quantify lipid accumulation using the dosed human and mouse adipocytes. HFPO dimer acid significantly increased lipid accumulation at 6 μM and 25 μM for the human HPA-s cells and mouse 3T3-L1 cells, respectively. HFPO dimer acid showed comparable or weaker adipogenesis activity than PFOA and HFPO-TA. Relative PPARγ messenger RNA (mRNA) levels were statistically significantly increased in human HPA-s cells exposed to 25 μM HFPO dimer acid and at 50 μM HFPO dimer acid in mouse 3T3-L1 cells.

Sun et al. (2019)

Three dimensional (3D) spheroids were used to evaluate the cytotoxicity of PFOA, HFPO dimer acid (referred to as "HFPO-DA" in this study) and PFO4DA (3,5,7,9-tetraoxadecanoic perfluoro acid) (Sun et al., 2019). HFPO dimer acid was diluted with serum-free DMEM/F-12 (Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12) medium and was reported as 95% pure. 3D spheroids were exposed to 100 μM of each of these three substances for 28 days. No changes in adenosine triphosphate (ATP) content or albumin secretion were observed. Lactic dehydrogenase and reactive oxygen species levels were significantly increased (compared with controls) after PFOA, HFPO dimer acid, or PFO4DA exposure. A 1.5- to twofold increase in *Scd1* expression (compared with control) was observed in PFOA, HFPO dimer acid, and PFO4DA exposure groups. PFOA exposure, but not HFPO dimer acid exposure, significantly increased PPARα expression compared to the control. For the apoptosis-related genes, PFOA exposure significantly increased expression of *caspase3*, *p53*, and *p21* compared to control, whereas HFPO dimer acid exposure produced no changes. For the oxidative stress genes, only PFOA significantly increased *Nqo1* expression, and PFOA, HFPO-dimer acid, and PFO4DA all significantly induced expression of *Gsta2* and *Ho-1*.

Xin et al. (2019)

Estrogenic effects of PFOA and HFPO dimer acid (referred to as "HFPO-DA" in this study) were evaluated in a series of *in vitro* assays (Xin et al., 2019). All tests were also performed on additional HFPO homologs (HFPO-TA and hexafluoropropylene oxide tetramer acid (HFPO-TeA)). HFPO dimer acid was prepared in DMSO at a concentration of 20 millimolar (mM) and was reported as 97% pure.

HFPO dimer acid binding affinity to the human ERα and ERβ ligand binding domains were compared to that of PFOA using a fluorescence polarization-based competitive fluorescence binding assay. HFPO dimer acid did not bind either ERα or ERβ (not detected; no IC_{50} (concentration at which 50% inhibition is observed) could be derived). PFOA displaced estrogen in a concentration-dependent manner, with IC_{50} values of 469.5 μM for ERα and 384.4 μM for ERβ.

The cytotoxic effects of HFPO dimer acid at concentrations ranging from 0.8 to 1,600 μM were determined in MVLN cells using the WST-1 assay. HFPO dimer acid produced no cytotoxicity, whereas PFOA inhibited cell viability at concentrations above 800 μM. MVLN cells were also exposed to PFOA or HFPO dimer acid at concentrations ranging from 1.6 to 800 μM, with or without E2 for 12 or 24 hours, and estrogenic/anti-estrogenic activity was assessed. Exposure to HFPO dimer acid did not result in any effects on ERs. PFOA exposure resulted in concentration-dependent antagonism of ERs and PFOA was also found to compete with E2 to activate ERs.

ELISA kits were used to measure E2, testosterone, and vitellogenin (VTG) in wildtype zebrafish larvae exposed to 0.4 or 1.6 μM PFOA or HFPO dimer acid for 168 hours post-fertilization. HFPO dimer acid and PFOA significantly increased E2, testosterone, and VTG compared to controls in all dose groups, except for VTG levels at 0.4 μM HFPO dimer acid.

Molecular docking and molecular dynamics simulations were performed using AutoDock 4.2 to compare binding interactions of HFPO dimer acid and PFOA with ERα and ERβ. The simulations illustrated that both HFPO dimer acid and PFOA fit into the binding cavity of ERα

and ERβ. The calculated energies from the simulations indicated that the order of the binding affinity for these compounds is HFPO-TeA > HFPO-TA > PFOA > HFPO dimer acid.

Behr et al. (2020)

The cytotoxicity, human nuclear receptor activation, and gene expression changes induced by HFPO dimer acid ammonium salt (referred to as "PMOH" (ammonium perfluoro(2-methyl-3-oxahexanoate)) in this study) was investigated *in vitro* using HEK293T and HepG2 cells. Seven other PFAS (PFOA, PFOS, PFHxA, perfluorobutanesulfonic acid (PFBS), PFBA, PFHxS, and 3H-perfluoro-3-((3-methoxypropoxy) propanoic acid (PMPP) were also analyzed in this study (Behr et al., 2020). The study authors do not report how the HFPO dimer acid ammonium salt was prepared.

The cytotoxicity of HFPO dimer acid ammonium salt was assessed in HepG2 cells. The cells were exposed to 50–500 μM of HFPO dimer acid ammonium salt for 24 hours, and cellular viability was determined using the MTT assay. Cell viability was not significantly decreased at any concentration.

Luciferase-based reporter gene assays were used to determine the ability of HFPO dimer acid ammonium salt to activate various human nuclear receptors that function in the regulation of lipid or xenobiotic metabolism. HEK293T cells were transfected with expression plasmids for hCAR, hFXR, hLXRα, hPPARα, hPPARδ, hPPARγ, hPXR, hRARα, or hRXRα. The cells were co-transfected with a luciferase reporter plasmid and the *Renilla*-luciferase construct pcDNA3-Rluc for normalization. Positive controls were included. Receptor activity was measured after 24 hours of exposure to 25, 50, or 100 μM of each chemical. Values were normalized to *Renilla reniformis* luciferase activities and compared to untreated cells. HFPO dimer acid ammonium salt significantly induced PPARα activation (sevenfold) at 25 μM and higher. HFPO dimer acid ammonium salt also significantly induced activation of PPARγ (2.4-fold) at 100 μM. The other human nuclear receptors were not significantly affected. Reporter gene assays for PPARα were repeated for PFOA and PMOH using concentrations up to 250 μM. Concentration-response curves were calculated and EC_{10} values were determined relative to a positive control (GW7647). HFPO dimer acid ammonium salt activated PPARα to a level of 10% at 5 μM, and a comparable activation was induced by PFOA at 50 μM.

HepG2 cells were exposed to concentrations up to 250 μM HFPO dimer acid ammonium salt for 24 hours, and the RNA was extracted for analysis of PPARα-dependent target gene expression. Untreated cells served as control. At 250 μM, PMOH significantly induced expression of *CPT1A* (1.7-fold), *HMGCS2* (2.8-fold), and *PLIN2* (1.4-fold). Compared to PFOA, the effects of PMOH were not as substantial. PFOA produced similar effects on the target genes as PPARα agonists GW7647 and WY14,643.

Although HFPO dimer acid ammonium salt was a more potent PPARα agonist than PFOA under the conditions of this study, it produced weaker effects on PPARα-dependent target gene expression.

Wen et al. (2020)

The epigenetic toxicities of HFPO dimer acid (referred to as "GenX" in this study) and PFOA were explored and compared *in vitro* using a liver hepatocellular carcinoma cell line (Wen et al., 2020). HepG2 cells were exposed to concentration gradients of the ammonium salt form of

PFOA (20–600 μM) or HFPO dimer acid (20–1,000 μM) for 48 hours. The test chemicals were first dissolved in DMSO (< 0.4% volume/volume), and vehicle controls were included. HFPO dimer acid was reported as 97% pure.

The MTT and neutral red assays were used to assess cell metabolism rates and viability. Following HFPO dimer acid exposure, cell metabolic activity was only slightly increased at all concentrations compared to control. Cell viability was increased from 20 to 200 μM, and then decreased linearly from 200 to 1,000 μM.

Following PFOA exposure, cell metabolic activity increased in a concentration-dependent fashion from 0 to 100 μM, peaked at 100 μM, and decreased in a concentration-dependent fashion from 100 to 400 μM. Cell viability decreased with increasing PFOA concentration (decreased by 87% at 600 μM).

Gene expression analysis was performed for 22 genes related to cell cycle, proliferation, apoptosis, and lipid metabolism. HepG2 cells were cultured in flasks and treated with 100–600 μM HFPO dimer acid for 48 hours, and the RNA was extracted for gene expression analysis. Overall, HFPO dimer acid did not have a strong impact on the genes examined; expression of most lipid metabolism and transport-related genes was either decreased or not significantly affected by HFPO dimer acid. In contrast, expression of lipid synthesis-related genes was mostly elevated, and expression of lipid transport genes was mostly decreased by PFOA.

Global methylation assays were performed using genomic DNA from HepG2 cells extracted immediately after the treatment period. Expression profiles of 10–11 translocation methylcytosine dioxygenases (*TET*s) and DNA methyltransferases (*DNMT*s) were also evaluated. In HFPO dimer acid-treated cells, global methylation (5mc) levels significantly decreased from 100 to 400 μM, and then increased from 600 to 800 μM. GenX caused decreased expression of *DNMT*s but had no clear effect on *TET*s. PFOA caused a significant, concentration-dependent decrease in global methylation (5-mC) levels from 20 to 400 μM, and significant concentration-dependent changes in *TET*s (*TET1* decreased whereas *TET2* and *TET3* increased with increasing PFOA concentration), but no significant trends in the expression of *DNMT*s.

Cannon et al. (2020)

The effects of HFPO dimer acid ammonium salt (referred to as "GenX" in this paper) on expression and activity of three ATP binding cassette (ABC) transporters at the blood-brain barrier were studied using rat brain capillaries exposed *ex vivo* to low nanomolar (nM) concentrations (Cannon et al., 2020). Rats were also exposed to 97% pure HFPO dimer acid ammonium salt *in vivo* followed by *ex vivo* measurement of transport activity. ATPase levels were measured *in vitro*, and protein levels were measured with Western blotting. The cytotoxicity of HFPO dimer acid ammonium salt was assessed using two human cell lines. HFPO dimer acid ammonium salt was prepared in fresh DMSO (0.1% volume/volume) prior to each experiment.

The brains from 4–6 male or female Hsd:Sprague Dawley rats (age 12–15 weeks) were harvested and capillaries were isolated from cortical gray matter. Capillaries were exposed to varying concentrations of HFPO dimer acid ammonium salt (0.01–1,000 nM) for 3 hours and P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), and multidrug resistance-

associated protein 2 (MRP2) transporter activities were measured with a confocal microscopy-based method. Hourly changes in transport activities were also measured during a 4-hour exposure to 100 nM HFPO dimer acid ammonium salt. In addition, reversibility assays were performed using 100 nM HFPO dimer acid ammonium salt exposure for 1 hour for P-gp and 2 hours for BCRP. After transport activities were measured, HFPO dimer acid ammonium salt was removed from the assay media and transport activities were measured at 0.5, 1, 2, and 3 hours after removal. In the main assays, P-gp transport activity was significantly lower in male capillaries exposed to 0.1–100 nM HFPO dimer acid ammonium salt and in female capillaries exposed to 1.0–100 nM HFPO dimer acid ammonium salt compared with controls. P-gp transport activity was significantly decreased beginning at 15 or 30 minutes of treatment with 100 nM and persisted to the 4-hour mark. BCRP transport activity was significantly lower in male capillaries exposed to 1.0–1,000 nM HFPO dimer acid ammonium salt and in female capillaries exposed to 0.1–1,000 nM, and a significant decrease in activity during a 4-hour exposure to 100 nM was observed beginning at the 1-hour time point for both sexes. MRP2 transport activity was not significantly affected by HFPO dimer acid ammonium salt. In the reversibility assays, P-gp transport activity in capillaries from both sexes was restored to control levels within 1 hour of HFPO dimer acid ammonium salt removal, but BCRP transport activity remained lowered for 2 hours after removal for both sexes.

A reconstituted transport assay system containing vesicle membranes and transport proteins was used to determine the effects of HFPO dimer acid ammonium salt on transport-associated ATPase activity *in vitro*. Purified P-gp and BRCP transport proteins were exposed to 0.001–1.0 μM HFPO dimer acid ammonium salt for 20 minutes and the enzymatic hydrolysis of ATP to inorganic phosphate was measured. The substrate was stimulated with paclitaxel for P-gp and sulfasalazine for BCRP. HFPO dimer acid ammonium salt did not alter ATPase activity associated with P-gp or BCRP transport either when the substrate was stimulated or when no substrate was added, indicating that HFPO dimer acid ammonium salt was not a substrate for either transporter using this particular *in vitro* reconstituted transport assay system.

Isolated brain capillaries pooled from male or female rats (n=6 rats/sex) were exposed to 100 nM HFPO dimer acid ammonium salt for 4 hours, and P-gp and BCRP protein levels were measured by Western blotting. No significant differences in P-gp or BCRP protein levels were identified for treated capillaries compared with control (vehicle-treated) capillaries for either sex.

Isolated brain capillaries from male or female rats (n=6 rats/sex) were also treated with HFPO dimer acid ammonium salt (1.0 or 100 nM) for 4 hours with or without the PPARγ inhibitor GW9662. HFPO dimer acid ammonium salt decreased P-gp transport activity at both concentrations for both sexes compared with controls. The addition of GW9662 blocked the reduction in activity in male capillaries at both concentrations, but only at 1.0 nM HFPO dimer acid ammonium salt in female capillaries. BCRP transport activity was lowered by treatment at both concentrations in both sexes, and co-treatment with GW9662 had no effect on the reduced BCRP transport activities for either sex.

Male and female Hsd:Sprague Dawley rats (5/sex/group) were administered a single oral gavage dose of 0, 10, 100, or 1,000 ng/kg (30 picomole (pmol)/kg, 300 pmol/kg, or 3 nanomole/kg) of HFPO dimer acid ammonium salt and sacrificed 5 hours later. Brains from each dose group were pooled, and capillaries were isolated for measurement of P-gp and BCRP transport activities. All

dose levels produced significant decreases in P-gp and BCRP transport activities in both sexes compared with controls.

Cell survival following HFPO dimer acid ammonium salt exposure was determined using the human ovarian cell line NCI/ADR-RES (with high P-gp expression) and human mammary epithelial cell line MX-MCF-7 (with high BCRP expression). The cells were first exposed to HFPO dimer acid ammonium salt alone at increasing concentrations of 10^{-9}–10^{-4} M for 72 hours, and the remaining cells were counted. No significant differences in survival were observed. Next, to determine if HFPO dimer acid ammonium salt affected the toxicity of known cytotoxic substrates for P-gp or BCRP, each cell line was exposed to 100 nM HFPO dimer acid ammonium salt for 72 hours in the presence of Adriamycin (for NCI/ADR-RES cells) or mitoxantrone (for MX-MCF-7 cells). Co-treatment of 100 nM HFPO dimer acid ammonium salt and 10 μM Adriamycin significantly reduced NCI/ADR-RES cell survival from 85% to 45%, and co-treatment of 100 nM HFPO dimer acid ammonium salt and 100 μM mitoxantrone significantly reduced MX-MCF-7 cell survival from 63% to 37%.

The results of these assays show that both *ex vivo* and *in vivo* exposure to low nM levels of HFPO dimer acid ammonium salt can inhibit P-gp and BCRP transport in rat brain capillaries. The effect of HFPO dimer acid ammonium salt on P-gp transport was shown to involve PPARγ.

4.6.3 Genotoxicity Studies

HFPO dimer acid ammonium salt was not observed to induce genetic mutations both with and without metabolic activation of the test substance by rat liver S9 fraction in two species of prokaryotes: *Escherichia coli* (strain WP2uvrA) and *Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA 1537) (DuPont-19713 RV1, 2008; DuPont-22734 RV1, 2008). An *in vitro* gene mutation test of the HFPO dimer acid ammonium salt in mouse lymphoma cells (strain L5178Y/TK+/-) was negative in the presence and absence of rat liver S9 fraction (DuPont-26129, 2008). HFPO dimer acid ammonium salt was observed to induce chromosomal aberrations in Chinese hamster ovary cells *in vitro* in the presence and absence of S9 activation (DuPont-19714 RV1, 2008; DuPont-22620 RV1, 2009). In *in vivo* mammalian studies, exposure to HFPO dimer acid ammonium salt by the oral route did not induce chromosomal mutations in the form of structural aberrations, numerical aberrations, or micronuclei nor DNA effects in the form of unscheduled DNA synthesis (DuPont-23219, 2007; DuPont-23220, 2007). A table summarizing the findings of the available genotoxicity studies is provided in appendix C.

5.0 Summary of Hazard

The available studies indicate adverse effects including liver, developmental, hematological, and immune effects occur following exposures in the range of 0.5–1,000 mg/kg/day GenX chemicals. Table 12 presents the available studies and their NOAELs and LOAELs. Discussion of the weight of evidence for hazard is presented following the table.

Table 12. Summary of Study NOAELs/LOAELs

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
28 Day Oral (Gavage) Toxicity Study in Rats (OECD, 2008a) DuPont-24447 (2008)	Medium	Males: 0, 0.3, 3, and 30 Females: 0, 3, 30, and 300	NOAEL = 0.3 LOAEL = 3	Hematological effects (↓ RBC count, hemoglobin, and hematocrit in males) Immune effects (↓ globulin, and ↑ A/G ratio in males)
28 Day Oral (Gavage) Toxicity Study in Mice (OECD, 2008a) DuPont-24459 (2008)	Medium/Low	Males and Females: 0, 0.1, 3, and 30	NOAEL = 0.1 LOAEL = 3	Liver effects (single-cell necrosis in males, ↑ relative liver weight in in males, and ↑ hepatocellular hypertrophy in males) Hematological effects (↓ hemoglobin and hematocrit in males) Immune effects (↓ globulin in females, and ↑ A/G ratio in both sexes)
28 Day Oral (Gavage) Immunotoxicity Study in Mice Rushing et al. (2017)	Medium	Males and Females: 0, 1, 10, and 100 Note: HFPO dimer acid	NOAEL = 10 LOAEL = 100	Immune effects (TDAR suppression in females, and ↑ lymphocytes in males)
90 Day Oral (Gavage) Toxicity Study in Rats (OECD, 1998) DuPont-17751-1026 (2009)	High	Males: 0, 0.1, 10, and 100 Females: 0, 10, 100, and 1,000	NOAEL = 0.1 LOAEL = 10	Hematological effects (↓ RBC count, hemoglobin, and hematocrit in males)

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
90 Day Oral (Gavage) Toxicity Study in Mice (OECD, 1998) DuPont-18405-1307 (2010); Reevaluation by NTP PWG Pathology (NTP, 2019)	High	Males and Females: 0, 0.1, 0.5, and 5	NOAEL = 0.5 LOAEL = 5	Liver effects (↑AST, ALT, and ALP in males; ↑ relative liver weight in males and females; and ↑ in constellation of liver lesions: cytoplasmic alteration, single-cell necrosis, focal necrosis, and hepatocellular apoptosis in males and females)
Combined Chronic Toxicity/ Oncogenicity Study in Rats (OECD, 2009) DuPont-18405-1238 (2013)	Medium	Males: 0, 0.1, 1, and 50 Females: 0, 1, 50, and 500	NOAEL = 1 LOAEL = 50	Liver effects (centrilobular necrosis in both sexes; ↑ ALP, ALT, and SDH in males; and ↑ centrilobular hepatocellular hypertrophy and cystic focal degeneration in males)
Oral (Gavage) Reproduction/ Developmental Toxicity Study in Mice (OECD, 2016a; modified according to the Consent Order) DuPont-18405-1037 (2010); Reevaluation by NTP PWG Pathology (NTP, 2019)	High	Males and Females: 0, 0.1, 0.5, and 5	NOAEL (F_0) = 0.1 LOAEL (F_0) = 0.5 NOAEL (F_1) = 0.5 LOAEL (F_1) = 5	Liver effects ((single-cell necrosis, focal necrosis, and cytoplasmic alteration), and ↑ relative liver weight in males and females); reproductive/developmental effects (↑ maternal GWG from GD0 through GD18) Developmental effects (↓ pup weights, and delays in the attainment of balanopreputial separation and vaginal patency)

Study	Overall study quality	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
Prenatal and Developmental Toxicity Study in Rats (OECD, 2001b) DuPont-18405-841 (2010)	Medium	Females: 0, 10, 100, and 1,000	NOAEL (F_0 and F_1) = 10 LOAEL (F_0 and F_1) = 100	Developmental effects (↑ early deliveries, ↓ fetal weights in both sexes, ↓ gravid uterine weight, and focal liver necrosis)
Reproductive and Developmental Toxicity in Rats Conley et al. (2019)	Medium	Females: 0, 1, 3, 10, 30, 62.5, 125, 250, and 500	NOAEL (F_0 and F_1) = 62.5 LOAEL (F_0 and F_1) = 125	Reproduction/developmental effects (↓ maternal GWG, , and indications of reduced body (females) and reproductive and non-reproductive organ weights in F_1 animals) Thyroid effects (↓maternal serum total T3 and T4 levels)
Reproductive and Developmental Toxicity in Mice Blake et al. (2020)	Medium	Females: 0, 2, and 10	NOAEL = NA LOAEL = 2	Reproductive/developmental effects (↑ mean abnormal placental lesions (including labyrinth atrophy, labyrinth congestion, labyrinth necrosis, early fibrin clot, and placental nodule), and ↑ maternal GWG)
Reproductive and Developmental Toxicity in Rats Conley et al. (2021)	High	Females: 0, 10, 30, 62.5, 125, or 250	NOAEL (F_0) = 30 LOAEL (F_0) = 62.5 NOAEL (F_1) = 10 LOAEL (F_1) = 30	Thyroid effects (↓maternal serum total T3 and T4 levels) Reproductive/developmental effects (↓ BW in F1 pups at PND0 and PND2)

5.1 Hepatic

The liver is a target organ for toxicity from oral exposure to HFPO dimer acid and its ammonium salt. Liver effects are observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals. Liver effects are also the endpoints that are observed at the lowest doses for these chemicals. 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 that PPARα activation is the only MOA. The increased relative liver weight and hepatocellular hypertrophy were only considered adverse when accompanied by effects such as necrosis, fibrosis, inflammation, and significantly increased serum levels for enzymes indicative of liver tissue damage (Hall et al., 2012).

Significant increases in liver weight relative to BW were observed in male and female Crl:CD(SD) rats and several strains of male and female mice treated with 0.5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 28–90 days (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017). These increases were observed in doses as low as 0.5 mg/kg/day in male Crl:CD-1 mice (26% increase) over 84–85 days (DuPont-18405-1037, 2010), and the greatest increases were observed when male (163%) and female (102.7%) Crl:CD-1 mice were administered 30 mg/kg/day for 28 days. Likewise, male Crl:CD(SD) rats exhibited increased relative liver weights of 19%–61% compared to control when administered 3 mg/kg/day–100 mg/kg/day for 28–90 days, while female rats' relative liver weights compared to control did not increase until much higher doses (12% at 300 mg/kg/day for 28 days and 85% at 1,000 mg/kg/day for 90 days) were administered. Comparatively, the one available chronic study in rats indicates that liver weight may increase and return to control levels after a time. For example, relative liver weights in male rats increased only 15% when administered 50 mg/kg/day for 1 year and did not exhibit a significant increase from control at 2 years. Likewise, female rat relative liver weights increased 67% and 42% after administration of 500 mg/kg/day for 1 and 2 years, respectively (DuPont-18405-1238, 2013).

Indications of liver damage were also reflected through increases in serum liver enzymes of Crl:CD-1 mice, particularly males, and Crl:CD(SD) rats administered HFPO dimer acid ammonium salt. For example, significant increases in ALT (420%–1,254%), AST (106%–478%), ALP (1,134%–1,221%), and SDH (1,134%–1,221%) were observed in male mice administered the ammonium salt at 5–30 mg/kg/day for 28–90 days. Female mice saw smaller increases in ALP (140%–143%) and SDH (32%–186%) compared to male mice administered the same dose. Overall, rats exhibited far fewer and smaller increases in serum liver enzyme levels following subchronic exposure than the mouse, with increases in AST (106%) and ALP (52%) at 100 mg/kg/day in male rats and AST (66%) in female rats at 1,000 mg/kg/day. In the chronic study, however, ALT (228%), ALP (180%), and SDH (140%) significantly increased in male rats only when administered 50 mg/kg/day for 1 year (DuPont-18405-1238, 2013).

Liver damage was confirmed microscopically in male and female mice and rats in several less-than-chronic studies (15–90 days) and one chronic study (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; Wang et al., 2017; Thompson et al., 2019; NTP, 2019). The most prevalent liver effects following both subchronic and chronic exposure to

HFPO dimer acid and/or its ammonium salt were hepatocellular hypertrophy (also referenced here as cytoplasmic alteration per NTP PWG's review) and single-cell and/or focal necrosis.

In both sexes of mice exposed either short term (28 days) or subchronically (30–90 days), hepatocellular hypertrophy was observed at 0.5 mg/kg/day, while male and female rats showed this effect at 3 mg/kg/day and 30 mg/kg/day, respectively. Interestingly, in the chronic study, male rats did not show any significant increases in hepatocellular hypertrophy when administered 0.1–50 mg/kg/day of HFPO dimer acid ammonium salt for 1 year, and only 10% of the rats exhibited minimal hypertrophy with 50 mg/kg/day administered for 2 years (DuPont-18405-1238, 2013). Conversely, female rats had significant hepatocellular hypertrophy at 500 mg/kg/day after 1 year (100%) and 2 years (93%).

Single-cell and focal necrosis were detected in all the available studies. The reanalysis of the liver pathology slides from DuPont 18405-1037 (2010) by Thompson et al. (2019) did not report necrosis in mice. This interpretation conflicts with the results from the original pathology conducted in DuPont 18405-1037 (2010) and the 2019 NTP PWG reanalysis (NTP, 2019) of DuPont 18405-1037 (2010). The incidence data as reported by NTP (see appendix D) were considered the appropriate measure of response in the liver from the reproductive/developmental study (DuPont-18405-1037, 2010) because the PWG analysis reflects more recent histopathological criteria for the grading of liver lesions and the PWG results were the consensus of eight pathologists.

In the subchronic toxicity studies in mice, males and females presented with single-cell and focal necrosis in doses as low as 0.5 mg/kg/day, which significantly increased at 5 mg/kg/day. Specifically, the incidence rates for single-cell and focal necrosis at 5 mg/kg/day were 100% and 83% in males and females, respectively, in DuPont 18405-1037 (2010) and 90% and 44% in males and females, respectively, in DuPont 18405-1307 (2010) (NTP, 2019). Apoptosis was observed in the 5 mg/kg/day-dose groups in these studies as well, but not in the 0.5 mg/kg/day-dose group (NTP, 2019). As noted in section 4.0 and appendix D in this assessment, the NTP PWG agreed that the dose response and constellation of liver lesions (i.e., hepatocellular hypertrophy, single-cell and focal necrosis and apoptosis) observed in DuPont 18405-1037 (2010) and DuPont 18405-1307 (2010) should be considered as adverse (NTP, 2019). Male and female rats exhibited hepatocellular necrosis at much higher doses in the available short-term study, with males exhibiting what was classified as general necrosis (30%) at 30 mg/kg/day and females presenting focal liver necrosis at 100 mg/kg/day (9%) and 1,000 mg/kg/day (23%). Interestingly, no liver necrosis was reported for either sex in the subchronic rat study (DuPont-17751-1026, 2009). It is possible that apoptosis could have been present in the other DuPont studies, but these studies might not have separated apoptotic lesions from other liver lesions reported (i.e., single-cell necrosis) since they were conducted prior to the histopathological guidance on separating apoptosis from single-cell necrosis (i.e., Elmore et al., 2016) and were not reanalyzed by the 2019 NTP PWG.

These findings suggest that mice are more sensitive to liver necrosis than rats in short-term and subchronic exposure scenarios. In the 2-year chronic rat study, centrilobular necrosis increased at 50 mg/kg/day and 500 mg/kg/day for males (7%) and females (4%), respectively, while single-cell necrosis was observed only in females (4%) at 500 mg/kg/day. Taken together, the male rat liver necrosis data appear to be inconsistent. Specifically, 30% of male rats have necrotic liver cells after 28 days of dosing with 30 mg/kg/day of HFPO dimer acid ammonium salt, yet no

necrosis is reported in male rats after 90 days of dosing with 0.1–100 mg/kg/day. However, necrosis returns in 50% of male rats after 1 year of dosing with 50 mg/kg/day to then be reduced to 7–13% incidence after 2 years of dosing.

Similarly, these data suggest that the pregnant rodent might be more susceptible than nonpregnant rodents to liver effects following exposures to GenX chemicals. Liver effects were reported in the pregnant dams in the available reproductive/developmental studies dosing during gestation (DuPont-18405-841, 2010; Conley et al., 2019; DuPont 18405-1037, 2010; Blake et al., 2020). All the studies reported increases in liver weight ranging from 12% to 34% in rats and 26% to 101% in mice over the gestational period. Conley et al. (2019) did not conduct liver histopathology, but both DuPont-18405-841 (2010) and Blake et al. (2020) reported hepatocellular hypertrophy and increased cell death as compared to control with increasing HFPO dimer acid ammonium salt concentration. Specifically, focal necrosis was observed in 2/22 (9%) and 5/22 (23%) pregnant rats after 15 days (GD6–GD20) of 10 mg/kg/day or 100 mg/kg/day of HFPO dimer acid ammonium salt, respectively, compared to 0 in the control group. Comparatively, nonpregnant female rats dosed from 28 to 90 days did not exhibit necrosis when treated with doses up to 1,000 mg/kg/day of HFPO dimer acid ammonium salt. Necrosis was observed in female rats only after 2 years of dosing with 500 mg/kg/day of HFPO dimer acid ammonium salt. Increased cell death (including both apoptosis and single-cell necrosis) or focal necrosis was observed in pregnant mice after 11 and 17 days (GD1.5–GD11.5 or 17.5) of 2 mg/kg/day or 10 mg/kg/day of HFPO dimer acid ammonium salt. Similarly, and as noted above, female mice dosed 14 days prior to mating and throughout gestation/lactation exhibited cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis after 53–64 days of dosing (NTP, 2019 reanalysis of DuPont 18405-1037, 2010). The incidence of single-cell and focal necrosis in the F_0 females was 6/24 (25%) and 20/24 (83%) in the 0.5- and 5-mg/kg/day-dose groups, respectively (NTP, 2019).

5.2 Hematological

The hematologic system could be a target of HFPO dimer acid ammonium salt toxicity as effects have been observed across studies of varying durations of oral exposure to the chemical. The primary effects observed are decreases in RBC number, hemoglobin, and percentage of RBCs in the blood, indicating that oral exposure to HFPO dimer acid ammonium salt might promote anemic conditions. In male mice and rats, the percent change in these effects from the controls was relatively small. For example, male Crl:CD-1 mice and Crl:CD(SD) rats treated with 3 mg/kg/day–100 mg/kg/day of HFPO dimer acid ammonium salt for 28–180 days had maximum decreases of 12%, 11%, and 12% in hemoglobin, erythrocyte count, and hematocrit, respectively (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008). Interestingly, in the available chronic study, no hematological effects were observed at the 12-month time point in male rats (DuPont-18405-1238, 2013). Female Crl:CD-1 mice and Crl:CD(SD) rats presented hematological effects at greater than 90 days and typically at higher doses than males, with one exception. Hemoglobin significantly decreased by 4% when female Crl:CD(SD) rats were administered 1 mg/kg/day of HFPO dimer acid ammonium salt for 90 days (DuPont-18405-1238, 2013). Otherwise, hematological effects occurred at doses greater than or equal to 50 mg/kg/day and the maximum decreases from control were 24%, 28%, and 20% for hemoglobin, erythrocyte count, and hematocrit, respectively (DuPont-18405-1238, 2013; DuPont-24447, 2008).

5.3 Renal

The kidney could also be a target organ for toxicity from oral exposure to HFPO dimer acid and/or ammonium salt; however, kidney effects typically presented at higher doses than the liver effects.

Significant increases in kidney weight relative to BW were observed in several less-than-chronic studies in Crl:CD-1 mice and Crl:CD(SD) rats treated with 0.1 mg/kg/day–1,000 mg/kg/day (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-24459, 2008; DuPont-24447, 2008). The maximum increase in kidney weight for male rodents was an increase of 16% compared to control in male rats treated with 100 mg/kg/day of HFPO dimer acid ammonium salt over 90 days. Likewise, the maximum kidney weight relative to BW increase in female rodents was 23% in female rats administered 1,000 mg/kg/day over 90 days (DuPont-17751-1026, 2009). Interestingly, increases in relative kidney weights were not observed in the same type of male rat when administered HFPO dimer acid ammonium salt for 1 or 2 years (DuPont-18405-1238, 2013). Relative kidney weight did increase in female Crl:CD(SD) rats by 25% and 14% when administered 500 mg/kg/day of HFPO dimer acid ammonium salt for 1 and 2 years, respectively (DuPont-18405-1238, 2013).

These increases in kidney weight were often associated with increases in BUN, which can be used as an indicator of renal damage. In several studies, urea nitrogen levels were significantly increased (16%–38%) in male mice and rats administered doses greater than or equal to 30 mg/kg/day of HFPO dimer acid ammonium salt for 28–180 days (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-24447, 2008; DuPont-24459, 2008). Female rats exhibited an increase in urea nitrogen levels (35%) only when administered 500 mg/kg/day of HFPO dimer acid ammonium salt for 1 year (DuPont-18405-1238, 2013). Kidney damage was equivocal microscopically in the less-than-chronic studies (28–90 days), and typically presented as increases in basophilic tubular cells and tubular epithelial hypertrophy or dilation without tubular degeneration and/or necrosis (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-24459, 2008; DuPont-24447, 2008).

In the chronic study, the increases in BUN and relative kidney weight noted above for female rats were associated with multiple microscopic observations of kidney damage when female rats were treated with HFPO dimer acid ammonium salt for 2 years. For example, at 50 mg/kg/day–500 mg/kg/day, female rats exhibited transitional cell hyperplasia, tubular dilation, pelvic and tubular mineralization, and papillary edema, which ultimately resulted in papillary necrosis at 500 mg/kg/day (DuPont-18405-1238, 2013).

To summarize, significant and dose-dependent increases in relative kidney weight occurred in rats at lower doses (e.g., 10 mg/kg/day) in a subchronic study (DuPont-18405-1307, 2010). Kidney hypertrophy, however, was not associated with microscopic damage of the kidney such as necrosis in this study. Additionally, there are instances in which kidney hypertrophy occurred at low doses in female mice (e.g., 0.1 mg/kg/day (DuPont-24459, 2008) or 5 mg/kg/day (DuPont-18405-1037, 2010)), but there was not a dose response in these datasets, and microscopic damage to the kidney tissues was not reported. Of the available studies, kidney hypertrophy was associated with significant microscopic damage only in female rats treated with 500 mg/kg/day of HFPO dimer acid ammonium salt for 2 years (DuPont-18405-1238, 2013). Thus, the observed kidney effects are potentially of concern. The biological significance,

however, of the observed hypertrophy and increases in BUN without microscopic evidence of kidney damage is not clear.

5.4 Reproductive/Developmental

Evidence in animals suggests HFPO dimer acid and/or ammonium salt could target the reproductive system and the developing fetus.

In a reproduction/developmental toxicity mouse study, there were no effects on mating, fertility, or copulation indices; mean days between pairing and coitus; mean gestation length; mean numbers of implantation sites; mean numbers of pups born; live litter size; percentage of males at birth; postnatal survival; or the general condition of pups (DuPont-18405-1037, 2010). Similarly, implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than control when pregnant mice were dosed with 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E17.5 (Blake et al., 2020). In the rat developmental toxicity study, however, early delivery on GD21 was observed in 18% and 41% of the dams at 100 mg/kg/day and 1,000 mg/kg/day, respectively, and the percentage of male (47%) and female (53%) pups born was significantly altered from control at 1,000 mg/kg/day (DuPont-18405-841, 2010). Conley et al. (2019) reported no significant effects from control on the number of fetuses or resorptions in pregnant rats dosed with 1–500 mg/kg/day HFPO dimer acid from GD14 through GD18. Conley et al. (2021) also reported no significant effects on the numbers of viable fetuses or resorptions in pregnant rats dosed with 1–125 mg/kg/day HFPO dimer acid from GD16 through GD20. However, pup survival was significantly reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day in pups born to dams dosed from GD8 to PND2. Specifically, pup survival percentages on PND2 were 100 ± 0, 96 ± 2, 97 ± 2, 87 ± 5, 38 ± 13, and 5 ± 5% in the 0–250 mg/kg/day groups, respectively.

Changes in maternal GWG were a consistently observed effect. In pregnant rats dosed during gestation and through PND2, maternal GWG significantly decreased 25%–70% compared to control at doses greater than or equal to 125 mg/kg/day of HFPO dimer acid ammonium salt (DuPont-18405-841, 2010; Conley et al., 2019, 2021). Conversely, pregnant mice dosed during gestation saw increases in maternal GWG ranging from 7% to 22% at doses as low as 0.5 mg/kg/day (DuPont-18405-1037, 2010; Blake et al, 2020). It is unclear why this response is different for mice and rats, but in Blake et al. (2020), the study authors hypothesize that it could be the result of differences in the exposure window or interspecies toxicokinetic differences in elimination rates. Specifically, the available rat studies dosed from GD6 through GD20 (DuPont-18405-841, 2010), GD14–GD18 (Conley et al., 2019), GD16-GD20 (Conley et al., 2021), or GD8-PND2 (Conley et al., 2021), while the mice were dosed earlier in gestation (GD1.5–GD17.5) in Blake et al. (2020) and 14 days prior to mating through LD21 in DuPont-18405-1037. Additionally, the elimination $T_{1/2}$ in urine (see section 2.3.5) for female mice (18 hours) is much longer than for female rats (8 hours) and there are also differences in the alpha and beta phase $T_{1/2}$ for female rats and mice (see Table 8).

Blake et al. (2020) presented data indicating that the placenta might be a target of GenX chemical exposure. Placental lesions were detected in 58% and 83% of mouse placentas evaluated after dosing with 2 and 10 mg/kg/day of HFPO dimer acid from E1.5 to E17.5, respectively, compared to 2% in the control group (Blake et al., 2020). The most frequent lesion detected was labyrinth atrophy, which was observed in 0/41 (0%), 15/31 (48%), and 16/35 (46%)

placentas in 0-, 2-, and 10-mg/kg/day-dose groups, respectively. Labyrinth congestion and early fibrin clots increased with increasing HFPO dimer acid doses. These placental lesions are indicative of a placental insufficiency phenotype (Blake et al., 2020). Additionally, placental weights increased in the 10-mg/kg/day-dose group and large placentas are associated with adverse health outcomes in neonates and adult offspring (Hutcheon et al., 2012; Risnes et al., 2009). It is unclear how these effects might impact reproductive and developmental outcomes.

In some of the available developmental studies, there was also a decrease in rodent pup weight that ranged from 9% to 24% when the pups were exposed to 5 mg/kg/day–1,000 mg/kg/day in utero (DuPont-18405-841, 2010; DuPont-18405-1037, 2010; Conley et al., 2019, 2021). The mouse pups showed delays in attaining balanopreputial separation and vaginal patency at 5 mg/kg/day of 2.6 days and 3.4 days, respectively, which could be related to the observed effects on BW during the preweaning period (DuPont-18405-1037, 2010). Additionally, the attainment of vaginal patency did not exhibit a dose-response relationship. The decrease in pup weight was associated with a decrease in gravid uterine weight by 10% and 25% at 100 mg/kg/day and 1,000 mg/kg/day, respectively, in the rat prenatal developmental toxicity study (DuPont-18405-841, 2010). Moreover, in a rat prenatal developmental study, a 14th rudimentary rib developed in 9% of the control fetuses, 10% of fetuses in the 10-mg/kg/day dose, 12% of fetuses in the 100-mg/kg/day dose, and 27% of the fetuses in the 1,000-mg/kg/day dose (DuPont-18405-841, 2010). Statistical analyses were not completed on the development of the 14th rudimentary rib in individual fetuses, but a statistically significant increase in the number of litters developing a 14th rudimentary rib was observed at the high dose. Conley et al. (2019) reported significant effects for the F_1 generation in their postnatal pilot study where F_0 pregnant rats were dosed with 125 mg/kg/day of HFPO dimer acid ammonium salt from GD14 through GD18. F_1 male pups had a decrease in right epididymis weight on a litter mean basis compared to control. Multiple significant effects were observed on an individual pup basis, including AGD and liver weight decreases in female F_1 offspring and paired testes, paired epididymides, right testis, right corpus/caput, right epididymis, left testis, and epididymal adipose tissue decreases in F_1 male mice. Similarly, F_1 male mice in the 5 mg/kg/day-dose group exhibited a decrease of 12% in the relative epididymis weight in a reproduction/developmental toxicity mouse study (DuPont-18405-1037, 2010).

Changes in thyroid hormones, which are important for neurodevelopment, were reported in Conley et al. (2019), Conley et al. (2021) and Blake et al. (2020). In pregnant rats (*n*=3) dosed with 0–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 through GD18 (Conley et al., 2019), maternal serum total T3 levels were decreased at greater than or equal to 30 mg/kg/day and total T4 levels at greater than or equal to 125 mg/kg/day. The decreases in maternal serum total T4 levels compared to control were -50%, -63%, and -76% in the 125-, 250-, and 500-mg/kg/day-dose groups, respectively. The decreases in maternal serum total T3 levels compared to control were -27%, -39%, and -48% in the 30-, 62.5-, and 125-mg/kg/day-dose groups, respectively. Maternal total T3 levels in the 250 and 500 mg/kg/day-dose groups were below the detection limit. Similar findings were reported in Conley et al. (2021) for pregnant rats (*n*=4-5) dosed with 0–250 mg/kg/day of HFPO dimer acid ammonium salt from GD16 through GD20 and GD8 through PND2. Notably, significant decreases in maternal total T4 (-35% for GD16-GD20 and -51% for GD8-PND2) were also observed in the 62.5 mg/kg/day dose groups in Conley et al. (2021). In Blake et al. (2020), placental thyroid hormones (rT3, T3, and T4) were quantified at GD17.5 from 2–3 pooled placental tissues of same-sex embryos. A

significant increase (60%) in T4 relative to control was reported for the 10-mg/kg/day HFPO dimer acid-dose group.

5.5 Immune System

In the one available study specifically addressing immunotoxicity, suppression of TDARs was measured through IgM antibody production in mice (Rushing et al., 2017). IgM antibody production was decreased by 7.3% in female C57BL/6 mice treated with 100 mg/kg/day of HFPO dimer acid. In male mice treated with the same dose of HFPO dimer acid, significant increases in the number of T lymphocytes were observed, but no suppression of TDARs.

In two studies of less-than-chronic duration (28–90 days), decreases in spleen weight relative to BW were observed in female mice and rats (DuPont-18405-1307, 2010; Rushing et al., 2017). For example, in C57BL/6 mice, relative spleen weights significantly decreased by 11% in females treated with 100 mg/kg/day of HFPO dimer acid for 28 days (Rushing et al., 2017).

Changes in early markers of potential immunotoxic effects were observed in multiple studies examining the oral toxicity of HFPO dimer acid and/or ammonium salt. The most prevalent indications were statistically significant decreases from control in serum globulin levels (6%–22%), which resulted in an increase in the serum A/G ratio (7%–58%) from the controls when both sexes of Crl:CD-1 mice and Crl:CD(SD) rats were treated with 1 mg/kg/day–500 mg/kg/day of HFPO dimer acid ammonium salt for 12 months or less (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008). Alterations in the serum levels of globulin can be associated with decreases in antibody production (FDA, 2002). To determine the biological significance of the apparent decrease in globulin production, however, immune function tests (such as TDAR) need to be conducted. Finally, female Crl:CD-1 mice exhibited a 21% and 18% decrease in spleen weight relative to BW when administered 0.5 mg/kg/day and 5 mg/kg/day of HFPO dimer acid ammonium salt for 90 days, respectively (DuPont-18405-1307, 2010). For HFPO dimer acid and/or ammonium salt, there were also two local lymph node assays (LLNAs) conducted in mice that showed equivocal results (DuPont-19897, 2006; DuPont-22616 RV1, 2007).

In summation, the results of the Rushing et al. (2017) TDAR assay in combination with the supportive findings of decreased globulin levels and spleen weight provide evidence that GenX chemicals can induce immune suppression in female mice.

5.6 Cancer

The single cancer bioassay for HFPO dimer acid ammonium salt showed increased incidence of liver tumors (females) and combined pancreatic acinar adenomas and carcinomas (males) in rats at the high doses only. Additionally, a statistically insignificant increase in the incidence of testicular interstitial cell adenoma was noted at the high dose. Although that result 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/day group cannot be ruled out," while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015). Given these uncertainties and the large number of early deaths in the study (see section 4.4), the existing evidence from this single chronic study is considered inadequate to justify a quantitative assessment. Further, the available data for HFPO dimer acid ammonium salt suggest that mice might be more sensitive to exposure to GenX chemicals than

rats. The available study (DuPont-18405-1238, 2013) only evaluated rats; there are no studies measuring cancer endpoints in mice. Given the evidence that the liver is the target organ for toxicity and primary organ for tumor development, the lack of data evaluating cancer in mice is a database deficiency. Thus, under EPA's *Guidelines for Carcinogen Risk Assessment* (EPA, 2005a), there is *Suggestive Evidence of Carcinogenic Potential* of oral exposure to GenX chemicals in humans, based on the female hepatocellular adenomas and hepatocellular carcinomas and male combined pancreatic acinar adenomas and carcinomas. No data are available to evaluate cancer risk via dermal or inhalation exposure.

6.0 Mode of Action

The available data indicate that multiple MOAs could be involved in the liver effects observed after GenX chemical exposure. The available studies provide support for a role for PPARα, cytotoxicity, mitochondrial dysfunction, and PPARγ. The potential MOA(s) for the observed reproductive and developmental effects (e.g., changes in GWG and placental lesions) are unknown. Additionally, no data support identification of a potential carcinogenic MOA for tumors in the pancreas and testes as being related to any of the proposed MOAs for the tumor development in either organ.

For some PFAS (e.g., PFOA), PPARα activation has been proposed as a potential MOA for some of the effects in the liver (i.e., liver tumors) (Klaunig et al., 2003, 2012; Maloney and Waxman, 1999). PPARα is primarily expressed in the liver, but also is present in the kidney, intestines, heart, and brown adipose tissue (Hall et al., 2012). Klaunig et al. (2003) describes the causal key events of the PPARα MOA for liver tumors as activation of PPARα, perturbation of cell proliferation and apoptosis, and selective clonal expansion. There are multiple effects associated with the PPARα MOA such as hepatocellular hypertrophy, peroxisome proliferation, expression of peroxisomal genes, Kupffer cell-mediated events, and increased liver weight. However, these associative effects might not be specific to the PPARα MOA (e.g., hepatocellular hypertrophy) or might not be causal to the development of liver tumors (e.g., peroxisome proliferation) (Klaunig et al., 2003). According to Klaunig et al. (2003), demonstration of PPARα agonism combined with microscopic evidence for peroxisome proliferation or increases in liver weight and one or more of the specific *in vivo* markers of peroxisome proliferation (e.g., induction of acyl-CoA oxidase or cytochrome P450 4A) are sufficient to establish a PPARα MOA.

For HFPO dimer acid and/or ammonium salt, there are data that demonstrate peroxisome proliferation in the liver. Activation of PPARα was measured in multiple 28-day studies in rodents (DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017). Using acyl-CoA oxidase activity as a measure, Rushing et al. (2017) showed increased activity compared to control in male C57BL/6 mice administered 10 mg/kg/day and 100 mg/kg/day of HFPO dimer acid (122% and 222%, respectively) and a 100% increase compared to control in C57BL/6 female mice at 100 mg/kg/day. Notably, there were no significant increases in acyl-CoA oxidase activity at 1 mg/kg/day, indicating that it might be a high dose effect.

The DuPont studies used β-oxidation activity and total cytochrome P450 content as markers of peroxisome proliferation in the livers of rats and mice (DuPont-24447, 2008; DuPont-24459, 2008). In Crl:CD-1 male mice, β-oxidation activity significantly increased compared to control at

doses of 0.1 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day of HFPO dimer acid ammonium salt by 57%, 744%, and 648%, respectively, and total cytochrome P450 content significantly decreased at 3 mg/kg/day and 30 mg/kg/day by 26% and 53%, respectively (DuPont-24459, 2008). β-oxidation activity significantly increased compared to control in female Crl:CD-1 mice at 3 mg/kg/day and 30 mg/kg/day by 495% and 823%, respectively, with no alterations in total cytochrome P450 content (DuPont-24459, 2008). In male Crl:CD(SD) rats, β-oxidation activity was significantly increased relative to control at dosages of 0.3 mg/kg/day, 3 mg/kg/day, and 30 mg/kg/day by 42%, 274%, and 772%, respectively, and total cytochrome P450 content was significantly increased by 23% at 30 mg/kg/day (DuPont-24447, 2008). In female rats dosed with 30 mg/kg/day and 300 mg/kg/day, β-oxidation activity was significantly increased compared to control to 49% and 198%, respectively, while total cytochrome P450 content remained unaltered (DuPont-24447, 2008).

Induction of genes associated with peroxisome proliferation in the liver was also demonstrated (Wang et al., 2017; Conley et al., 2019). Wang et al. (2017) demonstrates significant increases in hepatic mRNA levels of many PPAR targets (e.g., CD36 antigen, acyl-CoA oxidase 1, and cytochrome P450 family members) after administration of 1 mg/kg/day of HFPO dimer acid ammonium salt for 28 days. Relatedly, Conley et al. (2019) found upregulation of gene expression associated with PPARα signaling in maternal and fetal livers following *in vivo* exposure during GD14–GD18.

Additionally, significant increases in liver weight relative to BW were observed in male and female Crl:CD(SD) rats and several strains of male and female mice treated with 0.5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 28–90 days (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2017; NTP, 2019). Increases in liver weight were also reported in the pregnant dams in the available reproductive/developmental studies dosing during gestation (Blake et al., 2020; Conley et al., 2019; DuPont-18405-841, 2010; DuPont 18405-1037, 2010). Additionally, hepatocellular hypertrophy was observed at 0.5 mg/kg/day in both sexes of mice, while male and female rats showed these effects at 3 mg/kg/day and 30 mg/kg/day, respectively, in subchronic studies. Interestingly, in the chronic study, male rats showed only a 10% incidence of hepatocellular hypertrophy with dosing at 50 mg/kg/day for 2 years (DuPont-18405-1238, 2013). Conversely, female rats had significant hepatocellular hypertrophy at 500 mg/kg/day after 1 year (100%) and 2 years (93%).

There is evidence of perturbations to cell proliferation and apoptosis in the liver following short-term and subchronic exposure to HFPO dimer acid ammonium salt, particularly in the high-dose groups. In the 28-day mouse study, increased mitosis was observed in male (9/10) and female (5/10) mice in the high-dose groups only (30 mg/kg/day) and apoptosis was not reported (DuPont-24459, 2008). In the 90-day mouse study, increases in mitotic figures and apoptosis were reported in 7/10 and 10/10 male mice in the high-dose (5 mg/kg/day) group, respectively (NTP, 2019). No mitotic figures were detected in female mice, but an increase in apoptosis was observed in 3/9 mice (NTP, 2019). In the reproductive/developmental mouse study, mitotic figures were observed in 17/24 males and 2/24 females in the 5-mg/kg/day-dose group, but in no other dose groups (NTP, 2019). Similarly, apoptosis was reported in 21/24 males and 10/24 females in the 5-mg/kg/day high-dose group (NTP, 2019). Notably, decreases in the rates of apoptosis are typically observed with PPARα agonists, with Klaunig et al. (2003) describing

decreased rates of apoptosis as a "hallmark of liver growth seen in the early stages of treatment with PPARα agonists." Interestingly, increases in mitoses/mitotic figures and apoptosis are consistently restricted to the high-dose group in all available mouse studies; however, necrosis is observed in both the mid- and high-dose groups. These data suggest that PPARα's role in the observed liver effects may be dose dependent. In the 28-day rat study, mitosis/mitotic figures, hyperplasia, and apoptosis were not reported (DuPont-24447, 2008). In the 90-day rat study, mitosis/mitotic figures, hyperplasia, and apoptosis were not reported (DuPont-17751-1026, 2009). In the chronic rat study, mitotic figures and apoptosis were not reported, and hyperplasia was no different than control in the male and female rats in any dose group (DuPont-18405-1238, 2013). It is possible that the rat studies might not have separated apoptotic lesions from other liver lesions reported (i.e., single-cell necrosis) since these studies were conducted prior to the guidelines outlined in Elmore et al. (2016) and were not reanalyzed by the NTP PWG.

Although there is evidence for a PPARα MOA in the liver, particularly in the high-dose groups in the available studies, data indicate that liver toxicity extends beyond a single PPARα-based MOA. For example, liver necrosis was consistently observed in rodent toxicity studies with HFPO dimer acid ammonium salt and was reaffirmed by the NTP PWG's review of the 90-day subchronic study in mice and the reproductive and developmental toxicity study in mice (appendix D), which suggests that cytotoxicity is also a possible MOA. Felter et al. (2018) identified the following key events for establishing a cytotoxicity MOA:

1.) The chemical is not DNA reactive.
2.) Clear evidence of cytotoxicity by histopathology such as the presence of necrosis and/or increased apoptosis.
3.) Evidence of toxicity by increased serum enzymes that are relevant to humans.
4.) Presence of increased cell proliferation as evidenced by increased labeling index and/or increased number of hepatocytes.
5.) Demonstration of a parallel dose response for cytotoxicity and formation of tumors.
6.) Reversibility (ideally).

The available data for HFPO dimer acid support cytotoxicity as a potential MOA. For example, HFPO dimer acid does not appear to be DNA reactive *in vivo* (see section 4.6.3 and appendix C). It did not induce chromosomal mutations in the form of structural aberrations, numerical aberrations, or micronuclei or DNA effects in the form of unscheduled DNA synthesis (DuPont-23219, 2007; DuPont-23220, 2007). Secondly, clear evidence of cytotoxicity in the form of increased liver necrosis and apoptosis was confirmed microscopically in male and female mice and rats in several less-than-chronic studies (15–90 day) and one 2-year chronic study (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; Wang et al., 2017; NTP, 2019). There is also evidence of increased serum liver enzymes. Hall et al. (2012) identifies significant increases in ALT/AST, ALP, and bilirubin/bile acids as potentially clinically relevant. Additionally, other enzymes such as SDH might reflect alterations in liver function (Hall et al., 2012). For HFPO dimer acid, significant increases in ALT (420%–1,254%), AST (106%–478%), ALP (1,134%–1,221%), and SDH (1,134%–1,221%) were observed in male mice administered the ammonium salt at 5–30 mg/kg/day for 28–90 days. Female mice had smaller increases in ALP (140%–143%) and SDH (32%–186%) as compared to male mice administered the same dose over the same duration. Overall, rats exhibited far fewer and smaller increases in serum liver enzyme levels following subchronic exposure compared to the mouse,

with increases in AST (106%) and ALP (52%) at 100 mg/kg/day in male rats and AST (66%) in female rats at 1,000 mg/kg/day. In the chronic study, however, ALT (228%), ALP (180%), and SDH (140%) significantly increased in male rats only when administered 50 mg/kg/day for 1 year. Typically, an increase in bilirubin, when accompanied with increased bile acids, is a reliable index of liver toxicity (Hall et al., 2012). For HFPO dimer acid, however, a decrease in serum bilirubin is a consistent effect observed across multiple studies, especially in female rodents (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013; DuPont-18405-1307, 2010; Wang et al., 2017).

Data gaps exist for the other key events related to a cytotoxic MOA. Studies investigating if exposure to HFPO dimer acid result in increased labeling index and/or increased number of hepatocytes are unavailable. A 2-year chronic study in rats reported centrilobular and single cell necrosis in females in the 500-mg/kg/day high-dose group only (DuPont-18405-1238, 2013). Additionally, treatment-related liver tumors were also observed in the 500-mg/kg/day dose group (0/70 in control versus 11/70 in 500 mg/kg/day), which suggests a parallel response for cytotoxicity and formation of tumors. However, these effects were observed only in the high-dose group and dose selection in this study resulted in a large gap between the mid-dose (50 mg/kg/day) and high-dose (500 mg/kg/day). Therefore, the potential for a parallel dose response is unclear. Additionally, while liver necrosis exhibits a dose response in the 84/85 day modified reproductive developmental study (DuPont-18405-1037, 2010; NTP, 2019), there are no chronic studies in the mouse to determine if liver tumors form. The available data indicate that the mouse is the more sensitive to the liver effects resulting from HFPO dimer acid exposure.

Additionally, Blake et al. (2020) reports an increase in subcellular organelles consistent with peroxisomes and mitochondria in pregnant dam livers exposed to 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E11.5 or E17.5 using TEM. This increase in mitochondria is not typical of PPARα activation and suggests an alternate MOA such as mitochondrial alteration could also be operative for the liver effects resulting from exposure to HFPO dimer acid and/or ammonium salt. Further supporting this alternate MOA, a number of genes upregulated in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18 are specific to mitochondrial beta oxidation (*Cpt1a*, *Cpt1b*, *Cpt2*, *Acaa2*, *Acadl*, *Acadm*), mitochondrial ketogenesis (*Hmgcs2*), and mitochondrial electron transfer (*Etfdh*) (Conley et al., 2019).

Finally, a study of HFPO dimer acid in HEK293 embryonal kidney cells found activation of genes associated with the PPARγ signaling pathway (Li et al., 2019). Further supporting a role for the PPARγ signaling pathway, Conley et al. (2019) reports upregulation of genes in maternal and fetal livers exposed to 1–500 mg/kg/day of HFPO dimer acid ammonium salt from GD14 to GD18, which are associated with PPARγ signaling, including *Pck1*, *Aqp7*, and *Gk.* Additionally, Rosen et al. (2017) concluded that 11%–24% of the PFAS-induced increase in transcriptional activity is PPARα independent, depending on the PFAS. This study identified 67 genes that were similarly upregulated in wild type (129S1/Sv1mJ) and PPARα-null (129S4/SvJae-*Ppara*^{tm1Gonz}/J) mouse livers exposed to either 3 or 10 mg/kg/day of PFHxS, 1 or 3 mg/kg/day of perfluorononanoic acid, 3 mg/kg/day of PFOA, or 10 mg/kg/day PFOS for 7 days, indicative of PPARα independence. The authors note that genes typically associated with the activation of PPARα such as *Acox1* were similarly upregulated in wild type and PPARα-null mice livers, suggesting that these genes might not be specific indicators of PPARα activation. Interestingly,

Conley et al. (2019) found that five of the 67 genes identified as PPARα independent in the Rosen et al. (2017) study are also significantly upregulated in the liver of pregnant rats and their fetuses exposed to HFPO dimer acid at doses greater than or equal to 1 mg/kg/day (i.e., *Ehhadh*, *Slc22a5*, *Ech1*, *Cpt2*, and *Acox1*). *Slc22a5* and *Cpt2* are associated with mitochondrial fatty acid oxidation.

Taken together, the available data indicate that a PPARα MOA is plausible in the liver in response to GenX chemical exposure, especially at doses greater than 0.5 mg/kg/day; however, there are not yet enough data to conclude that PPARα 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 PPARα-null mice. It is worth noting that exposure to PFOA has been demonstrated to induce liver effects in PPARα-null mice, including hepatocellular hypertrophy (Minata et al., 2010). Additionally, available studies indicate that other MOAs (e.g., PPARγ, mitochondrial dysfunction, and cytotoxicity) are also plausible. The data are not adequate to conclude that any of the MOAs described here are the sole toxicologic MOA for HFPO dimer acid and/or ammonium salt in the liver and especially in other organ systems. For example, the potential MOA(s) for the observed reproductive and developmental effects (e.g., changes in GWG, placental lesions, reduced pup body weight, and reduced pup survival) are unknown, though Conley et al. (2021) provides mechanistic evidence that dysregulation of carbohydrate and lipid metabolism in the mother and developing offspring may be contributing to some of these effects. Of note, glycogen accumulation scores in pup livers were significantly lower compared to control in pups exposed to doses as low as 10 mg/kg/day of HFPO dimer acid ammonium salt from GD8-PND2. Additionally, no data support identification of a potential carcinogenic MOA for tumors in the pancreas or testes as being related to PPARα or any of the proposed alternative MOAs for the tumor development in either organ.

7.0 Dose-Response Assessment

7.1 Identification of Studies and Effects for Dose-Response Analysis

Several studies were evaluated further for identification of specific endpoints to carry forward for dose-response (BMD) modeling. EPA evaluated studies based on identification of adverse effects, duration of exposure, use of a control and two or more doses, and provision of NOAEL and/or LOAEL values. Data from available studies indicate that the liver is the most sensitive target of toxicity from exposure to GenX chemicals. Liver effects were observed in both male and female mice and rats at varying durations of exposures and doses. These effects occurred at the lowest doses and shortest durations of exposure to GenX chemicals.

Because liver effects such as increases in liver weight and hepatocellular hypertrophy (also referenced here as cytoplasmic alteration per NTP PWG's review) can be associated with activation of cellular PPARα receptors, EPA evaluated observed liver effects resulting from HFPO dimer acid ammonium salt exposure against the Hall criteria (Hall et al., 2012). These criteria indicate that increased liver weight and hepatocellular hypertrophy must be accompanied by histologic or clinical pathology indicative of liver toxicity to be considered adverse. Histologic or clinical pathology indicative of liver toxicity can include changes in liver enzyme concentrations in the serum, necrosis, inflammation, and degeneration. With these criteria in mind, EPA concluded that some of the observed liver effects such as single-cell and focal

necrosis, increased apoptosis, and increases in serum liver enzymes indicate toxicity of relevance to humans as opposed to PPARα-induced cell proliferation unique to rodents.

For the GenX chemicals database, many studies identified the mouse as the most sensitive species and the liver as a target organ for toxicity. Liver effects at low doses (e.g., less than or equal to 5 mg/kg/day) were identified in the 28 day oral (gavage) toxicity study in mice (DuPont-24459, 2008), the 90 day oral (gavage) toxicity study in mice (DuPont-18405-1307, 2010), and the oral (gavage) reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010). In these studies, increases in relative liver weight were accompanied by increases in hepatocellular hypertrophy, single-cell/focal necrosis and apoptosis.

EPA requested that NIEHS, NTP convene a PWG to provide independent, expert review of the liver tissues from the oral (gavage) reproduction/ developmental toxicity study in mice (DuPont-18405-1037, 2010) and the 90 day oral (gavage) toxicity study in mice (DuPont-18405-1307, 2010). Given the availability of longer duration studies demonstrating effects at low doses, the 28-day study in mice was not included in this review. The NTP PWG classified cell death according to the INHAND Organ Working Group's diagnostic criteria that describes how pathologists can distinguish between apoptosis and single-cell necrosis in standard H&E-stained tissue sections (Elmore et al., 2016). These criteria were unavailable at the time the DuPont studies were conducted and submitted to EPA.

The liver effects noted in the 28 day oral (gavage) toxicity study in mice (DuPont-24459, 2008) were not considered as a potential POD in support of the derivation of the RfD. The 28 day study did not use a dose range optimized for the identification of low-dose effects compared to the 90 day and reproduction/developmental toxicity studies (0, 0.1, 3, and 30 mg/kg/day-dose groups in the 28 day study versus 0, 0.1, 0.5 and 5 mg/kg/day in the 90 day and reproduction/developmental studies). For example, in DuPont-18405-1037 (2010), the LOAEL (i.e., the lowest dose at which an adverse effect is observed) of 0.5 mg/kg/day falls between the low and mid-doses of the dosing design used in DuPont-24459 (2008). Additionally, as described above, this short-term study was not reviewed by the NTP PWG because there were two longer duration studies in the most sensitive species.

The liver effects noted in the 90 day and reproduction/developmental toxicity studies (DuPont-18405-1307, 2010 and DuPont-18405-1037, 2010) were considered for determination of PODs in support of the derivation of RfDs. The NTP PWG concluded, that the dose response and constellation of lesions (i.e., cytoplasmic alteration (including hepatocellular hypertrophy), single-cell necrosis, focal necrosis, and apoptosis), rather than each lesion individually, represent adversity in these studies (appendix D). EPA interpreted the NTP PWG's definition that the constellation of liver lesions is adverse to apply to the dose group level, as opposed to individual animal level, given that the histopathology assessment represents a snapshot in time of a biological process within one portion of the liver that can vary across animals. Therefore, if multiple liver lesion types and progression of adverse liver effects (e.g., necrosis or apoptosis) were observed within a dose group, all animals in that dose group were included in the dose-response modeling. The constellation of liver lesions in the reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010) was selected for BMD modeling based on the incidence data as reported by the NTP PWG. Multiple liver lesions, including cytoplasmic alteration, single-cell, and focal necrosis, exhibited a dose response in both male and female mice in this study. These effects were observed at doses as low as 0.5 mg/kg/day. A constellation

of liver lesions observed in the 90-day toxicity study in mice (DuPont-18405-1307, 2010) were observed at higher doses (5 mg/kg/day) than in the reproduction/developmental toxicity study in mice (DuPont-18405-1037, 2010). The NTP PWG reported that 10 out of 10 male mice exhibited cytoplasmic alteration, compared to 0 in control at the 0.5-mg/kg/day dose in the 90-day toxicity study in mice (DuPont-18405-1307, 2010). Although NTP classified cytoplasmic alteration as part of the constellation of liver lesions considered adverse, no other liver lesions indicative of liver damage (i.e., single-cell or focal necrosis or apoptosis) were observed at the 0.5-mg/kg/day dose group in males. Consistent with the Hall criteria, EPA did not consider the cytoplasmic alteration findings alone as an adverse effect in the 0.5 mg/kg/day dose group but considered the constellation of liver lesions observed across the male mice in the high-dose group as adverse. Additionally, the female mice in this study did not exhibit a dose response for the constellation of liver lesions. For these reasons, the constellation of liver lesions observed in the 90-day toxicity study in mice were not selected for BMD modeling.

Additionally, the chronic rat 2-year cancer bioassay (DuPont-18405-1238, 2013) was not selected for the derivation of candidate RfDs for several reasons. Across all dosing groups in both male and female rats, just 25.4% of the test animals survived to their planned terminal necropsy with most of the animals experiencing unscheduled death/moribundity prior to the scheduled study termination at 104 weeks. Effects observed at low doses in this study include changes in serum albumin levels and the A/G ratio in male rats. For males, an increase in A/G ratio at 1 mg/kg/day at the 3-month time point and increases in both albumin and A/G ratio at the 12-month time point were observed, but these changes were not seen at 6 months. These changes, while indicative of an immune system effect, were deemed of unclear biological significance especially given these temporal inconsistencies. For these reasons, the changes in albumin and A/G ratio observed in DuPont-18405-1238 (2013) were not considered for determination of PODs in support of the derivation of the RfD. Liver effects were also observed in this study but did not occur at comparable doses to the oral reproductive/developmental toxicity study in mice. Also, the available chronic study evaluated only rats, and the data indicate that mice appear to be more sensitive. For example, mice presented with single-cell necrosis in doses as low as 0.5 mg/kg/day, with a large increase in response at 5 mg/kg/day in the oral reproductive/developmental toxicity study in mice (DuPont-18405-1037, 2010; NTP, 2019). Female mice also had a large increase in incidence compared to control at 5 mg/kg/day for both focal/multifocal and single-cell necrosis (DuPont-18405-1037, 2010; NTP, 2019). Conversely, the study authors did not report subchronic hepatocellular necrosis in the 90-day study of male and female rats. (DuPont-17751-1026, 2009). Hepatocellular necrosis is observed in the 2-year chronic rat study, but at higher doses (50 mg/kg/day for male rats and 500 mg/kg/day for female rats) as compared to the developmental/reproductive mouse study (0.5 mg/kg/day for male and female mice) (DuPont-18405-1238, 2013; NTP, 2019 reread of DuPont-18405-1037, 2010). While a chronic study is typically the preferred duration for development of lifetime RfD, in this case, the oral reproductive/developmental toxicity study indicates that adverse effects in the liver are observed in the parental mice at lower doses than those reported in the chronic study in rats. For these reasons, the adverse liver effects observed in DuPont-18405-1238 (2013) were not selected for determination of PODs in support of the derivation of the RfD.

Adverse health outcomes resulting from exposure from HFPO dimer acid or its ammonium salt are not limited to the liver. Studies in both rats and mice indicate that exposure to GenX chemicals during pregnancy and gestation results in adverse effects at low doses. Specifically,

Blake et al. (2020) determined that 58% and 83% of placentas evaluated at E17.5 were classified as abnormal in the 2- and 10-mg/kg/day HFPO dimer acid dose groups, respectively, with the number of abnormal placentas in the 10-mg/kg/day HFPO dimer acid dose group reaching statistical significance. Different placental lesions were recorded in the study, including labyrinth atrophy, labyrinth congestion, labyrinth necrosis, early fibrin clot, and the presence of placental nodules. Placental lesions were also evaluated against the proportion of placentas within a litter that were within normal limits to account for litter effects. The proportion of abnormal placentas was significantly higher at the 2- and 10-mg/kg/day dose groups relative to vehicle 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 LOAELs observed for liver effects (0.5 mg/kg/day). It is possible that the placental lesions occur at lower doses, especially given that 58% of placentas were classified as abnormal at the lowest dose tested, but these data are lacking. While the placental lesions observed are considered adverse, additional research is needed to understand if they would be seen at lower doses. Additionally, further research should evaluate the impact of GenX chemicals-induced placental lesions on development after gestation, including latent health outcomes. Blake et al. (2020) reported that these lesions did not impact some measured reproductive and developmental outcomes such as implantation sites, viable embryos, nonviable embryos, and resorptions. Because, however, a full two-generation reproductive toxicity study is not available for mice, the impact of placental lesions on development after gestation, including latent health outcomes, is unclear.

An increase in maternal GWG ranging from 13 to 22% was reported by DuPont-18405-1037 (2010) at doses as low as 0.5 mg/kg/day. Similarly, an increase in maternal GWG in mice at E17.5 at doses greater than or equal to 2 mg/kg/day (i.e., the lowest tested dose) was also reported by Blake et al. (2020) using a mixed-effect modeling approach that adjusts for repeated measures of relative maternal GWG, litter size, and embryonic day. Furthermore, Conley et al. (2019) evaluated maternal GWG in rats and observed a *decrease* in GWG following exposure to dosing greater or less than 250 mg/kg/day of HFPO dimer acid. A decrease in maternal GWG in rats was also reported in DuPont-18405-841 (2010), which suggests that the shift in maternal GWG might be species specific. Given the lack of mechanistic clarity for the maternal GWG endpoints in two similar species, the endpoint was not considered for determination of PODs in support of the RfD derivation. According to Blake et al. (2020), the inconsistency in maternal GWG response between rats (Conley et al., 2019; DuPont-18405-841, 2010) and mice (Blake et al., 2020; DuPont-18405-1037, 2010) might be due to differing statistical methods, interspecies elimination rates, and/or developmental exposure windows. 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 not selected for determination of PODs in support of the RfD derivation.

Immune and hematological effects were also observed at low doses; however, these endpoints are not as consistently observed as the liver effects. Additionally, there is some uncertainty regarding the biological significance of both the hematological and immune endpoints. For example, the observed changes in albumin and A/G ratio at dosing of 3 mg/kg/day (DuPont-24447, 2008; DuPont-24459, 2008) are considered early markers of potential immunotoxic effects. Evaluation of additional immune function assays, histopathology, and immune endpoints such as antibody levels, however, are not available. Currently little or no data exist on the

potential for GenX chemicals to impact aspects of immune function beyond the immunosuppression (e.g., allergic responses and autoimmunity). Furthermore, while considered adverse, the hematological effects were inconsistently observed, especially as study duration increased. For example, the hematological effects observed in the 28-day mouse study at 3 mg/kg/day were not observed in the 90-day subchronic study in mice, except for a 3% decrease in hemoglobin concentration at 5 mg/kg/day. No hematological changes were observed at the 0.1- or 0.5-mg/kg/day dose in the subchronic mouse study (DuPont-18405-1307, 2010). Likewise, the hematological effects observed in the subchronic rat study at low doses are not observed in the chronic rat study (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013). Specifically, decreases in hemoglobin, hematocrit, and RBC count that are observed at 10 mg/kg/day in the subchronic study are not observed after 12 months of dosing, which adds additional uncertainty to the significance of these effects (DuPont-18405-1238, 2013). For these reasons, hematological and immune endpoints from these studies were not considered further for determination of PODs in support of the derivation of the RfD.

7.2 Methods of Analysis

7.2.1 BMD Modeling

There are no biologically based dose-response (BBDR) models available for HFPO dimer acid and its ammonium salt. Thus, using the most current version of its Benchmark Dose Software at the time data were modeled, EPA evaluated a range of dose-response models thought to be consistent with underlying biological processes to determine how best to empirically model the dose-response relationship in the range of observed data (appendix E).

Consistent with EPA's *Benchmark Dose Technical Guidance* (EPA, 2012), the BMD and the 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 of comparison across endpoints, studies, and assessments. Using the pathology analysis from the NTP PWG, candidate PODs were estimated from all three doses (plus control) for DuPont-18405-1037 (2010) (Table 13).

Further details, including the BMD modeling output and graphical results for the selected models, are provided in appendix E of this assessment.

7.2.2 Dosimetric Adjustment of the Experimental Animal-Based POD to POD_{HED}

EPA guidance was followed to calculate a candidate POD_{HED} from the animal-based POD using a $BW^{3/4}$ allometric scaling approach (EPA, 2011b), which is derived from the relationship between body surface area and basal metabolic rate in adults. With infants and children, surface area and basal metabolic rates are very different than for adults with a slower metabolic rate. While this $BW^{3/4}$ allometric scaling is not appropriate for infants and children because of the limited toxicokinetic data available, the critical effect of liver single-cell necrosis observed in adult mice is not a developmental endpoint nor is it specific to early life stages. However, the exposure for the parental females in DuPont-18405-1037 (2010) took place during pregnancy. EPA indicates that:

> …exposure and internal dosimetry of pregnant, nursing, and growing animals may vary compared to adult animals, so use of the administered dose for toxicity studies involving

these periods is associated with relatively greater uncertainty, absent life stage-specific information (EPA, 2011b).

In this case, however, $BW^{3/4}$ allometric scaling relied on life stage-specific BW data from the pregnant or lactating dams as appropriate. The HFPO dimer acid ammonium salt POD_{HED}s from the experimental animal studies (DuPont-18405-1037, 2010) were adjusted via the dosimetric adjustment factor (DAF) equation below:

$$DAF = (BW_a^{1/4}/BW_h^{1/4}),$$

where:

- BW_a = animal BW
- BW_h = human BW

For the chronic reproductive/developmental toxicity study (DuPont-18405-1037, 2010), a BW_a value of 0.0372 kg was identified as the mean BW of the F_0 male mouse controls on study day 84 (the final day of animal dosing). The mean BW_a for the F_0 females in this study was 0.0349 kg taken from the controls upon sacrifice on LD21.

A BW_h of 80 kg for humans was selected based on National Health and Nutrition Examination Survey (NHANES) sampling data (EPA, 2011a). For adults more than 21 years of age, EPA updated the default BW assumption from 70 kg to 80 kg based on NHANES data from 1999 to 2006 as reported in Table 8.1 of EPA's *Exposure Factors Handbook* (EPA, 2011a). The updated BW represents the mean weight for adults ages 21 and older. The resulting DAF for the allometric scaling of doses from male mice to humans is 0.15 for DuPont-18405-1037 (2010). For the female mice, the DAF is 0.14 for DuPont-18405-1037 (2010). Applying the DAF to the identified PODs identified for liver effects in adult mice yields a POD_{HED} as follows:

$$POD_{HED} = \text{POD animal dose (mg/kg/day)} \times DAF$$

Table 13. Summary of Determination of POD_{HED}

Endpoint and reference	Species/ Sex	Model	BMR	BMD_{10} (mg/kg/day)	POD (mg/kg/day)	POD Type	DAF	POD_{HED}[a] (mg/kg/day)
HEPATIC								
Constellation of liver lesions in parental males (DuPont-18405-1037, 2010)[b]	Crl:CD1(ICR) mice F_0 parental male	Benchmark dose (ver. 3.1.2) Probit	10%	0.19	0.14	$BMDL_{10}$	0.15	0.02
Constellation of liver lesions in parental females (DuPont-18405-1037, 2010)[b]	Crl:CD1(ICR) mice F_0 parental female	Benchmark dose (ver. 3.1.2) Probit	10%	0.12	0.09	$BMDL_{10}$	0.14	0.01

Notes: N/A = not applicable.

[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).

[b]Calculations for DuPont 18405-1037 (2010) rely on pathology conclusions of the NTP PWG (Appendix D)

7.3 Derivation of Candidate RfD Values

To calculate the candidate RfD values, EPA applied UFs to the POD_{HED}s from the oral reproduction/developmental toxicity study in mice as described in this section. UFs were applied according to guidance in EPA's *Review of the Reference Dose and Reference Concentration Processes* (EPA, 2002).

An interspecies uncertainty factor (UF_A) of 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to account for uncertainty in extrapolating from laboratory animals to humans. The UF_A is generally presumed to include both toxicokinetic (i.e., absorption, distribution, metabolism, and elimination) and toxicodynamic (i.e., MOA) aspects. A POD_{HED} was derived from the BMDL using EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (EPA, 2011b). This guidance describes approaches for deriving POD_{HED}s from data from laboratory animals, with the preferred approach being PBPK modeling. For HFPO dimer acid and ammonium salt, no PBPK models have been developed or published. Other approaches described by the guidance include the use of chemical-specific data to inform the derivation of human equivalent oral exposures. In the absence of either PBPK models or chemical-specific information, a BW scaling to the ¾ power approach is applied to extrapolate toxicologically equivalent doses of orally administered agents from adult laboratory animals to adult humans. Although this scaling addresses most aspects of cross-species extrapolation of toxicokinetic processes, there is some residual uncertainty for toxicokinetics and uncertainty around toxicodynamic processes (EPA, 2011b). Thus, in the absence of chemical-specific data to quantify this uncertainty, a UF of 3 was applied.

An intraspecies uncertainty factor (UF_H) of 10 is applied to account for variability in the responses within the human populations because of both intrinsic (toxicokinetic, toxicodynamic, genetic, life stage, and health status) and extrinsic (lifestyle) factors that can influence the response to dose. No information to support a UF_H other than 10 was available to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics.

A LOAEL-to-NOAEL extrapolation uncertainty factor (UF_L) of 1 is applied because a BMDL is used as the basis for the POD_{HED} derivation. When the POD type is a BMDL, the current approach is to address this factor as one of the considerations in selecting a BMR for BMD modeling. In this case, the BMR of a 10% change for the modeled liver endpoints was selected under the assumption that it represents a minimal, but biologically significant, change for these effects.

A UF for extrapolation from a subchronic to a chronic exposure duration (UF_S) of 10 was applied for the derivation of the chronic RfD, but not of the subchronic RfD. The reproduction/ developmental study (DuPont-18405-1037, 2010) considered for dose-response analysis is shorter than the duration of a chronic study. Chronic studies typically employ repeated dosing for longer than 90 days or for more than 10% of the human life span (EPA, 2002). In DuPont-18405-1037 (2010), F_0 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, F_0 males in this study were dosed 70 days prior to mating and throughout mating through 1 day prior to scheduled termination, for a total of 84 to 85 days of exposure.

Because a 2-year chronic mouse study is unavailable and since female mice were dosed well below the 90-day exposure window typically employed in a subchronic study, the impact of a longer dosing duration on both the incidence and severity of liver effects in mice is unknown. This is important because duration of exposure appears to play a role in the progression and severity of liver effects resulting from GenX chemical exposure, as evidenced in female rats. Specifically, necrosis in female rats was not reported in the 28- or 90-day rat studies or the interim 1-year time point in the 2-year chronic rat study, which dosed the rats from 3 to 1,000 mg/kg/day. However, at the completion of the 2-year chronic rat study, centrilobular and single-cell necrosis are reported in the 500-mg/kg/day-dose group. Moreover, treatment-related liver tumors were observed in the 500-mg/kg/day rat dose group (0/70 in control versus 11/70 in the 500-mg/kg/day group). These data demonstrate progression of liver effects over the 2-year dosing period. Additionally, Blake et al. (2020) did not find clear evidence of changes in maternal liver serum enzymes (i.e., ALP, ALT or AST) or increases in liver necrosis as compared to control after 10-16 days of dosing at 2 mg/kg/day. Similarly, DuPont-24459 (2008) did not report single cell necrosis in female mice treated with 0.1 or 3 mg/kg/day after 28 days of dosing, though 4/10 mice displayed single cell necrosis in the 30 mg/kg/day dose group. However, DuPont-18405-1037 (2010) found liver necrosis in mice after 53-85 days of dosing at 0.5 mg/kg/day, indicating progression of liver effects as the duration of dosing increases. Because the mouse presents with liver necrosis at much lower doses and shorter durations (0.5 mg/kg/day at 53–85 days) than the rat and because the mode of action for these liver effects is uncertain (see section 6), it is critical to have a 2-year chronic study in the mouse to understand the progression of these liver effects. Specifically, a longer duration study would likely result in an increased frequency and/or magnitude of response and could also reveal additional adverse effects at lower doses than currently observed in the existing less-than-chronic mouse studies (DuPont-24459, 2008; DuPont-18405-1307, 2010; DuPont-18405-1037, 2010). For these reasons, EPA applied a UF of 10 to account for duration of exposure for the chronic RfD. For the subchronic RfD, a UF was not applied to account for duration as the study is of subchronic duration.

A database uncertainty factor (UF_D) of 10 was applied to account for database deficiencies. The database uncertainty factor is applied to account for a potentially lower reference value as a result of an incomplete characterization of a chemical's toxicity (EPA, 2002). The GenX chemicals database contains a number of toxicological studies including acute studies in both mice and rats, subchronic studies in mice and rats, a chronic study in rats, a one generation reproductive and developmental study in mice and gestational reproductive and developmental toxicity studies in mice and rats, as well as a single immunotoxicity study; however, when evaluating the available endpoints and studies to ensure comprehensive characterization of the potential toxicity, there are important deficiencies that need to be considered, particularly for understanding developmental toxicity. If data from the available toxicology studies raise suspicions of developmental toxicity and signal the need for developmental data on specific organ systems (e.g., detailed nervous system, immune system, carcinogenesis, or endocrine system), then the database factor should take into account whether or not these data are available and used in the assessment (EPA, 2002). For GenX chemicals, there are reproductive or developmental effects of concern in mice occurring at similar dose levels to the liver effects (changes in maternal GWG and placental lesions indicative of placental insufficiency) or ongoing research related to these and other endpoints or effects that have not been studied yet

(skeletal ossification, mammary gland development, altered metabolism in offspring, changes in thyroid hormones in the mouse).

For example, increases in maternal gestational weight gain and placental lesions were observed in mice at doses similar to the observed liver effects. In DuPont-18405-1037 (2010) mean maternal GWG, calculated from individual differences, significantly increased over the gestational period (0–18 days) by 18% and 22% in the 0.5- and 5-mg/kg/day-dose groups, respectively. The NOAEL for this effect would be 0.1 mg/kg/day which is the same as the liver effects. Additionally, Blake et al., 2020 found that maternal GWG was significantly increased compared to vehicle control at 2 mg/kg/day and 10 mg/kg/day at gestational day 17.5 using effect estimates from mixed effect models adjusting for repeated measures of relative GWG, litter size, and embryonic day. This is a consistent effect observed in two studies conducted by two different groups. Conley et al. (2019, 2021) also evaluated GWG in rats and observed a decrease following exposure to dosing greater than 125 mg/kg/day of HFPO dimer acid. A decrease in GWG in rats was also reported in DuPont-18405-841 (2010), which suggests that the shift in GWG might be species specific. Blake et al. (2020) also suggested that differing statistical methods, interspecies elimination rates, and exposure windows could explain these disparate results. In humans, altered GWG has been shown to adversely impact both mothers and infants. Effects including pregnancy-induced hypertension, gestational diabetes, postpartum weight retention, difficulty breast feeding, increased risk of stillbirth and infant mortality, and preterm birth have been associated with increased GWG (Rasmussen and Yaktine, 2009).

Secondly, Blake et al. (2020) reports a statistically significant increase (58%) in placental lesions over control at 2 mg/kg/day, the lowest dose used in this study. The placenta is critical to the transfer of nutrients, oxygen, and waste between mother and baby. Because of its role in maintaining pregnancy and programming latent health outcomes it is a relevant endpoint to evaluate maternal and embryo health. Placental insufficiency, as evidenced by effects such as those observed by Blake can result in reduced transfer of vital oxygen and nutrients. Additionally, deficiencies in placental development or function can result in hypertensive disorders of pregnancy which increases the risk of post-pregnancy hypertension, heart disease, and stroke in affected women, as well as increased risk for adverse cardiometabolic outcomes in adult offspring (Pinheiro et al., 2016). EPA notes that it is unclear how the placental lesions might impact reproductive and developmental outcomes. For example, implantation sites, viable embryos, nonviable embryos, and resorptions were not significantly different than control in Blake et al. (2020). Because, however, a full two-generation reproductive toxicity study is not available for mice, the impact of placental lesions on development after gestation or latent effects resulting from a placental insufficiency phenotype are unclear. Notably, Blake et al. (2020) also reported placental lesions for PFOA and, studies in humans have shown associations between PFOA exposure and health outcomes resulting from placental insufficiency such as pregnancy-induced hypertension or preeclampsia (EPA, 2016a).

As mentioned above, other database deficiencies include the absence of a full two-generation reproductive and developmental toxicity study to understand if latent effects occur as a result of exposure to GenX chemicals during development (e.g., adverse cardiometabolic outcomes in adult offspring associated with placental insufficiency). Additionally, Conley et al. (2021) reported that survival of pups born to dams dosed from GD8-PND2 was significantly reduced on PND1 and PND2 at doses ≥62.5 mg/kg/day. Pup body weight gain (birth to PND2) and PND2

body weight in the surviving pups were both reduced at doses ≥30 mg/kg/day. These effects were attributed to the hypoglycemia and elevated serum lipid levels newborns displayed, consistent with impaired fetal glycogen storage. Metabolic disturbance during fetal development is likely to lead to long-term negative metabolic outcomes in the offspring. These effects are among the most sensitive effects observed in the rat resulting from exposure to GenX chemicals and highlight the importance of having a full two-generation reproductive and developmental toxicity study.

Additionally, the evaluation of particular developmental endpoints during early organogenesis (i.e., GD0 to GD6) such as delayed skeletal ossification and mammary gland development in the mouse that have been observed following exposure to other PFAS like PFOA (EPA, 2016a,b) are lacking. For PFOA, the LOAEL for mammary gland developmental effects was 0.01 mg/kg/day, with no study NOAEL. There are no published studies looking at mammary gland development for GenX chemicals at this time. Similarly, studies that evaluate skeletal ossification in the more sensitive species, mice, do not exist for GenX chemicals. The LOAEL for reduced skeletal ossification was 1 mg/kg/day for PFOA (no study NOAEL) and studies looking at lower dose ranges were not available. These studies are especially important considering that Blake et al. (2020) demonstrated accumulation of HFPO dimer acid in whole mouse embryos from E1.5 to E11.5 to E1.5 to E17.5. The lack of studies evaluating these endpoints at or below doses included in the critical study identifies this as a significant gap in the understanding of the developmental toxicity of GenX chemicals.

In addition to the gaps in the database concerning reproductive and developmental toxicity, other database gaps are noted for GenX chemicals with respect to potential immune, hematological and neurological effects, which are outlined below. Additionally, there are no human toxicity data from epidemiological studies in the general population or worker cohorts evaluating the health effects of exposure to these GenX chemicals.

The immunotoxicity of GenX chemicals has not yet been fully elucidated. PFAS chemicals, including PFOS and PFOA, interact with the immune system in studies of both humans and animals (NTP, 2016; EPA, 2016a,b). The GenX chemical immunotoxicity database is less robust than PFOA and PFOS, but does include two LLNAs (DuPont-19897, 2006; DuPont-22616 RV1, 2007) and a 28-day immunotoxicity study (Rushing et al., 2017). Rushing et al. (2017) identified suppression of TDAR by a reduction in antigen-specific IgM antibody production in females and increased T cell numbers in males at the high dose only (100 mg/kg/day). The LLNA is typically used to identify potential skin-sensitizing chemicals through their ability to induce allergic immune response (OECD, 2010b). The LLNAs were conducted with HFPO dimer acid ammonium salt preparations of varied purity and yielded equivocal results (one positive (DuPont-19897, 2006) and one negative (DuPont-22616 RV1, 2007). Evaluations of additional immune function assays, histopathology, and immune endpoints such as antibody levels are not available. The combined GenX chemicals immunotoxicity dataset was found to be incomplete as it did not include sufficient measures of immunopathology, humoral immunity, cell-mediated immunity, nonspecific immunity, or host resistance, but the available studies are suggestive of a potential immune hazard. Data on the potential for these GenX chemicals to impact aspects of immune function beyond immunosuppression are lacking. Additional studies, therefore, would be useful to support a more conclusive determination of immunotoxic potential.

Finally, additional research is needed to help determine if the inconsistent hematological effects observed in many of the studies are adverse and to investigate potential neural effects of GenX chemical exposure. As mentioned above, the hematological effects observed in the 28-day mouse study at 3 mg/kg/day were not observed in the 90-day subchronic study in mice, except for a 3% decrease in hemoglobin concentration at 5 mg/kg/day. No hematological changes were observed at the 0.1- or 0.5-mg/kg/day dose in the subchronic mouse study (DuPont-18405-1307, 2010). Likewise, the hematological effects observed in the subchronic rat study at low doses are not observed in the chronic rat study (DuPont-17751-1026, 2009; DuPont-18405-1238, 2013). Cannon et al. (2020) demonstrated that HFPO dimer acid can modify the activity of transporters at the blood-brain barrier. Specifically, HFPO dimer acid inhibited P-gp and BCRP transport in rat brain capillaries. The potential neural effects that might result from inhibition of transport activity are unknown and require additional investigation.

Furthermore, Conley et al. (2019), Conley et al. (2021) and Blake et al. (2020) observed alterations in thyroid hormones in the pregnant dam after gestational exposure to GenX chemicals. Specifically, Conley et al. (2019, 2021) demonstrated significant decreases in maternal serum total T3 and T4 levels in the pregnant rat (e.g., a 51% decrease in total T4 in pregnant dams dosed with 62.5 mg/kg/day from GD8-PND2) while Blake et al. (2020) reported a significant increase in mouse placental total T4 levels relative to control. In the Blake et al (2020) study, there was a trend towards a significant effect of higher T4 in placentas exposed to 2 mg/kg/day GenX (38% increase) though not statistically significant. Maternal serum thyroid hormones could not be measured due to volume constraints in the study. The potential neurodevelopmental effects that might result from the disruption of these thyroid hormones are unknown and require additional investigation at lower doses.

Given the residual concerns for potentially more sensitive effects outlined above, a database uncertainty factor is considered necessary to account for the possibility that the currently available database for GenX chemicals may result in an under-protective point of departure. Specifically, a value of 10 was selected for the UF_D to account for the uncertainty surrounding reproductive or developmental effects of concern occurring at similar dose levels to the liver effects (maternal GWG, placental lesions indicative of placental insufficiency, changes in thyroid hormones) or effects that observed to occur with exposure to other PFAS (e.g., PFOA) but have not been studied or do not have published studies currently for GenX chemicals (skeletal ossification, changes in thyroid hormones, mammary gland development, and altered metabolism in the mouse).

The UFs described above were applied to the POD_{HED}s from section 7.2.2 to derive a candidate RfDs applicable to both subchronic and chronic exposures. Table 14 summarizes the results of this quantification for the subchronic scenario. The subchronic candidate RfDs range from 0.00003 mg/kg/day to 0.00007 mg/kg/day. Likewise, Table 15 summarizes the results of this quantification for the chronic scenario. The chronic candidate RfDs range from 0.000003 mg/kg/day to 0.000007 mg/kg/day. Each POD_{HED} is impacted by the doses used in the subject study, the endpoints monitored, and the animal species/gender studied. Thus, the array of outcomes, combined with knowledge of the individual study characteristics, helps to inform selection of a subchronic and chronic RfDs that will be protective for humans.

Table 14. Candidate Subchronic RfD Values

Endpoint and reference	POD_{HED}[a] (mg/kg/day)	POD Type	UF_L	UF_S	UF_A	UF_H	UF_D	UF_{TOT}	Candidate RfD value (mg/kg/day)
HEPATIC									
Liver constellation of lesions in parental male mice (DuPont-18405-1037, 2010)	0.02	$BMDL_{10}$	1	1	3	10	10	300	7×10^{-5}
Liver constellation of lesions in parental female mice (DuPont-18405-1037, 2010)	0.01	$BMDL_{10}$	1	1	3	10	10	300	3×10^{-5}

Note:
[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).

Table 15. Candidate Chronic RfD Values

Endpoint and reference	POD_{HED}[a] (mg/kg/day)	POD Type	UF_L	UF_S	UF_A	UF_H	UF_D	UF_{TOT}	Candidate RfD value (mg/kg/day)
HEPATIC									
Liver constellation of lesions in parental male mice (DuPont-18405-1037, 2010)	0.02	$BMDL_{10}$	1	10	3	10	10	3000	7×10^{-6}
Liver constellation of lesions in parental female mice (DuPont-18405-1037, 2010)	0.01	$BMDL_{10}$	1	10	3	10	10	3000	3×10^{-6}

Note:
[a] Calculated using $BW^{3/4}$ scaling (EPA, 2011b).

7.4 Selection of Overall RfD

The oral reproductive/developmental toxicity mouse study (DuPont-18405-1037, 2010) and its pathologic demonstration of liver effects in females (constellation of lesions including cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis) were selected as the critical study and effect, respectively, for deriving the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt. The RfD based on this grouping of effects occurred at the lowest dose and therefore provides the most health-protective RfD among the modeled endpoints based on the available data. The selection of the constellation of lesions is supported by the NTP PWG conclusion that the dose response and constellation of lesions (i.e., cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of the study. Because there is a negligible difference between the molecular weight of the HFPO dimer acid ammonium salt (347.08 grams per mol (g/mol)) and the free HFPO dimer acid (330.06 g/mol), the subchronic and chronic RfDs presented here are applicable for both chemicals.

Several of the other studies provide support for the selection of the DuPont-18405-1037 (2010) study as the critical analysis and the constellation of liver lesions as the critical effect (DuPont-24447, 2008; DuPont-24459, 2008; DuPont-18405-841, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013) on which to base the subchronic and chronic RfDs. The liver is the primary target organ for toxicity from oral exposure to HFPO dimer acid and its ammonium salt. Liver effects are observed in both male and female mice and rats at varying durations of exposures and doses of GenX chemicals. Specifically, changes in liver enzyme levels, histopathological lesions, and tumors are observed in both male and female mice and rats at varying durations of exposures (15 days to 2 years) and doses of these GenX chemicals (0.5–1,000 mg/kg/day).

7.4.1 Subchronic RfD

This section provides the calculation for the subchronic RfD. The values and rationale describing the input parameters for the RfD calculation can be found in sections 7.2 and 7.3, and appendix E.

$$\text{Subchronic RfD} = \frac{POD_{HED}}{Total\ UF}$$

$$= \frac{0.01 \frac{mg}{kg}/day}{300}$$

$$= \mathbf{3 \times 10^{-5}\ mg/kg/day\ or\ 0.03\ \mu g/kg/day}$$

where:

- POD_{HED} = 0.01 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPO dimer acid ammonium salt by gavage for 53–64 days (DuPont-18405-1037, 2010).
- Total UF = 300, including 10 for UF_H, 3 for UF_A, and 10 for UF_D.

7.4.2 Chronic RfD

This section provides the calculation for the chronic RfD. The values and rationale describing the input parameters for the RfD calculation can be found in sections 7.2 and 7.3, and appendix E.

$$\text{Chronic RfD} = \frac{POD_{HED}}{Total\ UF}$$

$$= \frac{0.01\frac{mg}{kg}/day}{3000}$$

$$= \mathbf{3 \times 10^{-6}\ mg/kg/day\ or\ 0.003\ \mu g/kg/day}$$

where:

- POD_{HED} = 0.01 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (constellation of liver lesions as defined by the NTP PWG) in parental female mice exposed to HFPO dimer acid ammonium salt by gavage for 53–64 days (DuPont-18405-1037, 2010).
- Total UF = 3000, including 10 for UF_H, 3 for UF_A, 10 for UF_S, and 10 for UF_D.

8.0 Effects Characterization

8.1 Uncertainty and Variability

The uncertainty and variability in an RfD are a function of both intrinsic and extrinsic factors. EPA has identified multiple short-term subchronic and chronic studies that provide dose-response information and were considered during the quantitative assessment of risk. The range of external dose NOAELs among these studies is 0.1 mg/kg/day–10 mg/kg/day. The LOAELs range from 0.5 mg/kg/day to 100 mg/kg/day.

The intrinsic uncertainties in the assessment reflect the fact that the NOAELs and LOAELs are derived using central tendency estimates for variables such as BW, food and drinking water intakes, and dose. The central tendency estimates are derived from small numbers of relatively genetically similar animals representing one or more strains of rats or mice living in controlled environments. The animals lack the heterogeneous genetic complexity, behavioral diversity, and complex habitats experienced by humans. These differences, to some extent, have been minimized using the modeled outcomes and use of allometric scaling to help inform the application of the UF.

While EPA has routinely used BW to allometrically scale toxicity data from animal test species to HEDs during the development of human health risk assessments, the applied methodology is not without limitation (EPA, 2011b). Allometric scaling using BW scaled to the ¾ power primarily addresses uncertainty associated with toxicokinetics, although the exact amount of uncertainty addressed by this method for any specific chemical is often not quantifiable. In following the recommended method to apply $BW^{3/4}$ scaling, it remains possible that the toxicokinetic uncertainty associated with GenX chemicals might be more or less than what is accounted for using this scaling methodology.

For all selected candidate studies, $BW^{3/4}$ scaling was found to be appropriate because GenX chemicals are not metabolized and have relatively short clearance times, especially compared to other longer chain PFAS chemicals such as PFOA (DuPont-18405-1017 RV1, 2011; EPA, 2011b; Gannon et al., 2016). The $BW^{3/4}$ scaling methodology is not appropriate, however, when using children's BWs. This limitation exists due to the absence of quantitative information describing the toxicokinetic and toxicodynamic differences between test animals and early life-stage humans (EPA, 2011b). Because the liver effects observed following exposure to GenX chemicals were in adult animals, the allometric scaling methodology was scaled to the average adult human BW.

Variability in the study outcomes is extrinsically a function of study design and the endpoints monitored. Studies of systemic toxicity monitor an array of endpoints that are not evaluated in studies of reproductive, developmental, neurological, and immunological toxicity. The reverse is true for the other types of toxicity studies compared to standard short-term and long-term systemic studies. Studies of systemic toxicity do not often examine neurological or immunological endpoints. Increases in liver weight were seen in many of the studies with dose-response information, and the histological evaluation of the liver supported a determination that the increase in liver weight when it is accompanied by necrosis can be considered as adverse rather than adaptive, according to the Hall et al. (2012) criteria. Increases in relative liver weight with confirmed liver necrosis were observed in DuPont-24447 (2008), DuPont-24459 (2008), DuPont-18405-1037 (2010), DuPont-18405-1307 (2010), and DuPont-18405-1238 (2013).

The subchronic and chronic RfDs are based on the POD_{HED} derived from the parental females from the oral reproductive/developmental toxicity study in mice with application of UFs to account for variability in the human population, database uncertainties, and possible differences in the ways in which humans and rodents respond to HFPO dimer acid and/or its ammonium salt that reaches their tissues (DuPont-18405-1037, 2010). Uncertainty associated with relying on a less-than-chronic study to derive a chronic RfD is addressed with a UF applied only for the chronic RfD calculation. The selected RfDs are based on the adverse liver effects observed in the parental female animals. Selection of this endpoint is expected to provide protection to both the sensitive life stages and the general population. The RfDs are supported by the outcomes from other studies based on different endpoints, including hematological, immune, and developmental effects (DuPont-24459, 2008; DuPont-17751-1026, 2009; DuPont-18405-1037, 2010). These supporting data from the HFPO dimer acid and its ammonium salt database increase confidence in the RfD.

8.2 Composition of Test Substance

Most of the available data for HFPO dimer acid and its ammonium salt with PMNs were submitted to EPA by DuPont, the manufacturer of GenX chemicals, under TSCA, as required pursuant to a consent order for these chemicals (EPA, 2009) or as required under TSCA reporting requirements (e.g., section 8(e) 15 U.S.C. § 2607.8(e)). In these submissions, DuPont provided information on the purity of the test substance used in each of the studies. Purity ranged from 84% to 88% across the toxicity studies considered in this assessment. DuPont provided a certificate of these analyses and noted that they were conducted under EPA GLP standards (40 CFR part 792). The major impurity identified is water (12.7%–13.3%). Trace amounts of PFOA were also identified in the test substance (3.4–150 parts per million). DuPont noted that test results were adjusted for purity based on the reported test article formulations. Based on the

information provided, administered doses of PFOA present as a contaminant in the formulations used by DuPont are low. For example, in the critical study chosen for the derivation of the RfDs, the dose of administered PFOA is 0.000075 mg/kg/day at the GenX chemicals NOAEL (0.1 mg/kg/day) (DuPont-18405-1037, 2010). For PFOA, NOAELs ranging from 0.01 mg/kg/day to 30 mg/kg/day have been identified for effects including developmental, liver, and immune endpoints (EPA, 2016a). Despite trace amounts of PFOA that might be present as an impurity, EPA recognizes the potential for this impurity to contribute to the observed toxicity at very high doses of GenX chemicals. At present, however, discerning the contribution of this low level of PFOA to observed toxicity is not possible. Thus, EPA concluded that the presence of PFOA at these low levels is not the primary driver of toxicity observed in the studies. Of note is that the same test substance (Lot H-28548) was used in the 90-day mouse and rat studies, the chronic rat study, and the oral reproductive and developmental toxicity and prenatal developmental toxicity studies (DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013). Additionally, the same test substance (Lot H-28397) was used in both the mouse and the rat 28-day studies (DuPont-24447, 2008; DuPont-24459, 2008). Despite differences in test substance purity, adverse effects were observed consistently across the DuPont studies. Many of the peer-reviewed studies did not report purity in their methods or formulations of HFPO dimer acid and ammonium salt (Behr et al., 2020; Blake et al., 2020; Rushing et al., 2017; Sheng et al., 2018; Wang et al., 2017).

Given the database for GenX chemicals, the quality of these studies—including adequacy of reporting of methods and results—and the weight of evidence for effects on the liver, hematological and immune systems, and reproductive and developmental endpoints, EPA concluded that the DuPont studies demonstrated adverse effects as a result of exposure to the HFPO dimer acid ammonium salt formulations and were appropriate for derivation of toxicity values for these chemicals.

8.3 Use of Data-Derived Extrapolation Factors

For HFPO dimer acid and/or ammonium salt, there are limited human half-life data (see section 8.4) and no BBDR or PBPK models available to evaluate toxicokinetic and toxicodynamic differences between humans and animals. Additionally, only a few repeat-dose studies are available on rats and mice that evaluate toxicokinetics. These studies indicate that there is little-to-no metabolism and that clearance is relatively rapid compared to other longer chain PFAS. MOA (both *in vivo* and *in vitro*) data are also inadequate. EPA considered the 2014 *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* in determining UF_A and UF_H (EPA, 2014c). Using the decision process described in Figure 2, EPA concluded that data are not adequate to support derivation of data-derived extrapolation factors. Specifically, given the lack of available models and data to address external dose and clearance in humans, default approaches to the application of UF_A and UF_H were employed, including BW scaling for oral exposure (EPA, 2011b). These approaches are described further in section 7.3.

8.4 Use of Data-Derived Dosimetric Adjustment Factor

EPA guidance recommends a hierarchical approach to deriving human equivalent oral exposures from animal studies, with the preferred approach being physiologically based toxicokinetic modeling. There are no such toxicokinetic models available for GenX chemicals. The next

preferred approach is to use chemical-specific information to derive a data-informed dosimetric adjustment factor (DAF). For GenX chemicals there are limited human data (outlined below) and a few repeat-dose studies available on rats and mice that evaluate toxicokinetics (see section 2.3.6).

In the one available human half-life study, twenty-five workers from a Chemours facility in the Netherlands volunteered blood samples before an off-work weekend and twenty-two workers provided a second sample at the start of the next shift (72-96 hours between sample collections) (Clark, 2021). Samples were sent to two independent laboratories. HFPO dimer acid concentrations ranged from below the level of detection (less than 0.5 µg/L) to 25 µg/L (Arbo Unie, 2020). Samples containing measurable amounts of HFPO dimer acid at both time points were used to calculate an average approximate half-life of 81 ± 55 hours, assuming an exponential rate of decay (Clark, 2021). The range was 42 to 333 hours with a median of 66 hours. Serum from eighteen of the twenty-two workers contained HFPO dimer acid at detectable levels (i.e., at or above the limit of detection) at both time points.

A letter summarizing the data and briefly outlining the methods used to calculate the human half-life was provided to TSCA in 2021 by Chemours (similar information can also be found on ECHA). However, EPA has not received the full study report and these data have not been peer reviewed. The dataset used by Chemours to calculate the half-life is limited to only 18 individuals. Chemours also provided EPA with an unpublished report containing the raw data (Arbo Unie, 2020); however, this report did not stratify the data based on sex or provide any additional details on the test subjects (including sex). Sex-stratification of the human worker data is potentially important because the critical effect in mice is more severe in females (DuPont-18405-1037, 2010). Because the information provided are insufficient, EPA did not use the human half-life data to estimate a data-informed DAF. Instead, EPA employed the default procedure of body weight scaling to the ¾ power (i.e., $BW^{3/4}$) to derive human equivalent oral exposures from animal studies in concordance with EPA guidance (EPA, 2011b; outlined in section 7.2.2).

Although the Chemours human half-life data are insufficient for use in the allometric scaling of animal to human dose for toxicity and risk assessment purposes, EPA conducted an exploratory analysis to determine the magnitude of the impact on the resulting POD_{HED} if this information was used to calculate a POD_{HED} in place of the default $BW^{3/4}$ DAF (which, as outlined above, is the agency's standard approach where acceptable data are not available) (Table 16).

Table 16. Comparison of POD_{HED} using different allometric scaling methods

Endpoint and reference	POD_{HED} (mg/kg/day) calculated using…	
	$BW^{3/4}$ DAF	Data Derived Human DAF
Liver constellation of lesions in parental males (DuPont-18405-1037, 2010)	0.02	0.06
Liver constellation of lesions in parental females (DuPont-18405-1037, 2010)	0.01	0.03

The method used to calculate the data-derived DAF is outlined in Section 6.1.1.2 of the Human Health Toxicity Values for Perfluorobutane Sulfonic Acid (CASRN 375-73-5) and Related Compound Potassium Perfluorobutane Sulfonate (CASRN 29420-49-3) (EPA, 2021a). Briefly, the ratio of elimination half-life in animals from which the POD is obtained ($t_{1/2A}$) to that in humans ($t_{1/2H}$) can be used to calculate the DAF, and the human equivalent dose (HED) can be calculated as follows:

$$POD_{HED} = POD \times \frac{t_{1/2A}}{t_{1/2H}}$$

For the comparison exercise in Table 16, the $t_{1/2A}$ used for GenX chemicals were the male and female mouse data from the beta elimination phase outlined in Table 8 and the $t_{1/2H}$ was the 81 hours calculated from the data outlined above (Arbo Unie, 2020; Clark, 2021). Although the Chemours human half-life data were found to be insufficient for this purpose (Chemours, 2021) describes the dataset as "limited"), this comparison demonstrates that the POD_{HED} calculated using either the $BW^{3/4}$ DAF or the Data Derived Human DAF are similar. This comparison exercise illustrates a degree of consistency between the $BW^{3/4}$ approach and the use of the only available human half-life dataset for deriving human equivalent oral doses for GenX chemicals.

8.5 Limited Data on Carcinogenicity

One study is available on evaluating carcinogenicity of HFPO dimer acid and its ammonium salt in rats (DuPont-18405-1238, 2013). In this study, liver and pancreatic tumors were noted at the highest doses tested. 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/day group cannot be ruled out" while also suggesting that Leydig cell tumor induction in rodents might have low relevance to humans (Caverly Rae et al., 2015).The available data for HFPO dimer acid ammonium salt suggest that mice might be more sensitive than rats to exposure to these GenX chemicals. Given the evidence that the liver is the target organ for toxicity and the primary organ for tumor development, additional research is needed using chronic duration exposures in mice. This uncertainty was not considered in the application of the UF_D because a noncancer toxicity value was developed for this assessment.

8.6 Internal Dosimetry Data for GenX Chemicals

EPA recognizes that there are similarities in the health effects observed across various PFAS. Specifically, GenX chemicals are linked to adverse effects on the liver, kidney, immune system, development, and cancer and these health effects have also been associated with PFOA exposure (EPA, 2016a,b). There are data available that demonstrate that the toxicokinetic profile for GenX chemicals is different than PFOA in that GenX chemicals are more rapidly excreted than PFOA and appear not to bioaccumulate like PFOA. These data lead one to question whether administering the same dose of these chemicals could result in a much lower internal dose for GenX chemicals than PFOA or PFOS and thus differences in potency between the two chemicals.

There are currently two studies evaluating the internal dose of the HFPO dimer acid and comparing it to the internal dose of either PFOA (Blake et al., 2020) or PFOS (Conley et al.,

2021). Specifically, Blake et al. (2020) evaluated internal dose of both chemicals in pregnant mice and their embryos. Concentrations of PFOA and HFPO dimer acid were measured in the maternal serum, maternal liver, amniotic fluid, and whole embryo dosed with 0, 1, or 5 mg/kg/day of PFOA or 2 or 10 mg/kg/day of HFPO dimer acid from E1.5 to E11.5 or E17.5. Although concentrations in the maternal serum were relatively similar, the total concentration of HFPO dimer acid is an order of magnitude less than PFOA in the maternal liver. Additionally, PFOA appears to accumulate in the liver from E11.5 to E17.5 in mice exposed to 1 mg/kg/day PFOA (48.3 ± 12.5 ug/mL to 181.1 ± 46.0 ug/mL); however, the concentration of HFPO dimer acid at 2 mg/kg/day is similar at both time points (5.45 ± 3.43 ug/mL to 4.56 ± 2.80 ug/mL). These differences are noteworthy because PFOA and HFPO dimer acid affected the maternal liver similarly in this study (e.g., increased liver weight and increased incidence of liver lesions) despite the concentration of HFPO dimer acid being an order of magnitude lower than PFOA and displaying no apparent accumulation between E11.5 and E17.5. The concentrations of PFOA and HFPO dimer acid are similar in the amniotic fluid and whole embryo in the 1- and 2-mg/kg/day-dose groups, respectively. These data suggest that a lower internal dose of HFPO dimer acid elicits the same effects on the liver as a higher internal dose of PFOA in the pregnant mouse. Additional research is needed to further elicit whether internal dosimetry is in fact different between these chemicals and to determine if these results are specific to the pregnant mouse.

Conley et al. (2021) compared the maternal serum levels of HFPO dimer acid and PFOS with respect to neonatal mortality. Conley et al. (2021) concluded that based on maternal serum concentrations, HFPO dimer acid (EC_{50} = 35.4 ug/mL) was ~2-fold more potent than PFOS (EC_{50} = 74.5 ug/mL). However, given that the molecular weight of PFOS (500 g/mol) is 34% greater than HFPO-DA (330 g/mol), the potency of PFOS (EC_{50} =148.9 uM) and HFPO dimer acid (EC_{50} = 107.1 uM) are very similar when correcting for molecular weight differences.

8.7 Effects on Bilirubin

A decrease in serum bilirubin is a consistent effect observed across multiple studies, especially in female rodents (DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013; Wang et al., 2017). This finding was surprising given that increased rather than decreased levels of serum bilirubin are typically indicative of liver damage, and multiple studies outlined above have confirmed microscopic liver damage (DuPont-18405-841, 2010; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013; Tietze, 2012). In female mice and rats, however, serum bilirubin levels were significantly decreased by 14%–50% relative to controls when the females were administered 5 mg/kg/day–1,000 mg/kg/day of HFPO dimer acid ammonium salt for 3–12 months (DuPont-17751-1026, 2009; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013). Additionally, male ICR mice treated with 1 mg/kg/day of HFPO dimer acid ammonium salt exhibited a significant 37% and 45% decrease in total and direct bilirubin, respectively, when compared to controls (Wang et al., 2017); this finding was not replicated in the other 28-day studies (DuPont-24447, 2008; DuPont-24459, 2008). The biological or mechanistic significance of this effect is unknown, yet its consistency across multiple studies is noteworthy.

8.8 Susceptible Populations and Life Stages

Data for the elucidation of differential susceptibility dependent on life stage (e.g., developing embryo/fetus, women of reproductive age, or pregnant women) are not available. Children are

frequently more vulnerable to pollutants than the average adult because of the differences in their behaviors and biology. These differences can result in greater exposure and/or unique windows of developmental susceptibility during the prenatal and postnatal periods for both the pregnant mother and the developing fetus. No human toxicity or epidemiological studies are available in the literature that address early developmental or reproductive life stages. Peer-reviewed literature and DuPont submitted data examining reproductive and developmental endpoints in both mice and rats (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-18405-841, 2010; DuPont-18405-1037, 2010) and summaries of these studies can be found in section 5.4 (Reproductive/Developmental). HFPO dimer acid ammonium salt can be transferred from a pregnant animal to the fetus during gestation and lactation (Blake et al., 2020; Conley et al., 2019, 2021; DuPont-18405-1037, 2010; Dupont-18405-849 RV1, 2011). When present, developmental and reproductive effects were found at doses similar to and higher than those associated with the selected critical effect: liver effects in females (constellation of lesions as defined by the NTP PWG to include cytoplasmic alteration, hepatocellular single-cell and focal necrosis, and hepatocellular apoptosis). The UF_H of 10 accounts for variability in the responses within human populations because of both intrinsic (including life stage) and extrinsic (lifestyle) factors that can influence the response to dose. No information to characterize interindividual and age-related variability in the toxicokinetics or toxicodynamics is available. Thus, the RfDs provided in sections 7.4.1 and 7.4.2 (Subchronic RfD and Chronic RfD) are applicable to all life stages. When reviewing data pertinent to the hazard potential of GenX chemicals, EPA adhered to the requirements of its 2013 reaffirmation of the Policy on Evaluating Health Risks to Children (EPA, 2013).

There is some sex-specific variation in the toxicokinetics of these two GenX chemicals in rodents. Toxicokinetic data from DuPont calculate clearance times from the urine and plasma, which is defined by DuPont as the time when 98.4% of the anion from the HFPO dimer acid ammonium salt was cleared from the urine or plasma. These data show the HFPO dimer acid and its ammonium salt clearance time in the plasma to be considerably faster for female rodents than for male rodents (see the summary in section 2.3.6 (Clearance and Half-Life Data). For example, Dupont-25300 (2008) identified 143 hours as the clearance time for HFPO dimer acid ammonium salt in male mice at 10 mg/kg and 139 hours for 30 mg/kg. In female mice, the clearance values were 57 and 62 hours for the low dose and the high dose, respectively. However, this difference was not as pronounced in mice in the $T_{1/2}$ estimates. Specifically, the alpha (distribution) phase $T_{1/2}$s were 5.8 and 4.6 hours for male and female mice, respectively, and the beta (elimination) phase $T_{1/2}$s were 36.9 hours and 24.2 hours for male and female mice, respectively. It is unknown if or how these observed sex-specific toxicokinetic differences in rodents contribute to the toxic response.

The available data suggest that the pregnant rodent might be more susceptible to liver effects following exposure to GenX chemicals during gestation. Liver effects were reported in the pregnant dams in the available reproductive/developmental studies dosed during gestation (Blake et al., 2020; Conley et al., 2019; DuPont-18405-841, 2010; DuPont 18405-1037, 2010). All the studies reported increases in liver weight ranging from 12% to 34% in rats and 26% to 101% in mice over the gestational period. Conley et al. (2019) did not conduct liver histopathology, but both DuPont-18405-841 (2010) and Blake et al. (2020) reported hepatocellular hypertrophy and increased cell death as compared to controls with increasing HFPO dimer acid ammonium salt concentration. Specifically, focal necrosis was observed in 2/22 (9%) and 5/22 (23%) pregnant

rats after just 15 days (GD6–GD20) of 10 mg/kg/day or 100 mg/kg/day of HFPO dimer acid ammonium salt, respectively, compared to 0 in the control group. Comparatively, nonpregnant female rats dosed from 28 to 90 days did not exhibit necrosis when treated with doses up to 1,000 mg/kg/day of HFPO dimer acid ammonium salt. Necrosis was observed in nonpregnant female rats only after 2 years of dosing with 500 mg/kg/day of HFPO dimer acid ammonium salt. Increased cell death (including both apoptosis and single-cell or focal necrosis) was observed in pregnant mice after 11 and 17 days (GD1.5–GD11.5 or GD17.5) of 2 mg/kg/day or 10 mg/kg/day of HFPO dimer acid ammonium salt. Similarly, and as noted above, female mice dosed 14 days prior to mating and throughout gestation and lactation exhibited cytoplasmic alteration, apoptosis, single-cell necrosis, and focal necrosis after 53–64 days of dosing (NTP, 2019 reread of DuPont 18405-1037, 2010). The incidence of single-cell and focal necrosis in the F_0 females was 6/24 (25%) and 20/24 (83%) in the 0.5- and 5-mg/kg/day-dose groups, respectively (NTP, 2019). A chronic study in mice is not available to compare to the gestational exposures in female pregnant mice, and comparisons to the 90-day subchronic study in mice is potentially limited by sample size (n = 9) in the 0.1 and 0.5 mg/kg/day-dose groups.

Susceptible populations include groups who have relatively high exposure to GenX chemicals. While data are currently unavailable, there is the potential for highly exposed populations. For example, formula fed infants, who have high daily water ingestion relative to body weight, have the potential for relatively high exposure to GenX chemicals when GenX chemicals are present in tap water and this tap water is used to reconstitute formula. As a second example, workers and their families who work at and/or live near facilities that use the GenX processing aid technology have the potential for greater exposure levels and duration of exposure. Finally, communities living in close proximity to facilities using the GenX processing aid technology have the potential for increased exposure as evidenced by the detection of GenX chemicals in drinking water, surface water, soil and rainwater samples collected close to the facility (see section 1.3).

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Appendix A: Literature Search Strategy

This appendix presents the details of the literature search strategy U.S. Environmental Protection Agency (EPA) used to identify primary, peer-reviewed literature pertaining to hexafluoropropylene oxide (HFPO) dimer acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6) and its ammonium salt (CASRN 62037-80-3). The literature searches were conducted using the databases listed in Table A-1.

The initial literature searches for these GenX chemicals were conducted in July 2017 (acid) and January/February 2018 (ammonium salt). Subsequent literature searches were conducted from 2018 to March 2020. The searches were conducted using CASRN, synonyms, and additional relevant search strings (see Table A-2). Because the results of this core search were so limited, additional databases were identified and searched for physiochemical property information, health effects, toxicokinetics, and mechanistic information (see Table A-3 and Table A-4). Combined, these initial literature searches returned 27 studies for HFPO dimer acid and HFPO dimer acid ammonium salt after duplicates across the two chemicals were deleted. The literature searches conducted after publication of the public comment draft in November 2018 resulted in 48 additional studies for HFPO dimer acid and HFPO dimer acid ammonium salt after duplicates were deleted.

As previously stated, the available data for GenX chemicals come primarily from studies submitted under the Toxic Substances Control Act (TSCA). Those studies were combined with the results of the search of the publicly available peer-reviewed literature for evaluation for relevance to the assessment. The submitted studies and literature identified by the search of publicly available sources are available through EPA's Health & Environmental Research Online (HERO) website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627. Potential relevance was based primarily on a title and abstract screen. Table A-5 presents the inclusion/exclusion criteria applied to conducting the literature searches. An additional 48 studies from peer-reviewed literature were identified during the updated literature searches conducted in February 2019, October 2019, and March 3, 2020. These studies were subjected to title and abstract screening to determine relevancy according to the inclusion/exclusion criteria outlined in Table A-6. Relevancy was confirmed by review of the full text of studies included in the title abstract screen. 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. These supplemental categories are outlined in Table A-7.

Table A-1. Summary of Core Database Search Results

Search date	PubMed	WOS	Toxline	TSCATS via Toxline/NLM	Other sources	Combined dataset after duplicate removal
HFPO dimer acid (CASRN 13252-13-6)						
7/24/17	3	12	0	0	3	16
7/17–2/19	6	11	0	0	0	11
2/19–10/19	9	8	0	0	9	16
10/19–3/20	7	4	N/A[a]	0	1	9
HFPO dimer acid ammonium salt (CASRN 62037-80-3)						
1/18 and 2/18	9	12	0	0	3	18
2/18–2/19	8	13	0	0	1	15
2/19–10/19	15	11	0	0	2	20
10/19–3/20	7	3	N/A[a]	0	1	8

Note: N/A = not applicable; NLM = National Library of Medicine; TSCATS = Toxic Substances Control Act Test Submissions; WOS = Web of Science.

[a] Toxline was no longer available in March 2020.

Table A-2. Database Search Strings

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
PubMed	13252-13-6[rn] OR "2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid"[tw] OR "Perfluoro(2-methyl-3-oxahexanoate) "[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)- "[tw] OR "Perfluorinated aliphatic carboxylic acid"[tw] OR "Perfluoro(2-methyl-3-oxahexanoic) acid"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid"[tw] OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid"[tw] OR "perfluoro-2-(propyloxy)propionic acid"[tw] OR "perfluoro-2-methyl-3-oxahexanoic acid"[tw] OR "perfluoro-2-propoxypropanoic acid"[tw] OR "perfluoro-2-propoxypropionic acid"[tw] OR "perfluoro-α-propoxypropionic acid"[tw] OR "propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-"[tw] OR "propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-"[tw] OR (GenX AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw])) OR (("2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic"[tw] "Perfluorinated aliphatic carboxylic"[tw] OR "Perfluoro(2-methyl-3-oxahexanoic)"[tw] OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic"[tw] "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic"[tw] OR "perfluoro-2-(propyloxy)propionic"[tw] OR "perfluoro-2-methyl-3-oxahexanoic"[tw] OR "perfluoro-2-propoxypropanoic"[tw] OR "perfluoro-2-propoxypropionic"[tw] OR "perfluoro-α-propoxypropionic"[tw]) AND (acid[tw] OR acids[tw]))	(62037-80-3[rn] OR "62037-80-3"[tw] OR "Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate"[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt"[tw] OR "Perfluorinated aliphatic carboxylic acid, ammonium salt"[tw] OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt"[tw] OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate"[tw] OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)"[tw] OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid"[tw] OR "Ammonium perfluoro-2-methyl-3-oxahexanoate"[tw] OR "FRD-902"[tw] OR "GenX-H3N"[tw] OR "HFPO-DA"[tw] OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt"[tw] OR "Undecafluoro-2-methyl-3-oxahexanoic acid"[tw] OR ((GenX[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw])) OR (("Undecafluoro-2-methyl-3-oxahexanoic"[tw] OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)"[tw] OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)"[tw] OR "Perfluorinated aliphatic carboxylic"[tw]) AND (salt[tw] OR salts[tw] OR acid[tw] OR acids[tw])))) OR (((Undecafluoro AND oxahexanoic) OR (Ammonium AND perfluoro AND oxahexanoic) OR (Tetrafluoro AND heptafluoropropoxy) OR "Perfluorinated aliphatic carboxylic"[tw] OR "Perfluorinated aliphatic carboxylic"[tw]) AND (salt[tw] OR salts[tw] OR acid[tw] OR acids[tw]))

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
WOS	TS="2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid" OR TS="Perfluoro(2-methyl-3-oxahexanoate)" OR TS="Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-" OR TS="Perfluorinated aliphatic carboxylic acid" OR TS="Perfluoro(2-methyl-3-oxahexanoic) acid" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid" OR TS="2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid" OR TS="perfluoro-2-(propyloxy)propionic acid" OR TS="perfluoro-2-methyl-3-oxahexanoic acid" OR TS="perfluoro-2-propoxypropanoic acid" OR TS="perfluoro-2-propoxypropionic acid" OR TS="perfluoro-α-propoxypropionic acid" OR TS="propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR TS="propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR (TS="GenX" AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) OR ((TS="2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic" OR TS="Perfluorinated aliphatic carboxylic" OR TS="Perfluoro(2-methyl-3-oxahexanoic)" OR TS="2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic" OR TS="2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic" OR TS="perfluoro-2-(propyloxy)propionic" OR TS="perfluoro-2-methyl-3-oxahexanoic" OR TS="perfluoro-2-propoxypropanoic" OR TS="perfluoro-2-propoxypropionic" OR TS="perfluoro-α-propoxypropionic") AND TS=(acid OR acids))	TS=("Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt" OR "Perfluorinated aliphatic carboxylic acid, ammonium salt" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt" OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate" OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid" OR "Ammonium perfluoro-2-methyl-3-oxahexanoate" OR "FRD-902" OR "GenX-H3N" OR "HFPO-DA" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt" OR "Undecafluoro-2-methyl-3-oxahexanoic acid") OR ((TS=GenX AND (TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated)))) OR ((TS=("Undecafluoro-2-methyl-3-oxahexanoic" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)" OR "Perfluorinated aliphatic carboxylic" OR "Perfluorinated aliphatic carboxylic")) AND (TS=(salt OR salts OR acid OR acids))) **Timespan:** All years. **Indexes:** SCI-EXPANDED, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, CCR-EXPANDED, IC.

Database	HFPO dimer acid (CASRN 13252-13-6)	HFPO dimer acid ammonium salt (CASRN 62037-80-3)
Toxline	(13252-13-6[rn] OR "2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic acid" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic acid" OR "Perfluoro(2-methyl-3-oxahexanoate) " OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)- " OR "Perfluorinated aliphatic carboxylic acid" OR "Perfluoro(2-methyl-3-oxahexanoic) acid" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid" OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid" OR "perfluoro-2-(propyloxy)propionic acid" OR "perfluoro-2-methyl-3-oxahexanoic acid" OR "perfluoro-2-propoxypropanoic acid" OR "perfluoro-2-propoxypropionic acid" OR "perfluoro-α-propoxypropionic acid" OR "propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR "propionic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-" OR (GenX AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA)) OR (("2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propionic" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-Propanoic" OR "Perfluorinated aliphatic carboxylic" OR "Perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic" OR "2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic" OR "perfluoro-2-(propyloxy)propionic" OR "perfluoro-2-methyl-3-oxahexanoic" OR "perfluoro-2-propoxypropanoic" OR "perfluoro-2-propoxypropionic" OR "perfluoro-α-propoxypropionic") AND (acid OR acids))) AND ((aneupl [org] OR biosis [org] OR cis [org] OR dart [org] OR pubdart [org] OR emic [org] OR epidem [org] OR fedrip [org] OR heep [org] OR hmtc [org] OR ipa [org] OR riskline [org] OR mtgabs [org] OR niosh [org] OR ntis [org] OR pestab [org] OR ppbib [org]) AND NOT pubmed [org] AND NOT pubdart [org])	(62037-80-3[rn] OR "Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)-, ammonium salt" OR "Perfluorinated aliphatic carboxylic acid, ammonium salt" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid, ammonium salt" OR "Ammonium 2-(perfluoropropoxy)perfluoropropionate" OR "Ammonium Perfluoro(2-methyl-3-oxahexanoate)" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic) acid" OR "Ammonium perfluoro-2-methyl-3-oxahexanoate" OR "FRD-902" OR "GenX-H3N" OR "HFPO-DA" OR "Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt" OR "Undecafluoro-2-methyl-3-oxahexanoic acid" OR "GenX" OR (("Undecafluoro-2-methyl-3-oxahexanoic" OR "Ammonium perfluoro(2-methyl-3-oxahexanoic)" OR "2,3,3,3-Tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)" OR "Perfluorinated aliphatic carboxylic" OR "Perfluorinated aliphatic carboxylic") AND (salt OR salts OR acid OR acids))) AND ((aneupl [org] OR biosis [org] OR cis [org] OR dart [org] OR pubdart [org] OR emic [org] OR epidem [org] OR fedrip [org] OR heep [org] OR hmtc [org] OR ipa [org] OR riskline [org] OR mtgabs [org] OR niosh [org] OR ntis [org] OR pestab [org] OR ppbib [org]) AND NOT pubmed [org] AND NOT pubdart [org])
TSCATS1	13252-13-6[rn] AND (TSCATS [org])	62037-80-3[rn] AND (TSCATS [org])

Notes: PFAS = per- and polyfluoroalkyl substances; PFOA = perfluorooctanoic acid; PFOS = perfluorooctane sulfonate; TSCATS = Toxic Substances Control Act Test Submissions; WOS = Web of Science.

Table A-3. Processes Used to Augment the Search of Core Databases for HFPO Dimer Acid (CASRN 13252-13-6)

System used	Selected key reference(s) or sources
TSCATS[a]	TSCA Test Submissions 2.0; website now retired (https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm)
	Chemical Data Access Tool (CDAT); website now retired (https://java.epa.gov/oppt_chemical_search/)
	ChemView (https://java.epa.gov/chemview)
Resources searched for physiochemical property information	Agency for Toxic Substances and Disease Registry (ATSDR) (https://www.atsdr.cdc.gov/) Australian National Industrial Chemicals Notification and Assessment Scheme (NICNAS) (https://www.nicnas.gov.au/chemical-information) CAMEO Chemicals (https://cameochemicals.noaa.gov/) Canada DSL List (http://webnet.oecd.org/CCRWEB/Search.aspx) Chemical Risk Information Platform (CHRIP) (http://www.nite.go.jp/en/chem/chrip/chrip_search/systemTop) ChemIDplus (https://chem.nlm.nih.gov/chemidplus/) ChemSpider (http://www.chemspider.com/) CRC Handbook of Chemistry and Physics (http://hbcponline.com/faces/contents/ContentsSearch.xhtml;jsessionid=9408875156F724E0E945D3A6D0454891) ECHA Information on Chemicals (https://echa.europa.eu/) eChemPortal (https://www.echemportal.org/echemportal/index.action) Hazardous Substances Data Bank (HSDB) https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) HSNO Chemical Classification and Information Database (CCID) updated link[b] (https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/) IARC Monographs (http://www.inchem.org/pages/iarc.html) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris) J-Check (http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en) Kirk-Othmer Encyclopedia of Chemical Technology updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/0471238961) NIEHS (https://www.niehs.nih.gov/) OSHA Occupational Chemical Database (https://www.osha.gov/chemicaldata/) PubChem (https://pubchem.ncbi.nlm.nih.gov/search/index.html) SRC Fate Pointers (http://esc.syrres.com/fatepointer/search.asp) Ullmann's Encyclopedia updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/14356007) EPA ACToR (https://actor.epa.gov/actor/home.xhtml) EPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) EPA ChemView (https://java.epa.gov/chemview) EPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents

System used	Selected key reference(s) or sources
Resources searched for health effects, toxicokinetics, and mechanistic information	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA OEHHA (http://www.oehha.ca.gov/risk.html) CPSC (http://www.cpsc.gov) ECHA (http://echa.europa.eu/information-on-chemicals) eChemPortal[c] (http://www.echemportal.org/echemportal/) EFSA Europe (http://www.efsa.europa.eu/) Environment Canada (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) European Union Risk Assessment Reports (https://ec.europa.eu/jrc/en/publications-list) Federal Docket (http://www.regulations.gov) Health Canada (https://www.canada.ca/en/health-canada.html) IARC (http://monographs.iarc.fr/ENG/Classification/index.php) ITER (http://www.tera.org/iter/) Japan Existing Chemical Data Base (http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp) NICNAS (http://www.nicnas.gov.au/chemical-information) NIEHS (http://www.niehs.nih.gov/) NTP (http://ntpsearch.niehs.nih.gov/home) OEHHA Toxicity Criteria Database (http://www.oehha.ca.gov/tcdb/index.asp) EPA NSCEP (https://www.epa.gov/nscep) FDA (http://www.fda.gov/) WHO (http://www.who.int/ipcs/assessment/en/)

Notes: TSCATS = Toxic Substances Control Act Test Submissions

[a] Only relevant TSCATS studies from these interfaces were added to the HERO project page.

[b] The URL has been updated (as listed here) since the literature search; during the search, a previous URL was used.

[c] eChemPortal includes the following databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, EPA-IRIS, EPA-SRS, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, and UK CCRMP Outputs.

Table A-4. Processes Used to Augment the Search of Core Databases for HFPO Dimer Acid Ammonium Salt (CASRN 62037-80-3)

System used	Selected key reference(s) or sources
TSCATS[a]	Chemical Data Access Tool (CDAT); website now retired (https://java.epa.gov/oppt_chemical_search/)
	ChemView (https://java.epa.gov/chemview)
Resources searched for physiochemical property information	ATSDR (https://www.atsdr.cdc.gov/) CAMEO Chemicals (https://cameochemicals.noaa.gov/) Canada DSL List (http://webnet.oecd.org/CCRWEB/Search.aspx) ChemIDplus (https://chem.nlm.nih.gov/chemidplus/) CRC Handbook of Chemistry and Physics (http://hbcponline.com/faces/contents/ContentsSearch.xhtml;jsessionid=9408875156F724E0E945D3A6D0454891) ECHA Information on Chemicals (https://echa.europa.eu/) eChemPortal (https://www.echemportal.org/echemportal/index.action) Hazardous Substances Data Bank (HSDB) (https://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB) HSNO Chemical Classification and Information Database (CCID) updated link[b] (https://www.epa.govt.nz/database-search/chemical-classification-and-information-database-ccid/) IARC Monographs (http://www.inchem.org/pages/iarc.html) Integrated Risk Information System (IRIS) (https://www.epa.gov/iris) J-Check (http://www.safe.nite.go.jp/jcheck/search.action?request_locale=en) Kirk-Othmer Encyclopedia of Chemical Technology updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/0471238961 NICNAS (https://www.nicnas.gov.au/chemical-information) NIEHS (https://www.niehs.nih.gov/) OSHA Occupational Chemical Database (https://www.osha.gov/chemicaldata/) PubChem (https://pubchem.ncbi.nlm.nih.gov/search/index.html) SRC Fate Pointers (http://esc.syrres.com/fatepointer/search.asp) Ullmann's Encyclopedia updated link[b] (https://onlinelibrary.wiley.com/doi/book/10.1002/14356007) EPA ACToR (https://actor.epa.gov/actor/home.xhtml) EPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) EPA ChemView (https://java.epa.gov/chemview) EPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents

System used	Selected key reference(s) or sources
Resources searched for health effects, toxicokinetics, and mechanistic information	ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA - OEHHA (http://www.oehha.ca.gov/risk.html; http://www.oehha.ca.gov/tcdb/index.asp) CPSC (http://www.cpsc.gov) ECHA (http://echa.europa.eu/information-on-chemicals) eChemPortal[c] (http://www.echemportal.org/echemportal/) EFSA Europe (http://www.efsa.europa.eu/) Environment Canada (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) EPA-NSCEP (https://www.epa.gov/nscep) European Union Risk Assessment Reports (https://ec.europa.eu/jrc/en/publications-list) Federal Docket (http://www.regulations.gov) Google (Quick search only www.google.com) Health Canada (https://www.canada.ca/en/health-canada.html) IARC (http://monographs.iarc.fr/ENG/Classification/index.php) ITER (TERA database) (http://www.tera.org/iter/) Japan Existing Chemical Data Base (JECDB) (http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp) NICNAS (http://www.nicnas.gov.au/chemical-information) NIEHS (http://www.niehs.nih.gov/) NTP (http://ntpsearch.niehs.nih.gov/home) FDA (http://www.fda.gov) WHO (http://www.who.int/ipcs/assessment/en/)

Notes: TSCATS = Toxic Substances Control Act Test Submissions

[a] Only relevant TSCATS studies from these interfaces were added to the HERO project page.

[b] The URL has been updated (as listed here) since the literature search; during the search, a previous URL was used.

[c] eChemPortal includes the following databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, EPA-IRIS, EPA-SRS, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, and UK CCRMP Outputs.

Table A-5. Inclusion-Exclusion Criteria for HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt Studies

PECO Parameter	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog • Alternative animal models in standard laboratory conditions (e.g., *Xenopus*, zebrafish, minipig) • Human or animal cells, tissues, or organs (not whole animals); bacteria; nonmammalian eukaryotes; other nonmammalian laboratory species	• Ecological species
Exposure	• Exposure is to HFPO dimer acid and/or its ammonium salt • Exposure via oral, inhalation, dermal, intraperitoneal, or intravenous injection routes • Exposure is measured in air, dust, drinking water, diet, gavage, or injection vehicle, or via a biomarker of exposure (PFAS levels in whole blood, serum, plasma, or breast milk) • Exposure is via cells in culture or subcellular matrices	• Study population is not exposed to HFPO dimer acid and/or its ammonium salt • Exposure is to a mixture only without evaluating HFPO dimer acid and/or its ammonium salt individually
Outcome	• Studies that include a measure of one or more health effect endpoints, including effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer • *In vivo* and/or *in vitro* studies related to toxicity mechanisms or physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action • Qualitative or quantitative description of absorption, distribution, metabolism, elimination, and toxicokinetic and/or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD) • Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations	

PECO Parameter	Inclusion criteria	Exclusion criteria
Other	• Structure and physiochemical properties • Reviews and regulatory documents	Not on topic, including:[a] • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant • Bioremediation, biodegradation, or chemical or physical treatment of HFPO dimer acid and/or its ammonium salt, including evaluation of wastewater treatment technologies and methods for remediation or contaminated water and soil • Ecosystem effects, studies in ecological species that are not relevant to health effects in humans • Studies of environmental fate and transport of HFPO dimer acid and/or its ammonium salt compounds in environmental media • Analytical methods for detecting/measuring HFPO dimer acid and/or its ammonium salt compounds in environmental media and use in sample preparations and assays • Studies describing the manufacture and use of HFPO dimer acid and/or its ammonium salt compounds • Not chemical-specific (studies that do not involve testing of HFPO dimer acid and/or its ammonium salt compounds) • Studies that describe measures of exposure to HFPO dimer acid and/or its ammonium salt compounds without data on associated health effects

Notes: PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; PBTK/TD = physiologically based toxicokinetic and toxicodynamic; PFAS = pre- and polyfluoroalkyl substances.

[a] Although these criteria were used for the peer-reviewed literature, the current document describes environmental fate data submitted by DuPont (now the Chemours Company). A subsequent targeted search for bioconcentration and bioaccumulation data was also conducted. In addition, a summary of occurrence data is also provided in the current document to give context to the toxicity values.

Table A-6. Inclusion-Exclusion Criteria for HFPO Dimer Acid and HFPO Dimer Acid Ammonium Salt Studies after the Public Comment Draft

PECO Parameter	Inclusion criteria	Exclusion criteria
Population	• Humans • Standard mammalian animal models, including rat, mouse, rabbit, guinea pig, hamster, monkey, dog	• Ecological species (*supplemental tag —non-PECO model*) • Alternative animal models in standard laboratory conditions (e.g., *Xenopus*, zebrafish, minipig) (*supplemental tag—non-PECO model*) • Human or animal cells, tissues, or organs (not whole animals); bacteria; nonmammalian eukaryotes; other nonmammalian laboratory species (*supplemental tag—mechanistic*)
Exposure	• Exposure is to HFPO dimer acid and/or its ammonium salt • Must include 2 or more levels of exposure to HFPO dimer acid and/or its ammonium salt (if not stated, include at title/abstract screening) • **Humans**: Exposure is measured in air, dust, drinking water, diet, or gavage or injection vehicle, or via a biomarker of exposure (PFAS levels in whole blood, serum, plasma, or breast milk) • Any exposure length is acceptable • **Animals**: Exposure via oral route only • Any exposure length for an animal study is acceptable for reproductive or developmental exposures • Exposure duration for all other animal study designs require an exposure duration of 28 days or more (if not stated, include at title/abstract screening)	• Study population is not exposed to HFPO dimer acid and/or its ammonium salt • There is only 1 exposure group (*supplemental tag—single-dose group in study*) • Exposure is to a mixture only without evaluating HFPO dimer acid and/or its ammonium salt individually (*supplemental tag—mixture study*) • Exposure via inhalation, dermal, intraperitoneal, or intravenous injection routes (*supplemental tag—non-oral route of administration*) • Exposure is via cells in culture or subcellular matrices (*supplemental tag—mechanistic*) • Acute exposures (< 28 days) in animal studies (*supplemental tag—acute/short-term duration exposures*)
Comparator	• A concurrent control group exposed to vehicle-only treatment or an untreated control • A comparison or referent population exposed to HFPO dimer acid and/or its ammonium salt at lower levels (or no exposure/exposure below detection limits) or for shorter periods of time • Biological monitoring (e.g., whole blood, serum, plasma, or breast milk) that can be used to establish a range of exposure	• Case reports and case series (*supplemental tag—case report or case series*)

PECO Parameter	Inclusion criteria	Exclusion criteria
Outcome	• Studies that include a measure of one or more health effect endpoints, including effects on reproduction, development, developmental neurotoxicity, liver, thyroid, immune system, nervous system, genotoxicity, and cancer • Qualitative or quantitative description of absorption, distribution, metabolism, elimination, and toxicokinetic and/or toxicodynamic models (e.g., PBPK, PBTK, PBTK/TD)	• *In vivo* and/or *in vitro* studies related to toxicity mechanisms, physiological effects/adverse outcomes, and studies useful for elucidating toxic modes of action (*supplemental tag—mechanistic*) • Studies addressing risks to infants, children, pregnant women, occupational workers, the elderly, and any other susceptible or differentially exposed populations (*supplemental tag—susceptible population*)
Other Exclusion Criteria		• Not on topic, including: • Structure and physiochemical properties (*supplemental tag—structure and physiochemical properties*) • Reviews and regulatory documents (*supplemental tag—other assessments or records with no original data*) • Abstract only, inadequately reported abstract, or no abstract and not considered further because study was not potentially relevant (*supplemental tag—conference abstract*) • Ecosystem effects, studies in ecological species that are not relevant to health effects in humans (*supplemental tag—non-PECO model*) • Bioaccumulation of the target chemical in fish (*supplemental tag—bioaccumulation data in fish*) • Studies of environmental fate and transport of HFPO dimer acid and/or its ammonium salt compounds in environmental media or food (*supplemental tag—environmental fate or occurrence*) • Studies that describe measures of exposure to HFPO dimer acid and/or its ammonium salt compounds without data on associated health effects (*supplemental tag—exposure characteristics*) • Bioremediation, biodegradation, or chemical or physical treatment of HFPO dimer acid and/or its ammonium salt, including evaluation of wastewater treatment technologies and methods for remediation or contaminated water and soil • Analytical methods for detecting/measuring HFPO dimer acid and/or its ammonium salt compounds in environmental media and use in sample preparations and assays • Studies describing the manufacture and use of HFPO dimer acid and/or its ammonium salt compounds • Not chemical specific (studies that do not involve testing of HFPO dimer acid and/or its ammonium salt compounds)

Notes: **PBPK = physiologically based pharmacokinetic; PBTK = physiologically based toxicokinetic; PBTK/TD = physiologically based toxicokinetic and toxicodynamic; PFAS = per- and polyfluoroalkyl substances.**

Table A-7. Supplemental Tags for the GenX Chemicals Literature Search

Category	Evidence
Mechanistic studies	Studies reporting measurements related to a health outcome that inform the biological or chemical events associated with phenotypic effects, in both mammalian and non-mammalian model systems, including *in vitro*, *in vivo* (by various routes of exposure), *ex vivo*, and *in silico* studies. When possible, mechanistic studies will be sub-tagged as pertinent to cancer, non-cancer, or unclear/unknown.
Non-mammalian model systems	Studies in non-mammalian model systems (e.g., fish, birds, *C. elegans*).
ADME and toxicokinetic	Studies designed to capture information regarding absorption, distribution, metabolism, and excretion, including toxicokinetic studies. Such information might be helpful in updating or revising the parameters used in existing PBPK models.
Acute/short-term duration exposures	Animal studies of less than 28 days.
Single-dose group	Studies that used only a single-dose group were tagged as supplemental due to the GenX chemicals database having several multi-dose group studies.
Exposure characteristics	Studies that include data unrelated to toxicological endpoints, but which provide information on exposure sources or measurement properties of the environmental agent (e.g., demonstrate a biomarker of exposure).
Susceptible populations	Studies that identify potentially susceptible subgroups (e.g., studies that focus on a specific demographic, life stage, or genotype).
Mixture studies	Studies not considered PECO-relevant because they do not contain an exposure or treatment group assessing only the chemical of interest.
Non-oral routes of exposure	Studies not addressing routes of exposure that fall outside the PECO scope, and include inhalation and dermal exposure routes
Case studies or case series	Case reports and case series will be tracked as potentially relevant supplemental information.
Records with no original data	Records that do not contain original data such as other agency assessments, informative scientific literature reviews, editorials, or commentaries.
Conference abstracts	Records that contain insufficient documentation to support study evaluation and data extraction.

Category	
Bioaccumulation in fish	BAFs were mentioned in the public comment draft assessment.

Notes: ADME = absorption, distribution, metabolism, and excretion; BAFs = bioaccumulation factors; PBPK = physiologically based pharmacokinetic; and PECO = population, exposure, comparator, and outcome.

Appendix B: Acute and 7-Day Study Summaries

This appendix summarizes studies evaluating acute exposure to hexafluoropropylene oxide (HFPO) dimer acid or HFPO dimer acid ammonium salt by the oral, dermal, and inhalation routes of exposure and investigating dermal and eye irritation.

Oral Toxicity. In a study of the HFPO dimer acid ammonium salt (no Test Guideline (TG) cited), a single dose of 1.5, 12, 130, 1,000, 2,250, 3,400, 5,000, 7,500, 11,000, 12,963, or 17,000 milligrams per kilogram (mg/kg) of HFPO dimer acid ammonium salt was administered by stomach tube to young male rats. The approximate lethal dose (ALD) was determined to be 7,500 mg/kg. Discomfort, gasping, and tonic convulsions were observed before death at lethal doses (7,500 mg/kg and higher). Discomfort, increased water intake, inactivity, polyuria, and initial weight loss were observed in rats at the three highest sublethal doses (2,250 mg/kg, 3,400 mg/kg, and 5,000 mg/kg). Slightly enlarged livers with enlarged hepatocytes and pronounced cell membranes were also observed in rats at the three highest sublethal doses. Slight-to-moderate degenerative changes in the pancreas were also observed in doses at 2,250 mg/kg and higher. No effects were observed at doses of less than or equal to 1,000 mg/kg (DuPont-2-63, 1963).

In another study evaluating toxicity of HFPO dimer acid ammonium salt by the oral route of exposure (no TG identified), a single dose of 670, 2,300, 3,400, 5,000, 7,500, or 11,000 mg/kg of HFPO dimer acid ammonium salt (purity > 99%) was administered to 7-week-old male rats (1/dose group). Rats were evaluated for clinical signs of toxicity over a 14-day observation period. No clinical signs of toxicity were observed in the rat dosed at 670 mg/kg. Rats dosed at 2,300 and 3,400 mg/kg exhibited weight loss (17% and 14%, respectively); ruffled fur; and a wet, yellow-stained perineum at 1 day postexposure. The rats dosed at 2,300 and 3,400 mg/kg no longer exhibited these effects at 2 days and 4 days postexposure, respectively. Rats dosed with greater than or equal to 5,000 mg/kg died by 1 day after dosing. The rat dosed with 11,000 mg/kg exhibited lethargy, low carriage, and low posture before its death. The ALD was determined to be 5,000 mg/kg (DuPont-770-95, 1996).

A single dose of HFPO dimer acid ammonium salt (82.6% purity) was administered by oral gavage to 10- to 11-week-old female rats at a dose of 175, 550, 1750, or 5,000 mg/kg (1–3 rats/group) in a study conducted according to Organization of Economic Cooperation and Development (OECD) TG 425 (Up-and-Down Procedure) (OECD, 2008c). Rats were then evaluated for clinical signs of toxicity over a 14-day observation period. All rats exhibited clinical signs of toxicity such as hair loss, lethargy, high posture, stained fur/skin, clear ocular discharge, prostrate posture, partially closed eyes, or salivation. With the exception of hair loss, clinical signs disappeared by 2 days postexposure. All three rats dosed at 5,000 mg/kg died within 2 days after dosing. Grossly observable evidence of organ or tissue damage in these rats included discoloration of lungs (rat #1651), discoloration of lungs and mandibular lymph nodes (rat #1746), and discoloration of lungs and liver (rat #1975). No visible lesions were observed in females dosed at 175 mg/kg, 550 mg/kg, or 1,750 mg/kg. With the exception of rats dosed at 5,000 mg/kg, increases in body weight (BW) were observed in all rats over the course of the study. The oral median lethal dose (LD_{50}) was estimated to be 3,129 mg/kg for female rats (DuPont-22932, 2007).

Oral toxicity of HFPO dimer acid ammonium salt was also evaluated in male rats in a study conducted according to OECD TG 425 (OECD, 2008c). A single dose of HFPO dimer acid ammonium salt (86% purity) was administered by oral gavage to 9- to 11-week-old male rats at a dose of 175, 550, 1,750, or 5,000 mg/kg (three rats). Rats were then evaluated for clinical signs of toxicity over a 14-day observation period. All rats exhibited clinical signs of toxicity such as lethargy, wet fur, stained fur/skin, decreased muscle tone, low posture, or lung noise. One rat dosed at 1,750 mg/kg and all three rats dosed at 5,000 mg/kg died either the day dosed or by the day after dosing. Grossly observable evidence of organ or tissue damage in rats dosed at 5,000 mg/kg included expanded lungs and discolored stomach, discoloration and cloudiness of eyes, and stained skin. With the exception of rats dosed at 5,000 mg/kg, increases in BW were observed in all rats over the course of the study. The oral LD_{50} was determined to be 1,750 mg/kg for male rats (DuPont-25438 RV1, 2008).

Another study evaluated oral toxicity of HFPO dimer acid in both male and female rats in a study conducted according to OECD TG 425 (OECD, 2008c). A single dose of HFPO dimer acid (98% purity) was administered to 9- to 11-week-old rats. Males were dosed at 175, 550, 1,750, or 5,000 mg/kg (2–6 rats/group). Female rats were also dosed at 175, 550, 1,750, or 5,000 mg/kg (1–4 rats/group). Clinical signs were not observed in rats dosed at 175 mg/kg or in one male rat dosed at 550 mg/kg. The rest of the rats in this study exhibited clinical signs of toxicity. Clinical signs of toxicity in male rats observed up to 5 days after dosing included lung noise, absent feces, lethargy, not eating, stained fur/skin, wet fur, labored breathing, decreased muscle tone, prostrate posture, tremors, clear oral discharge, diarrhea, ataxia, and/or high posture. Clinical signs in female rats were observed for up to 3 days after dosing and included wet fur, stained fur/skin, ataxia, labored breathing, cold to touch, clear ocular or oral discharge, lethargy, lung noise, absent feces, not eating, and/or rubbing face on the bottom of the cage (DuPont-25875, 2008).

All rats dosed at 5,000 mg/kg died by the day after dosing. Among rats dosed at 1,750 mg/kg, two males and three females died by the day after dosing. One male rat dosed at 550 mg/kg (rat #274) was sacrificed in extremis on the fourth day after dosing following a 23% reduction in BW. Gross findings were detected in three male rats dosed at 5,000 mg/kg, in four rats dosed at 1,750 mg/kg, and in one rat dosed at 550 mg/kg. Small testes and epididymis were observed in rat #274. A discolored, glandular stomach was observed in two of the male rats dosed at 1,750 mg/kg. Gross findings for male rats dosed at 5,000 mg/kg included a glandular stomach; a glandular, discolored stomach (rats #640, #796, and #821); and discolored skin (rat #796). Gross findings for female rats dosed at 1,750 mg/kg included a glandular, discolored stomach (rats #478, #527, and #626); discolored lymph nodes (rat #527); and discolored skin (#527). The female rat dosed at 5,000 mg/kg exhibited wet skin; a discolored esophagus with foamy fluid; and a thick, discolored stomach. Increases in BW were observed in animals that survived until the end of the study. The oral LD_{50} was estimated to be 1,730 mg/kg for male rats and 1,750 mg/kg for female rats (DuPont-25875, 2008).

Another study conducted according to OECD TG 425 (OECD, 2008c) evaluated toxicity of HFPO dimer acid by the oral route of exposure in female mice. A single dose of HFPO dimer acid ammonium salt (86% purity) was administered to 8- to 9-week-old female mice at a dose of 175, 550, or 1,750 mg/kg (1–3 mice). No clinical signs of toxicity were observed in mice dosed at 175 mg/kg or in two mice dosed at 550 mg/kg. One mouse dosed at 550 mg/kg, however,

exhibited wet fur on the day of dosing. All three mice dosed at 1,750 mg/kg died on the day of dosing. Discoloration of lungs and an ovarian cyst were observed in a mouse dosed at 550 mg/kg. Skin stain was also observed in two mice dosed at 1,750 mg/kg. These observations were considered by study authors to be nonspecific and not indicative of test substance related. With the exception of mice dosed at 1,750 mg/kg, increases in BW were observed in all mice over the course of the study. The oral LD_{50} was estimated to be 1,030 mg/kg for female mice (DuPont-24126, 2007).

Dermal Toxicity. In a study evaluating toxicity through dermal absorption (no TG identified), 5,000 mg/kg of HFPO dimer acid ammonium salt (purity > 99%) was applied directly onto the shaved, intact skin of two young adult male New Zealand white rabbits for a period of 24 hours. One rabbit exhibited necrosis from days 2–6 post-application in a small area of treated skin. The necrotic area sloughed off by day 7, and alopecia was then observed in this area until the study was completed. Moderate erythema was observed in both rabbits at 1 day post-application and was still observed up to 3 days post-application. Erythema persisted until 13 days post-application, with the degree of severity decreasing over time. Both rabbits exhibited scaling and sloughing of skin 6–13 days after application. Increases in BW were observed for both rabbits at the conclusion (day 14) of the study. The ALD was determined to be higher than 5,000 mg/kg (DuPont-839-95, 1996).

The dermal toxicity of HFPO dimer acid ammonium salt (86% purity) was also evaluated in rats in a study conducted according to OECD TG 402 (OPPTS 870.1200) (OECD, 2017). A single dose of 5,000 mg/kg (five males and five females) was applied directly onto the shaved, intact skin for 24 hours. Rats were then observed daily for 14 days posttreatment. All female rats exhibited mild erythema on the test site 1 day post-application. Erythema was no longer detectable by the second day after application. Erythema was not observed in male rats. Hyperkeratosis was observed in four male and four female rats. Ulceration was observed in one male and two female rats. All dermal effects cleared up by 13 days posttreatment. Increases in BW were observed for male and female rats by the conclusion (day 14) of the study. The LD_{50} of the compound was determined to be higher than 5,000 mg/kg (DuPont-24113, 2007).

Inhalation Toxicity. The toxicity of HFPO dimer acid ammonium salt by the inhalation route of exposure was evaluated in 8-week-old male and female rats (no TG identified) (DuPont-17751-723, 2009). One group of five male and five female rats were exposed to an aerosol atmosphere containing 5,200 milligrams per cubic meter (mg/m^3) of HFPO dimer acid ammonium salt (84% purity) to determine the inhalation median lethal concentration (LC_{50}). Two other groups of three male and three female rats were exposed to HFPO dimer acid ammonium salt at concentrations of 0, 13, and 100 mg/m^3 in air to evaluate respiratory tract pathology. All rats were exposed nose-only for a single 4-hour period. Rats exposed to 0, 13, and 100 mg/m^3 of HFPO dimer acid ammonium salt in air were evaluated for clinical signs of toxicity for 2 days following exposure and rats exposed to 5,200 mg/m^3 were evaluated for a period of 14 days following exposure. Respiratory tract tissues (lung, larynx/pharynx, trachea, and nose) of the 0-, 13-, and 100-mg/m^3 exposure groups were also evaluated microscopically. According to study authors, no clinical signs of toxicity were observed for any animals at any exposure in this study. However, following the 100 mg/m^3 exposure, all rats displayed a red nasal discharge immediately after exposure. Rats exposed to 5,200 mg/m^3 exhibited red discharge from eyes, nose, and mouth as well as red stains on skin/fur immediately after exposure. Red discharge and staining were absent

within 1 or 2 days after exposure. Rats in the 5,200-mg/m^3 exposure group lost 2.5% to 6.8% of their original BW for 1 or 2 days after exposure but exhibited normal weight gain for the remainder of the experiment. The LC_{50} was determined to be greater than 5,200 mg/m^3 (DuPont-17751-723, 2009).

Dermal Irritation. The dermal irritation of HFPO dimer acid ammonium salt (86% purity) was evaluated in three male New Zealand white rabbits in a study conducted according to OECD TG 404 (OPPTS 870.2500) (OECD, 2002). A 0.5-mL aliquot of the compound was applied to an area of shaved skin for a period of 4 hours. Very slight erythema was observed in one rabbit following removal of the compound. At 60 minutes post-application, very slight erythema was observed in one rabbit and well-defined erythema was observed in the other two rabbits. Erythema had cleared by 24 hours postexposure (DuPont-24030, 2007).

Eye Irritation. In an OECD TG 405 (OPPTS 870.2400) (OECD, 2020a) study evaluating eye irritation of HFPO dimer acid ammonium salt (86% purity), a 0.1-mL aliquot of compound was administered to one eye of a young adult male New Zealand white rabbit. Necrosis, characterized by brown and white discoloration of the conjunctival membrane of the treated eye, was observed at 1, 24, and 28 hours after application. Corneal opacity, iritis, conjunctival chemosis, and discharge were also observed. Fluorescein stain examination of the treated eye indicated corneal injury (DuPont-24114, 2007).

Seven-Day Toxicity Studies. Four 7-day studies are available for HFPO dimer acid or ammonium salt in rats or mice. The toxicity of HFPO dimer acid ammonium salt (86.6% purity) by the oral route of exposure was evaluated in 6-week-old male and female rats (DuPont-24009, 2008). Five rats of each sex were exposed to 0, 30, 300, or 1,000 mg/kg HFPO by oral gavage for 7 days. No clinical signs of toxicity were observed in either sex at any dose level tested. A significant decrease in BW was observed on test day 7 in males exposed to 1,000 mg/kg versus control. Significant decreases in red blood cells (RBCs), hemoglobin, and hematocrit were observed in male rats at 300 milligrams per kilogram per day (mg/kg/day) and in both male and female rats at 1,000 mg/kg/day. A significant increase in red cell distribution width, reticulocytes, and neutrophils was also observed in female rats exposed to 1,000 mg/kg/day. Decreases in serum lipids and globulins were observed in males at all dosage groups as well as in females at 300 and 1,000 mg/kg/day. Increased alanine aminotransferase, urea nitrogen, and glucose as well as decreased sorbitol dehydrogenase, creatinine, and calcium were observed at doses of 300 and/or 1,000 mg/kg/day. Increases in liver weight were observed in males at all doses and in females at 1,000 mg/kg/day and corresponded with increases in B-oxidation and/or increases in P450 enzyme activity. Mild-to-minimal hepatocellular hypertrophy was also observed in both sexes at 1,000 mg/kg/day. Decreases in heart weight were observed in males at 1,000 mg/kg and increases in kidney weight were observed in females at 1,000 mg/kg/day: No microscopic changes were observed in these organs.

In another study evaluating toxicity of HFPO dimer acid (99% purity) by the oral route of exposure, 6-week-old male and female rats (5/sex) were exposed to 0, 30, 100, and 300 mg/kg of HFPO dimer acid by gavage over a period of 7 days (DuPont-24116, 2008). No clinical signs of toxicity were observed. Significant decrease in RBC count and a significant increase in red cell distribution width were observed in females at 300 mg/kg/day. Significant decreases in hemoglobin and hematocrit were observed in male rats at 300 mg/kg/day. A significant increase in mean corpuscular cell volume was observed in males at 30 mg/kg/day. Decreases in serum

lipids were detected in all dosed male groups versus control. Increased alkaline phosphatase and urea nitrogen and decreased bilirubin, creatinine, total protein, globulin, and calcium were observed at 30 and/or 300 mg/kg/day. Increased liver weight was observed in males at all doses and in females at 300 mg/kg/day. Microscopic examination of livers detected hepatocellular hypertrophy in all treated males and females. Lesions observed in males and females were mild and minimal, respectively. A statistically significant increase in β-oxidation was detected in females exposed to 300 mg/kg/day versus control.

A 7-day study was conducted in 6-week-old male mice to evaluate toxicity of HFPO dimer acid ammonium salt (86.6% purity) by the oral route of exposure (DuPont-24010, 2008). Doses of 0 or 30 mg/kg/day were administered over a period of 7 days. By test day 7, BWs were significantly higher in exposed males versus controls. A twofold increase in liver weight relative to control was detected in exposed males. No grossly observable lesions in the liver were observed. Microscopic changes in the liver observed at 30 mg/kg/day included minimal single-cell necrosis of hepatocytes, moderate hepatocellular hypertrophy, and moderate increases in mitotic figures. Minimal vacuolation of hepatocytes was also observed in one treated mouse.

Another 7-day gavage study was conducted in 6-week-old male mice to evaluate toxicity of HFPO dimer acid (99% purity) by the oral route of exposure (DuPont-25281, 2008). Doses of 0 or 30 mg/kg/day were administered over a period of 7 days. By test day 7, BWs were significantly higher in exposed males versus controls. A twofold increase in liver weight was detected in exposed males versus control. Microscopic changes to the liver of exposed animals included minimal single-cell necrosis of hepatocytes, moderate hepatocellular hypertrophy, and moderate increases in mitotic figures. Minimal vacuolization was also observed in 2/5 treated mice.

Appendix C: Genotoxicity Study Summary

Table C-1 provides a summary of the available genotoxicity data for hexafluoropropylene oxide (HFPO) dimer acid and/or its ammonium salt.

Table C-1. Genotoxicity Study Summary

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-19713 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD TG 471) (OECD, 2020b)	*Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (strain WP2*uvr*A)	HFPO dimer acid ammonium salt (85% purity) 33.3, 66.7, 100, 333, 667, 1,000, 3,333, and 5,000 µg/plate for preliminary toxicity test 333, 667, 1,000, 3,333, and 5,000 µg/plate for toxicity-mutation test Negative control (sterile water) and positive control (benzo[a]pyrene, 2-nitrofluorine, 2-aminoanthracene, sodium azide, acridine mutagen Institute of Cancer Research (ICR)-191, or 4-nistroquinoline-N-oxide) also included in study	With S9	Negative.
				Without S9	Negative.
DuPont-22620 RV1 (2009)	*In vitro* Mammalian Chromosome Aberration Test (OECD TG 473) (OECD, 1997a)	Chinese hamster ovary cells (CHO-K_1 line)	HFPO dimer acid ammonium salt (83% purity) 49, 98, 244, 489, 977, 1954, and 3391 µg/mL for preliminary toxicity test* 977, 1954, and 3391 µg/mL for the 4-hour nonactivated and activated test conditions* 489, 977, and 1954 µg/mL for the 20-hour nonactivated test condition* Negative control (sterile water) and positive control (mitomycin C or cyclophosphamide) also included in study * Doses have been corrected to account for 83% HFPO dimer acid ammonium salt purity.	With S9	Positive at 3,391 µg/mL* in 4-hour activated test conditions.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-23219 (2007)	*In vivo* Unscheduled DNA Synthesis Test in Mammalian Cells (OECD TG 486) (OECD, 1997b)	Primary hepatocytes harvested from male rats (5/dose group)	HFPO dimer acid ammonium salt (83% purity) 1, 10, 100, 1,000, and 2,000 mg/kg for preliminary toxicity test 500, 1,000, and 2,000 mg/kg/day for Unscheduled DNA Synthesis Test Negative control (distilled water) and positive control (dimethylnitrosamine) also included in study	Negative–No significant increase in the mean number of net nuclear grain counts in hepatocytes at 2–4 or 12–16 hours after dosing.	
Dupont-26129 (2008)	*In vitro* Mammalian Cell Gene Mutation Test (OECD TG 476) (OECD, 1997c)	L5178Y/TK$^{+/-}$ Mouse lymphoma cells	HFPO dimer acid ammonium salt (87% purity) 0.5, 1.5, 5, 15, 50, 150, 500, 1,500, and 3,500 µg/mL for both non-activated and S9-activated cultures at both 4-hour and 24-hour exposures for preliminary toxicity assay 500, 750, 1,000, 1,500, and 2,000 µg/mL for nonactivated cultures with a 4-hour exposure 150, 250, 500, 600, and 750 µg/mL for S9-activated cultures with a 4-hour exposure 250, 500, 600, 750, and 1,000 µg/mL for nonactivated cultures with a 24-hour exposure Negative control (sterile, distilled water) and positive control (methyl methanesulfonate or 7,12-dimethylbenz(a)anthracene) also included in study	With S9	Negative.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
Dupont-19714 RV1 (2008)	*In vitro* Mammalian Chromosome Aberration Test (OECD TG 473) (OECD, 1997a)	Chinese hamster ovary cells (CHO-K_1 line)	HFPO dimer acid ammonium salt (85% purity) 0.3, 1, 3, 10, 30, 100, 300, 1,000, and 3,471 µg/mL for preliminary toxicity test 100, 500, 1,000, 2500, and 3,471 µg/mL for the chromosome aberration assay for the 4-hour nonactivated, 4-hour S9-activated, and 20-hour nonactivated test conditions Cytogenetic evaluations were conducted at 1,000, 2,500, and 3,471 µg/mL for the 4-hour nonactivated and 4-hour S9-activated test conditions and at 100, 500, and 1,000 µg/mL for the 20-hour nonactivated test condition	With S9	The percentage of cells with structural aberrations in the test substance-treated groups was not increased above that of the vehicle control at any concentration. The percentage of cells with numerical chromosome aberrations at 2,500 and 3,471 µg/mL in the 4-hour S9-activated test conditions was increased in a dose-dependent manner above that of the vehicle control. The change was outside the historical control range and considered biologically relevant.

Study	Assay	Strain/Species	Dosing	Activation	Results
			Negative control (sterile water) and positive control (mitomycin-C or cyclophosphamide) also included in study	Without S9	In the 20-hour nonactivated test condition, substantial toxicity was observed at 3,471 µg/mL and a substantial reduction in mitotic index relative to vehicle control was observed in the mitotic index relative to vehicle control. The percentage of cells with structural aberrations in the test substance-treated groups was not increased above that of the vehicle control at any concentration. An increase in the percentage of cells with numerical chromosome aberrations was observed at 3,471 µg/mL in the 4-hour nonactivated condition relative to vehicle control.
DuPont-22734 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD TG 471) (OECD, 2020b)	*Salmonella typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *Escherichia coli* (strain WP2*uvr*A)	HFPO dimer acid ammonium salt (82.6% purity) 32.5, 65.2, 97.7, 325, 652, 977, 3,256, and 4,885 µg/plate for the toxicity-mutation assay* 325, 652, 977, 3256, and 4885 µg/plate for the mutagenicity test* * Doses have been correct to account for 82.6% HFPO dimer acid ammonium salt purity.	With S9	Negative.
				Without S9	Negative.

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-23220 (2007)	*In vivo* Micronucleus and Chromosome Aberration Assay (OECD TGs 474 and 475) (OECD, 2014, 2016b)	Primary bone marrow cells harvested from male and female ICR mice (2 males or 5 of each sex/dose for preliminary toxicity study) (5 of each sex/dose for toxicity study) (5 of each sex/dose for Micronucleus and Chromosome Aberration Assay)	HFPO dimer acid ammonium salt (82.6% purity) 1, 10, 98, 975, and 1,950 mg/kg by oral gavage for preliminary toxicity study* 1170, 1365, 1560, and 1,755 mg/kg by oral gavage for toxicity study* 317, 634, and 1,268 mg/kg by oral gavage for Micronucleus and Chromosome Aberration Assay* Positive control (colchicine) and negative control (sterile water) also included in the study * Doses have been corrected to account for 82.6% HFPO dimer acid ammonium salt purity.	Negative–No statistically significant increases in the incidence of micronucleated polychromatic erythrocytes or structural or numerical chromosomal aberrations in bone marrow of male and female ICR mice at doses up to and including the maximum tolerated dose (1,268 mg/kg).	

Notes: DNA = deoxyribonucleic acid; µg/mL = micrograms per milliliter; µg/plate = micrograms per plate; mg/kg = milligrams per kilogram; mg/kg/day = milligrams per kilogram per day; OECD = Organization for Economic Cooperation and Development; TG = test guideline.

Appendix D: NTP PWG Final Report on the Pathology Peer Review of Liver Findings

FINAL REPORT

December 4, 2019

PATHOLOGY PEER REVIEW
OF LIVER FINDINGS

H-28548: SUBCHRONIC TOXICITY 90 DAY GAVAGE STUDY IN MICE
(PROJECT ID: DUPONT-18405-1307)
&
AN ORAL (GAVAGE) REPRODUCTION/DEVELOPMENTAL TOXICITY SCREENING
STUDY OF H-28548 IN MICE
(STUDY NUMBER WIL-189225)
(STUDY SPONSOR NUMBER: DUPONT-18405-1037)

Prepared by:

Susan A. Elmore, MS, DVM, DACVP, DABT, FIATP
Amy Brix, DVM, PhD, DACVP

INTRODUCTION

The study report, summary tables and individual animal findings, along with hematoxylin and eosin stained microscope slides used in the Subchronic Toxicity 90 Day Gavage Study In Mice (Project ID: DuPont-18405-1307) and An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 In Mice (Study Number WIL-180225) (Project ID: DuPont-18405-1037) were received by the NTP reviewing pathologist. The slides for review each contained two liver lobes presumed to be the left and median lobes. The data and slides of the liver were reviewed per NTP standards (Sills et al, 2017), and the results are summarized in this report. The experimental design for this study is as follows:

DUPONT-18405-1307 **SUBCHRONIC TOXICITY STUDY**		
DOSAGE (mg/kg/day)	**MICE**	
	MALE	**FEMALE**
0	10	10
0.1	10	10
0.5	10	10
5	10	10

DUPONT-18405-1037 **REPRODUCTIVE/DEVELOPMENTAL TOXICITY STUDY**		
DOSAGE (mg/kg/day)	**F_0 MICE**	
	MALE	**FEMALE**
0	25	25
0.1	25	25
0.5	25	25
5	25	25

SUMMARY OF ORIGINAL STUDY RESULTS

DUPONT-18405-1307 (Subchronic Toxicity 90 Day Gavage Study)

The following information is excerpted from the Final Report entitled "H-28548: Subchronic Toxicity 90 Day Gavage Study in Mice," dated February 19, 2010:

> In 5 mg/kg/day male and female dose groups, increases were observed in the incidence of single cell necrosis, mitotic figures, and/or pigment. The liver effects at 5 mg/kg/day correlated with clinical chemistry effects and were considered test substance related and adverse. Other test substance-related effects were observed in the livers of 0.5 and 5 mg/kg/day males and 5 mg/kg/day females, including increases in absolute and/or relative liver weight, enlarged and/or discolored livers, and centrilobular hepatocellular hypertrophy. The liver effects observed in 0.5 mg/kg/day males were considered to be non-adverse adaptive responses as they were not correlated with clinical or microscopic pathology evidence of liver toxicity.

DUPONT-18405-1037 (Reproduction/Developmental Toxicity Screening Study)

The following information is excerpted from the Final Report entitled "An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice," dated December 29, 2010:

> In male and female mice given 5 mg/kg/day, mild to moderate hepatocellular hypertrophy was observed microscopically. The hepatocellular hypertrophy was characterized by cytoplasmic eosinophilic stippling that is consistent with peroxisome proliferation and was associated with correlative increases in liver weights. Other microscopic changes in the liver at 5 mg/kg/day included increases in single cell necrosis, mitotic figures, pigment, and focal necrosis (females only). In male and female mice given 0.5 mg/kg/day, the incidence and severity of hepatocellular hypertrophy, as well as the correlative liver weight changes was reduced. Other lesions at the 0.5 mg/kg/day dose level were limited to minimal single cell necrosis in 5 of 24 males.

SLIDE REVIEW WORK SHEETS (SRWS)

The Slide Review Work Sheets are presented in appendix A (Dupont-18405-1307 Subchronic Toxicity Study) and appendix B (Dupont-18405-1037 Reproduction/ developmental Toxicity Screening Study). These work sheets list, in animal ID number order, the original study pathologist's findings, along with the reviewing pathologist's comments. Entries other than "Agree" under the reviewing pathologist's comments indicate a disagreement with the study pathologist's (SP's) diagnosis. In each instance, space is provided to record remarks made during the Slide Review.

FINDINGS OF THE SLIDE REVIEW

DUPONT-18405-1307 (Subchronic Toxicity 90 Day Gavage Study)

The slides reviewed during this quality assessment were of adequate quality and had no artifacts that interfered with making diagnoses. The liver was reviewed from all animals for all lesions. It was requested by the NTP pathologist that the reviewing pathologist use the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al, 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al, 2016). The study pathologist diagnosed hepatocellular hypertrophy which included the morphologic change of eosinophilic stippling commonly observed with peroxisome proliferators. The reviewing pathologist agreed that there was hypertrophy of the hepatocytes, but often regarded the severity to be less than recorded by the study pathologist. In addition, the reviewing pathologist recommended adding the diagnosis cytoplasmic alteration to account for the brightly eosinophilic, frequently granular, appearance of the cytoplasm of hepatocytes. After reviewing these lesions with the NTP pathologist, the NTP pathologist recommended using the term cytoplasmic alteration to encompass both hypertrophy and eosinophilic change to the hepatocytes, as she considered them part of the same process. The reviewing pathologist agreed with most occurrences and severities of single cell necrosis. However, the reviewing pathologist also observed apoptosis, and recommended adding the diagnosis of "apoptosis, hepatocellular" when present. Descriptions of individual lesions recorded during this review are listed below. The summary incidences are found in Table 1.

DUPONT-18405-1037 (Reproduction/Developmental Toxicity Screening Study)

The slides reviewed during this quality assessment were of adequate quality and had no artifacts that interfered with making diagnoses. The liver was reviewed from all animals for all lesions. For the most part, the reviewing pathologist agreed with the study pathologist's diagnoses and severities. It was requested by the NTP pathologist that the reviewing pathologist use the terminology of the INHAND document containing standardized terminology of the liver (Thoolen et al, 2010) except where it would be superseded by the terminology published by the INHAND committee with reference to cell death/necrosis/apoptosis (Elmore et al, 2016). The study pathologist diagnosed hepatocellular hypertrophy which included the morphologic change of eosinophilic stippling commonly observed with peroxisome proliferators. The reviewing pathologist agreed that there was hypertrophy of the hepatocytes, but used the terminology "cytoplasmic alteration" at the request of the NTP pathologist, based upon review of the slides from the 18405-1307 subchronic study. The reviewing pathologist agreed with most occurrences and severities of single cell necrosis. However, the reviewing pathologist also observed apoptosis, and recommended adding the diagnosis of "apoptosis, hepatocellular" when present. The reviewing pathologist recorded additional occurrences of mixed cell infiltrates in most groups of animals. Descriptions of individual lesions recorded during this review are listed below. The summary incidences are found in Table 2.

DESCRIPTIONS OF LESIONS

Single cell necrosis (Figures 1 & 2) consisted of individual hepatocytes that had pale, granular, vacuolated or eosinophilic cytoplasm; nuclei were either swollen or pyknotic and karyorrhectic.

The cells frequently appeared fragmented and were often surrounded by degenerative inflammatory cells. Inflammatory cells were not documented separately as they were considered a response to the necrosis. All of the lesions were considered minimal in severity, which was recorded when 1-10 cells were observed in ten 20X fields. Single cell necrosis was not recorded unless at least two affected cells were observed in the entirety of the liver sections examined; if 2 or more necrotic cells were observed, counting of ten 20X fields was done to determine severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

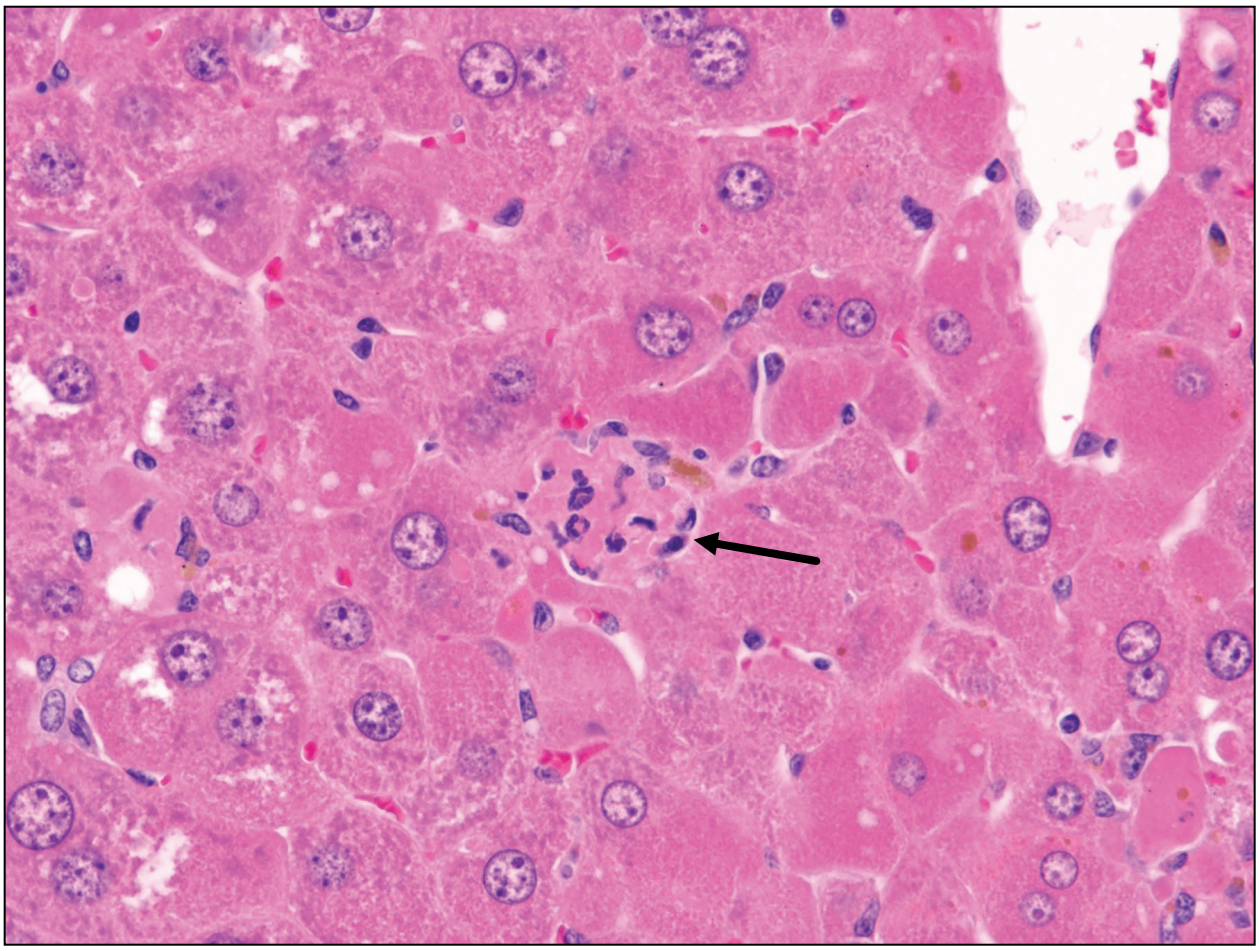

Figure 1. Single cell necrosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The necrotic cell (arrow) is fragmented and surrounded by inflammatory cells.

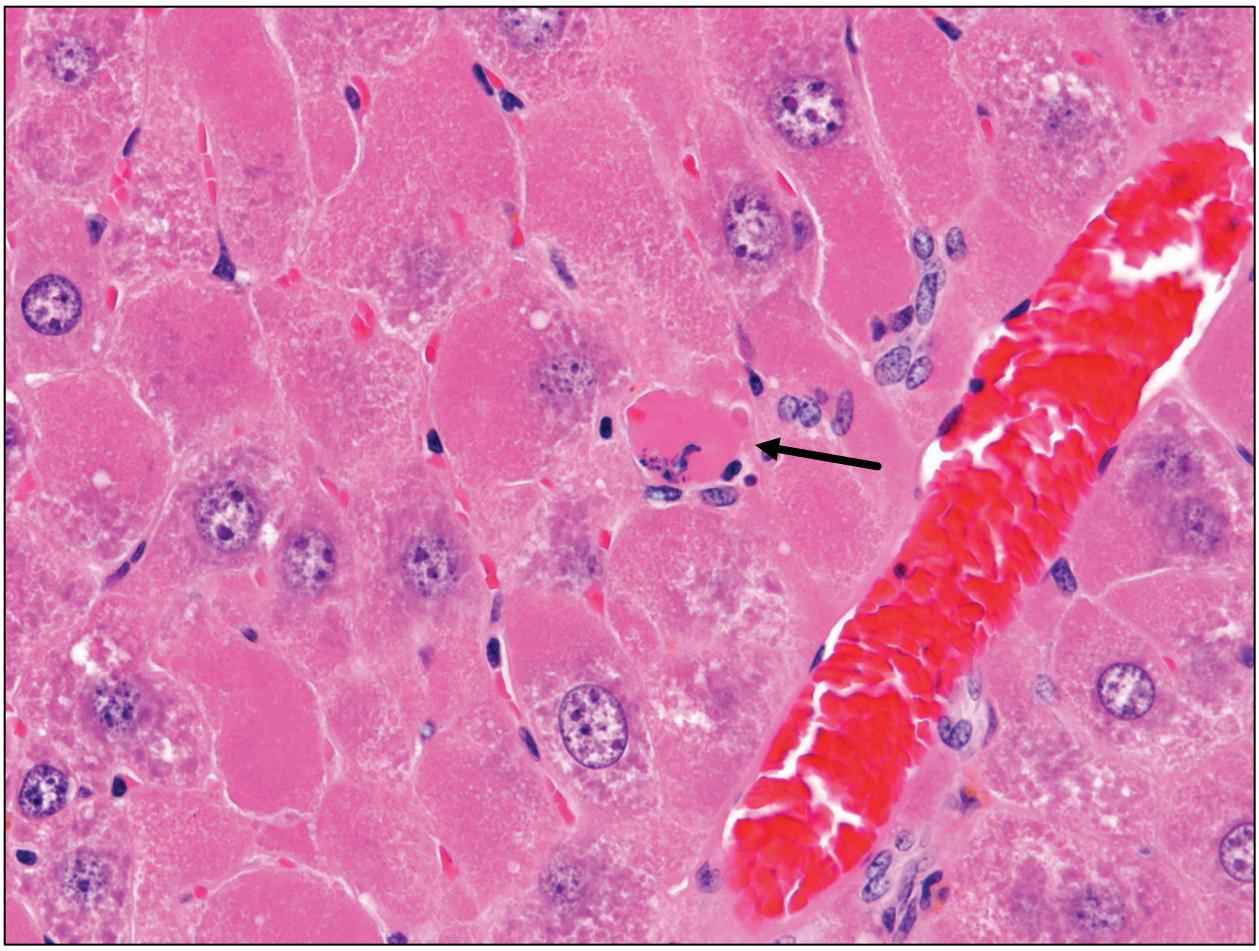

Figure 2. Single cell necrosis in the liver of a Group 4 male mouse (animal 405) from the 18405-1307 subchronic study. The necrotic cell (arrow) is swollen, and has brightly eosinophilic cytoplasm and a karyorrhectic nucleus.

Apoptosis; hepatocellular was recorded when individual hepatocytes were observed that had characteristics, as described in the article by Elmore (Elmore et al, 2016), of apoptosis. Briefly, affected cells were typically shrunken, with hyper-eosinophilic cytoplasm and condensed, pyknotic or karyorrhectic nuclei. The cells were round and often small; occasionally they were phagocytosed by surrounding cells. There was a lack of associated inflammatory cells with apoptotic hepatocytes. Grading was done based upon the Thompson article (Thompson et al, 2018) to be consistent with the reviewing pathologist's grading criteria. All of the lesions were considered minimal to mild in severity, which was recorded when 1-10 cells, or 11-40 cells, respectively, were observed in ten 20X fields. Apoptosis was not recorded unless at least two affected cells were observed in the entirety of the liver sections examined; if 2 or more apoptotic cells were observed, counting of ten 20X fields was done to determine severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

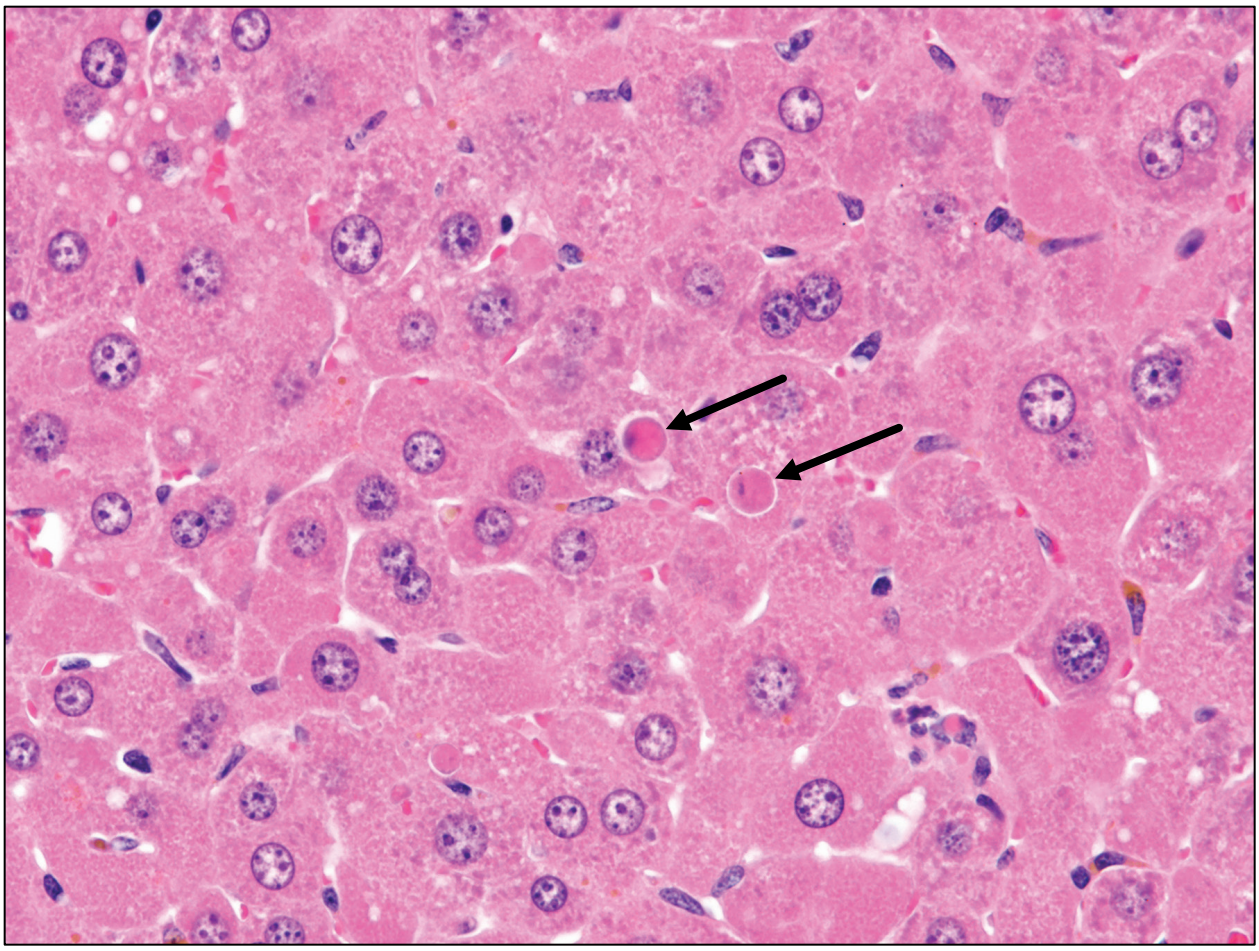

Figure 3. Apoptosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The apoptotic hepatocytes are small, rounded, and brightly eosinophilic (arrows).

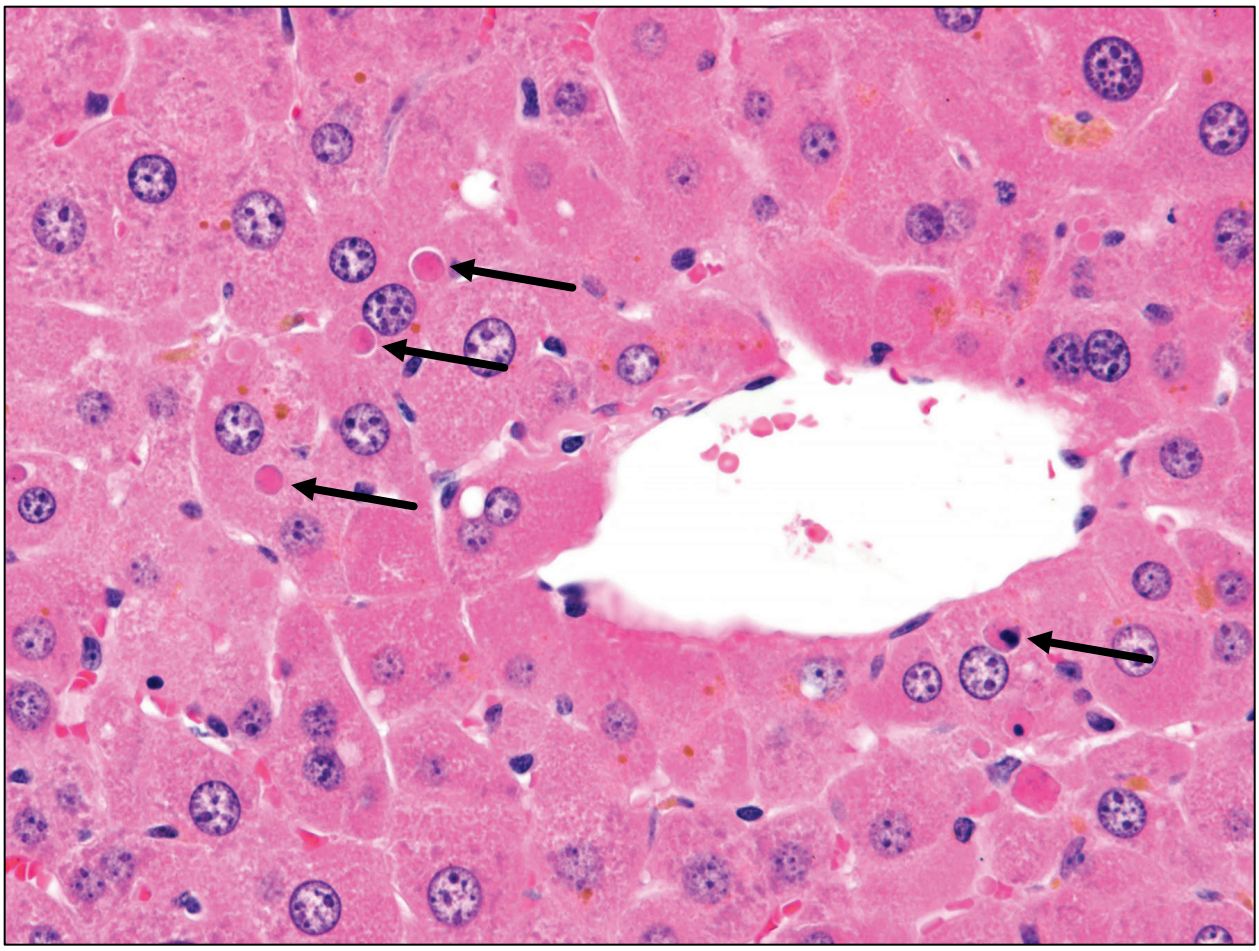

Figure 4. Apoptosis in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. Evidence of apoptosis is provided by small round eosinophilic remnants of hepatocytes (arrows).

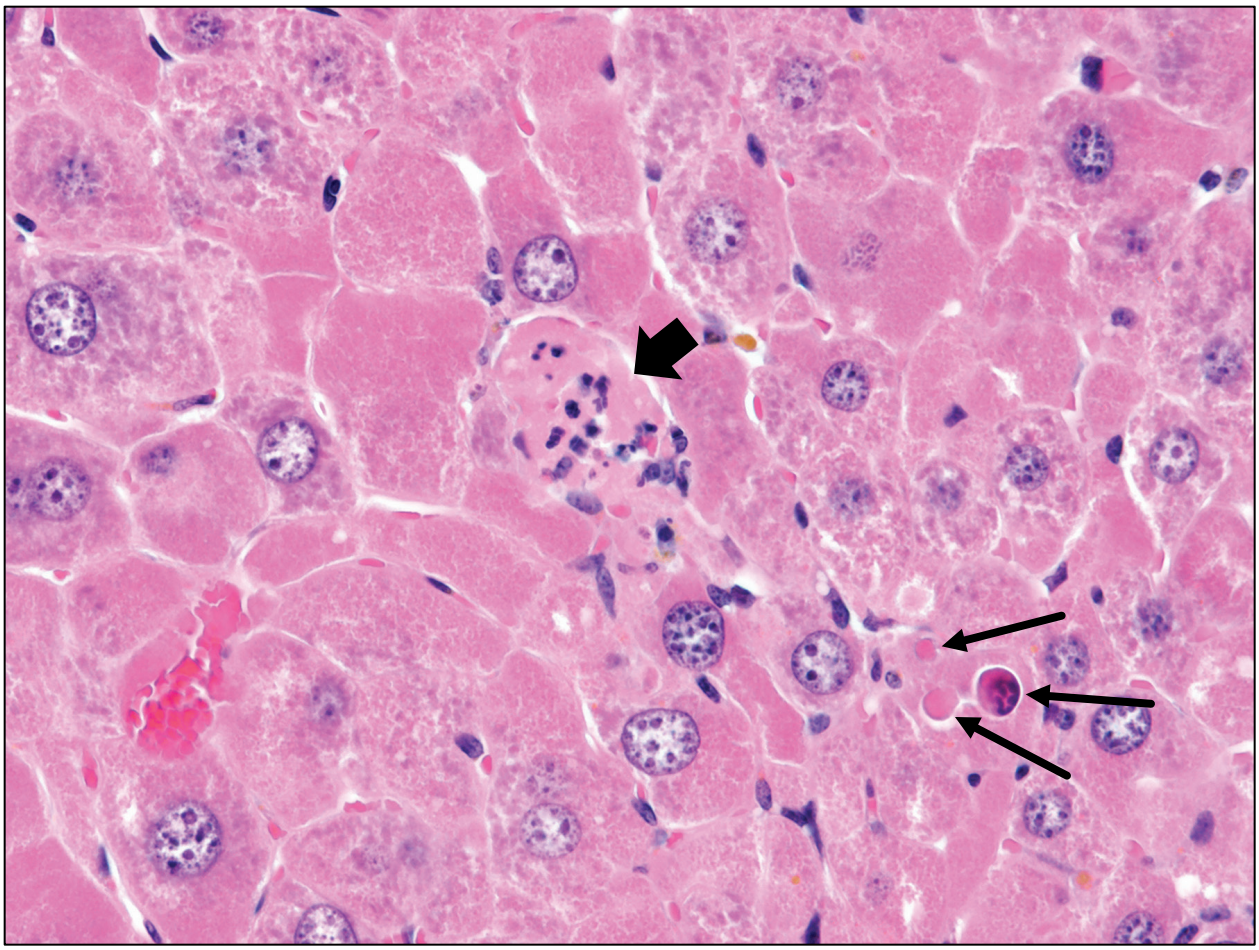

Figure 5. Apoptosis (long arrows) and single cell necrosis (short arrow) in the liver of a Group 4 male mouse (animal 405) from the 18405-1307 subchronic study.

Focal necrosis consisted of a localized area of coagulative necrosis. Generally, there was a loss of cellular detail of the affected hepatocytes; rarely there was a small amount of mineral (dystrophic) associated with the areas of necrosis. Inflammatory cell infiltrates typically ringed the region of necrotic hepatocytes. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

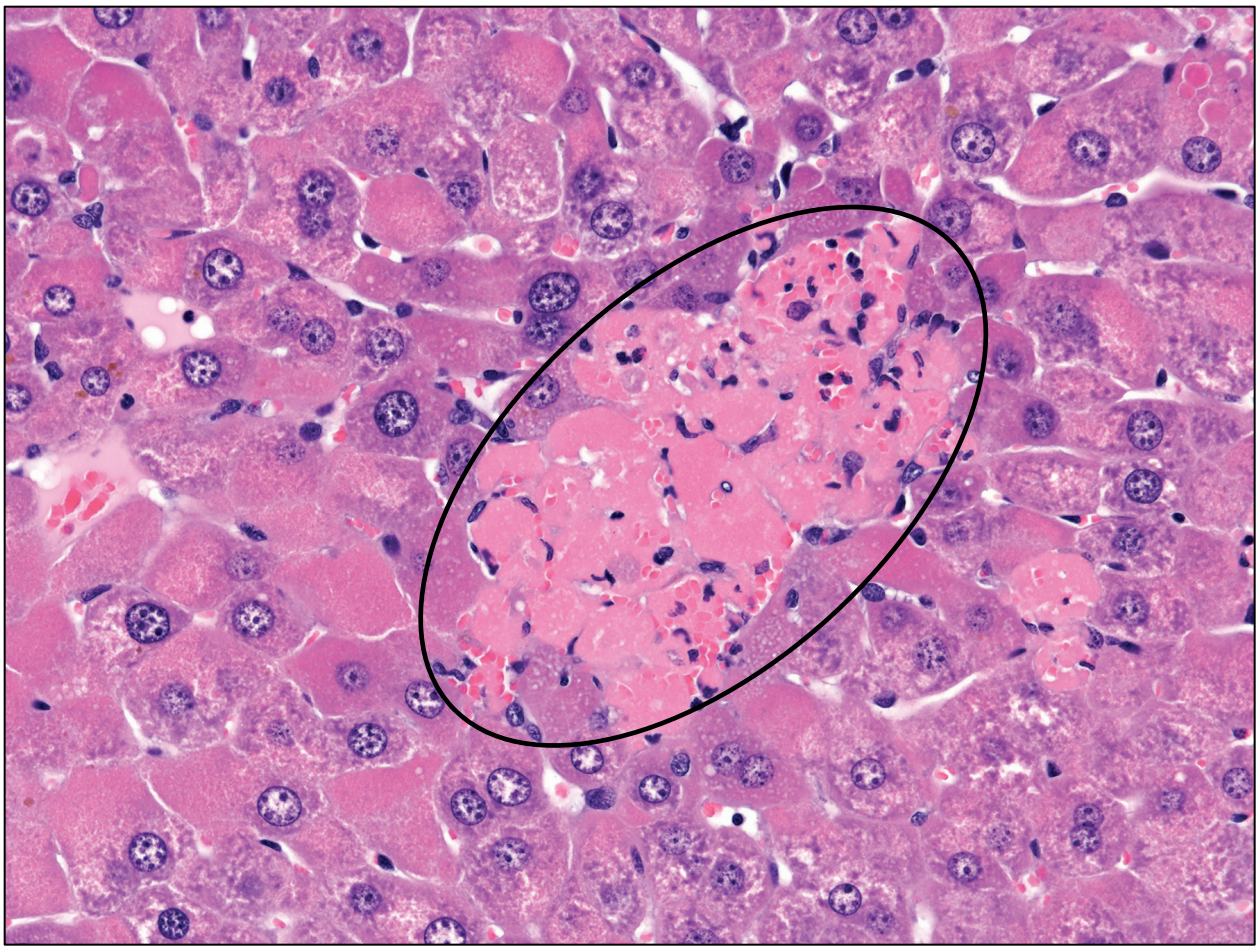

Figure 6. Focal necrosis (outlined) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. There is a small area of contiguous hepatocytes that are necrotic. Many of the cell borders are indistinct.

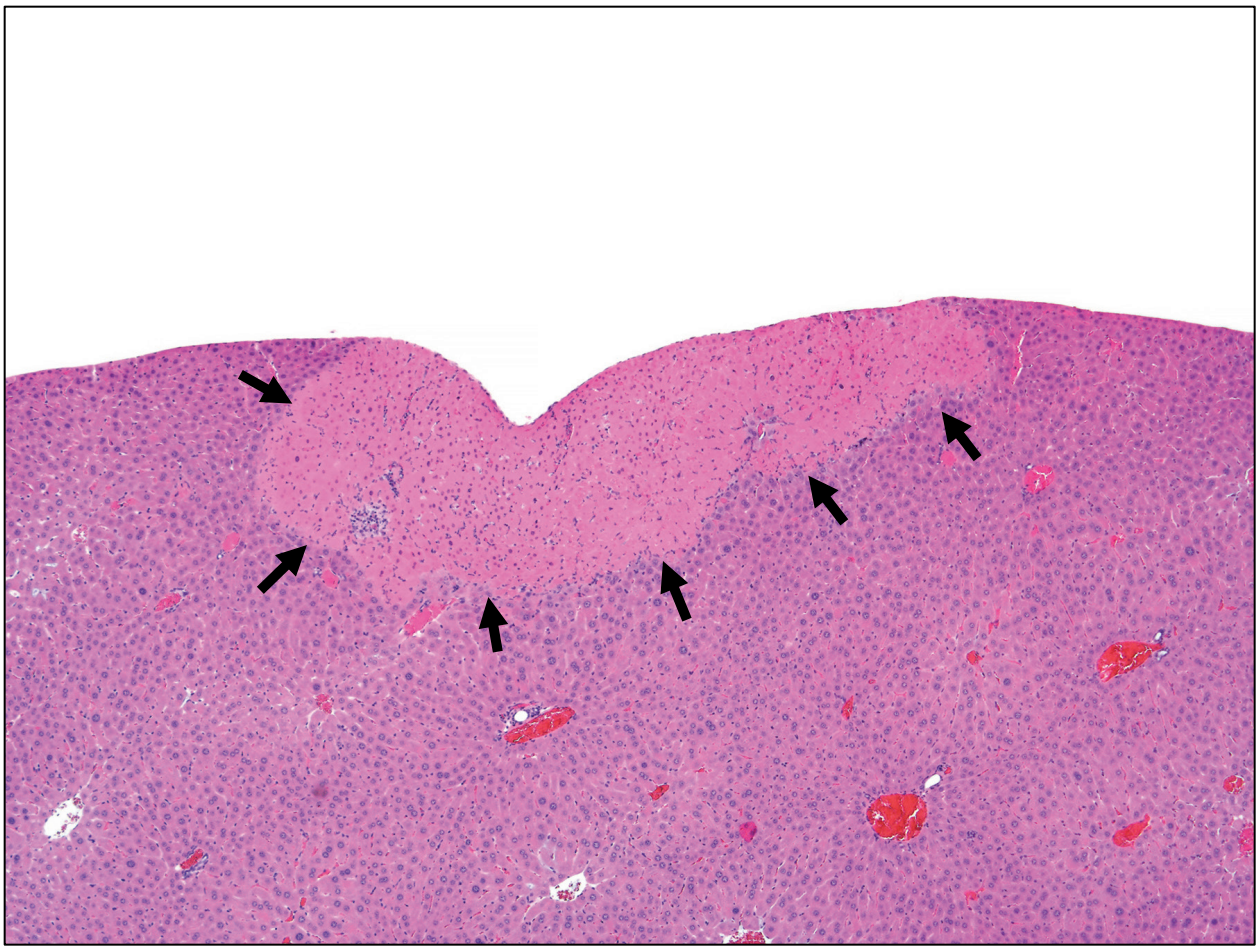

Figure 7. Focal necrosis (area delineated by arrows) in the liver of a Group 4 male mouse (animal 456) from the 18405-1307 subchronic study. This example of focal necrosis is more extensive than that shown in figure 6.

Cytoplasmic alteration was characterized by a bright eosinophilia to the cytoplasm of hepatocytes, usually accompanied by a slight increase in cell, and sometimes nuclear, size. The cytoplasm usually had a granular appearance to it, although with greater severities, the cytoplasm lost its granular appearance, and was just filled with smooth, homogeneous, brightly eosinophilic material. Severity grading was subjectively based on the number of hepatocytes involved and the amount of material within the affected hepatocytes. Minimal (+1) cytoplasmic alteration was recorded when there was an eosinophilic granular appearance to the hepatocytes in the centrilobular region of most hepatic lobules. With mild (+2) cytoplasmic alteration, more of each hepatic lobule was involved, so that many of the hepatic lobules appeared to be completely affected, rather than having alteration limited to the centrilobular area. All the hepatocytes appeared to be affected with moderate (+3) cytoplasmic alteration and those in the centrilobular area usually had lost the granular appearance to the cytoplasm and instead had a more solid, brightly eosinophilic appearance to it. Many of the hepatocytes with moderate cytoplasmic alteration were also larger than normal, and some also had larger than normal nuclei. These latter changes were not recorded separately, but were considered part of the cytoplasmic alteration. Marked (+4) cytoplasmic alteration was a diffuse change, with most of the hepatocytes distended by increased amounts of brightly eosinophilic cytoplasm that lacked granularity or definition,

similar to what was seen with moderate cytoplasmic alteration, but affected the entire hepatic lobule rather than just the centrilobular part. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

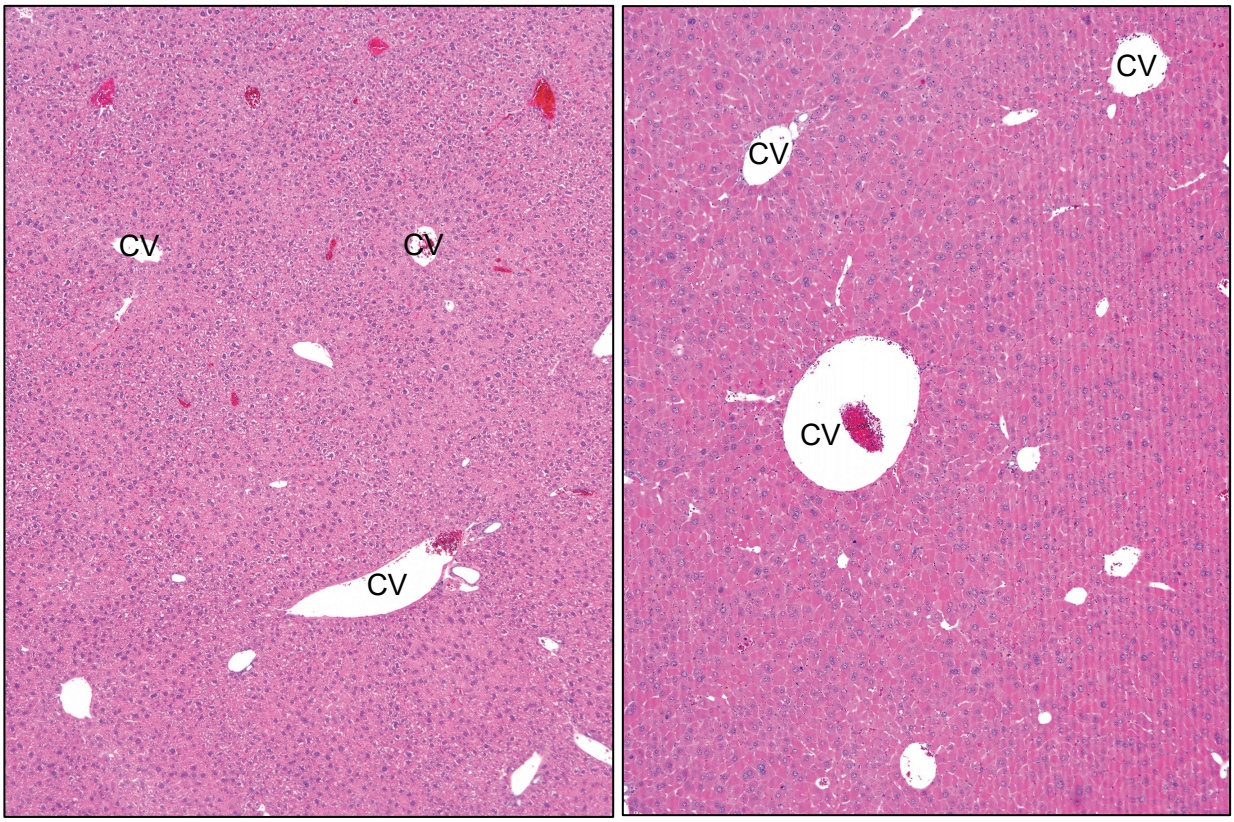

Figure 8. Control liver from a Group 1 mouse (animal 101) on the left; cytoplasmic alteration in the liver of a Group 4 male mouse (animal 401) on the right; from the 18405-1307 subchronic study. At this low magnification, the Group 4 mouse liver appears brightly eosinophilic when compared to the Group 1 (control) liver. The nuclei are also spaced further apart from each other, consistent with hypertrophied cells. CV=central vein.

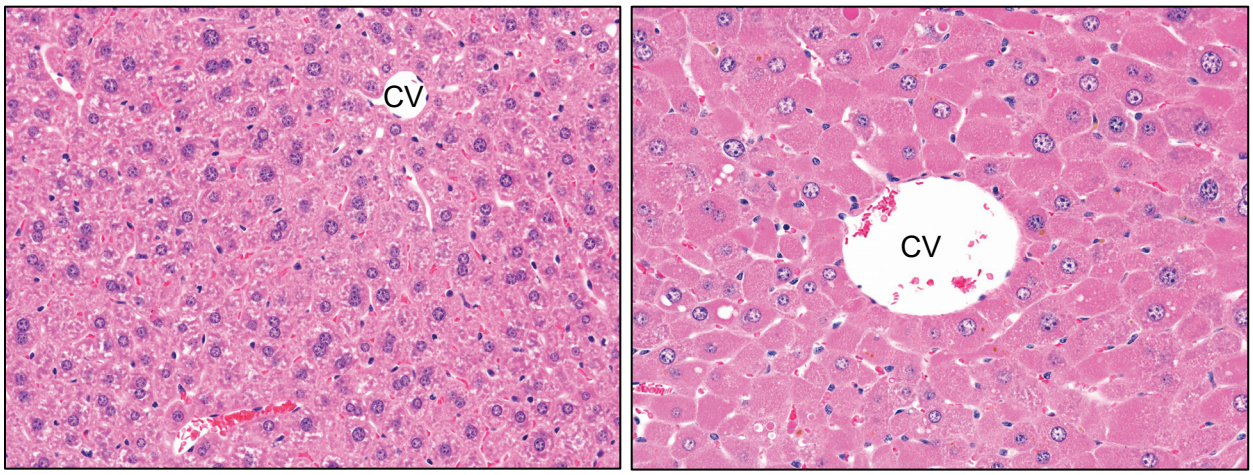

Figure 9. Control liver from a Group 1 mouse (animal 105) on the left; cytoplasmic alteration in the liver of a Group 4 male mouse (animal 401) on the right; from the 18405-1307 subchronic study. This higher magnification photo reveals the eosinophilic granular nature of the cytoplasm in the Group 4 mouse. CV=central vein.

Mixed cell infiltrate was characterized by the presence clusters of inflammatory cells within the hepatic parenchyma. They were often found randomly scattered throughout the liver, and less commonly in the periportal or centrilobular areas. The infiltrates were composed primarily of lymphocytes with fewer macrophages and plasma cells. Neutrophils were a small component of many of the foci, and were a major component of a few of them. Occasionally, a necrotic hepatocyte could be found within the focus of inflammatory cells. Mixed cell infiltrate was used as a diagnostic term as it is the term preferred in INHAND (Thoolen et al, 2010). This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies. Mixed cell infiltrates are common background lesions in mice, although they may be exacerbated with treatment.

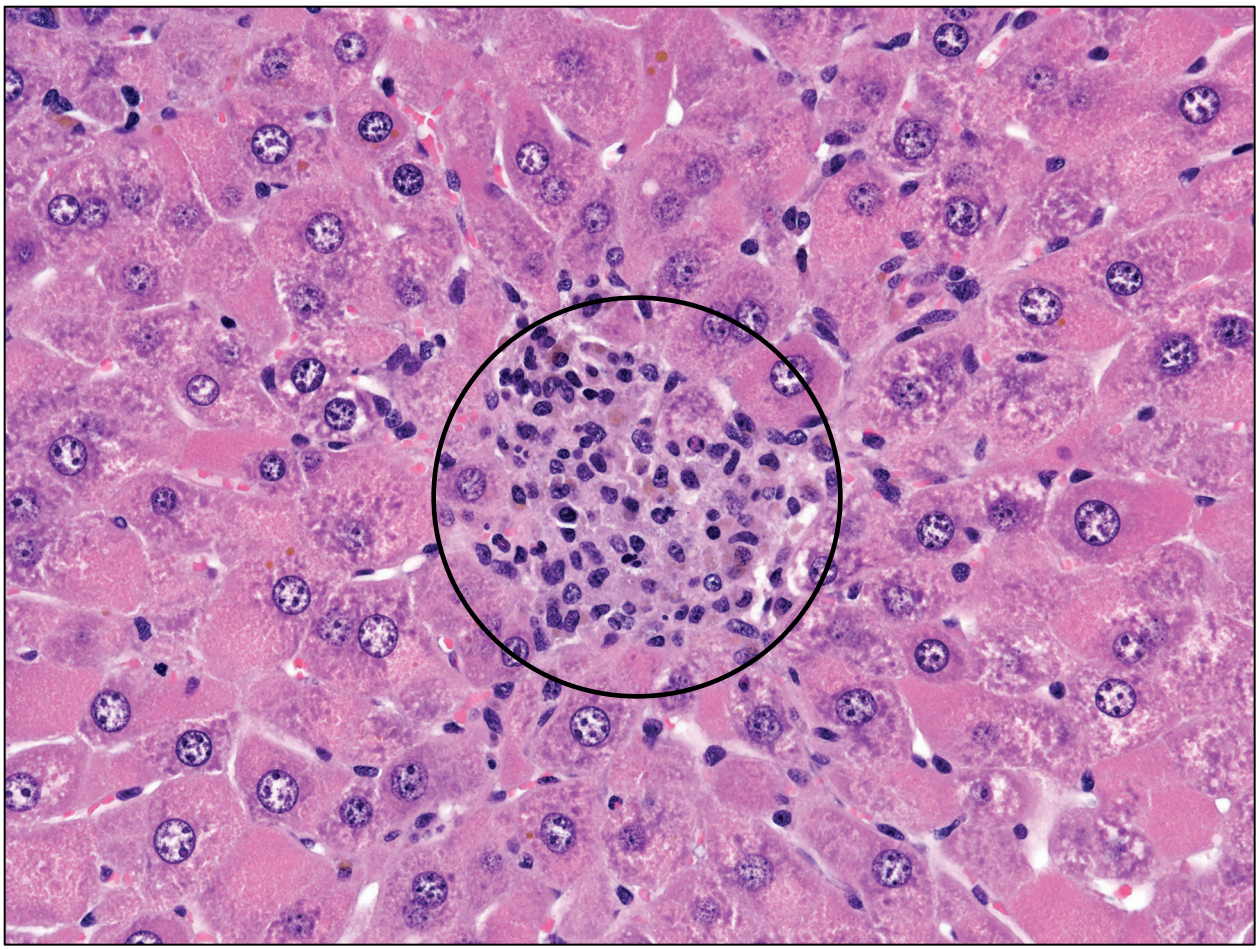

Figure 10. Mixed cell infiltrates (circled) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. Different types of inflammatory cells, including macrophages (some containing pigment consistent with cell breakdown product), lymphocytes, plasma cells, and neutrophils are present in a focal area. Some of these areas also contained an occasional necrotic hepatocyte.

Mitotic figures were considered to be present when there were an increased number of mitotic figures observed in the sections of liver examined. Typically, if 3 or more mitotic figures were observed, ten 20X fields were counted for the number of mitotic figures, and severity scores were based upon how many mitotic figures were counted: Minimal if 1-10 cells were observed in ten 20X fields; mild if 11-40 cells were observed in ten 20X fields. All the occurrences of mitotic figures were considered of minimal or mild severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

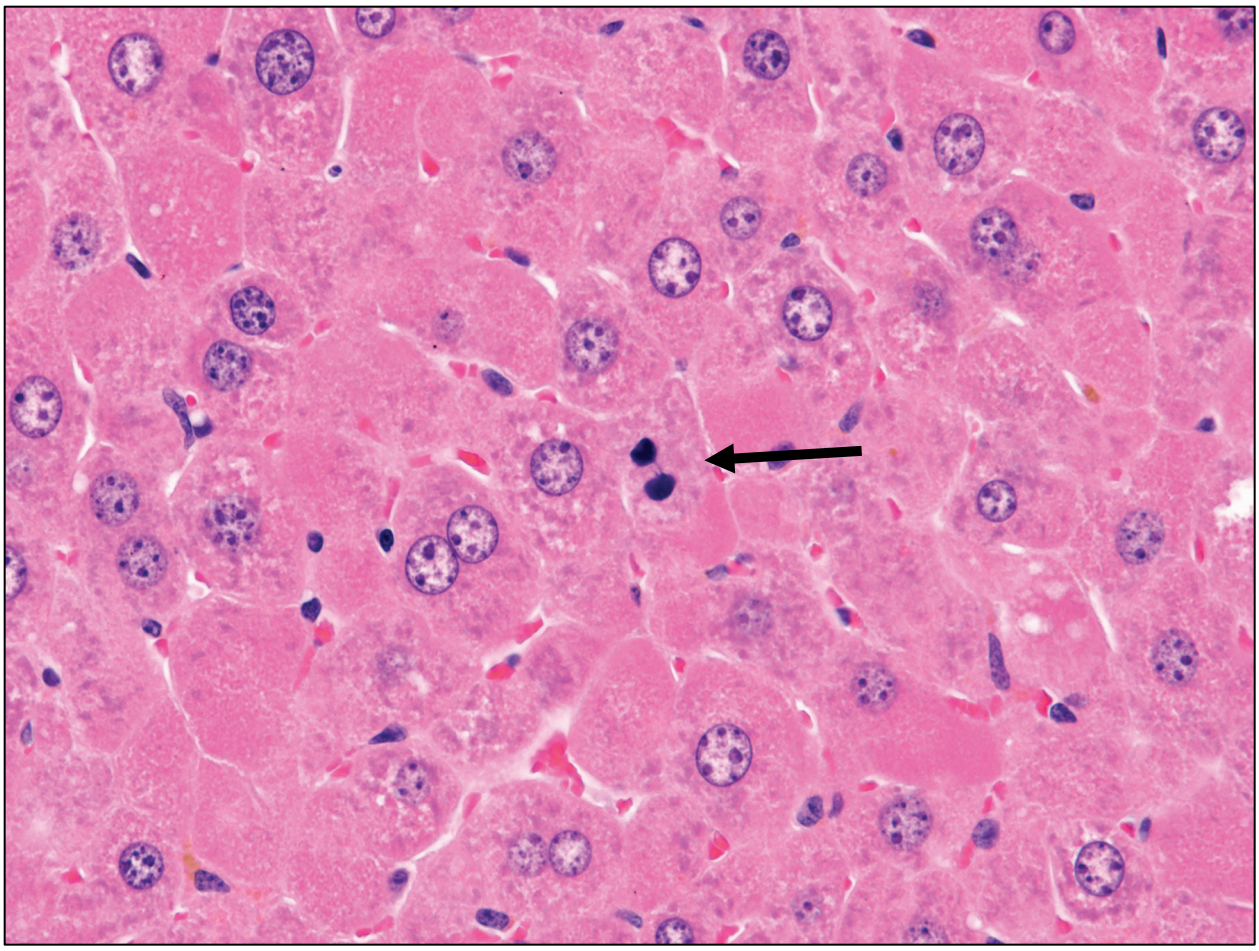

Figure 11. A mitotic figure (arrow) in the liver of a Group 4 male mouse (animal 7744) from the 18405-1037 reproduction/developmental study. Although mitotic figures can be found in the livers of normal mice, there were increased numbers observed in the livers of some animals in this study.

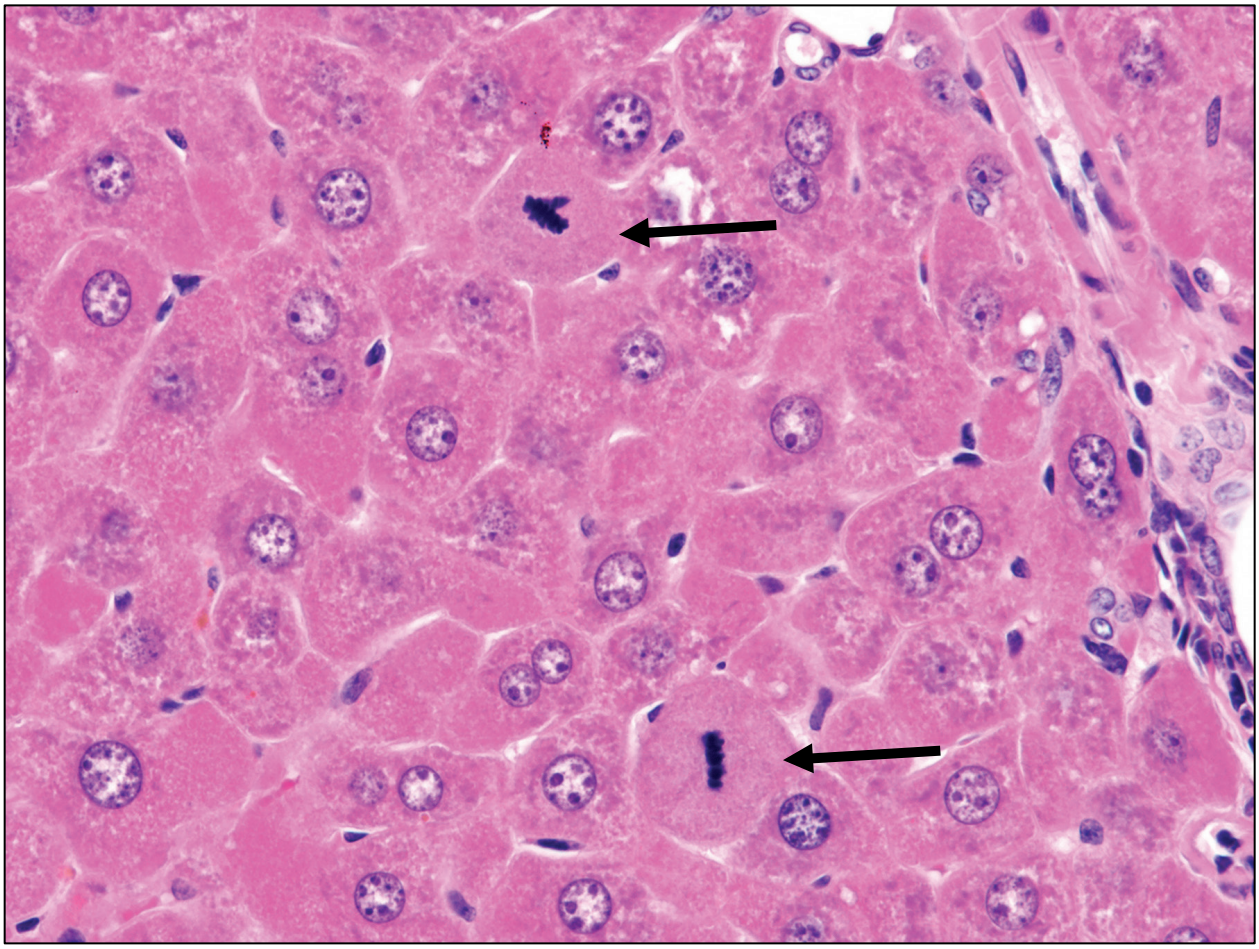

Figure 12. Mitotic figures (arrows) in the liver of a Group 4 male mouse (animal 409) from the 18405-1307 subchronic study. Increased mitotic figures were observed in both the 18405-1307 subchronic and the 18405-1037 reproduction/developmental studies.

Pigment increased; was characterized by golden brown pigment that was found primarily in bile canaliculi, Kupffer cells, but occasionally in hepatocytes as well. All the occurrences were of minimal severity. This lesion was observed in both the 18405-1307 subchronic & the 18405-1037 reproduction/developmental studies.

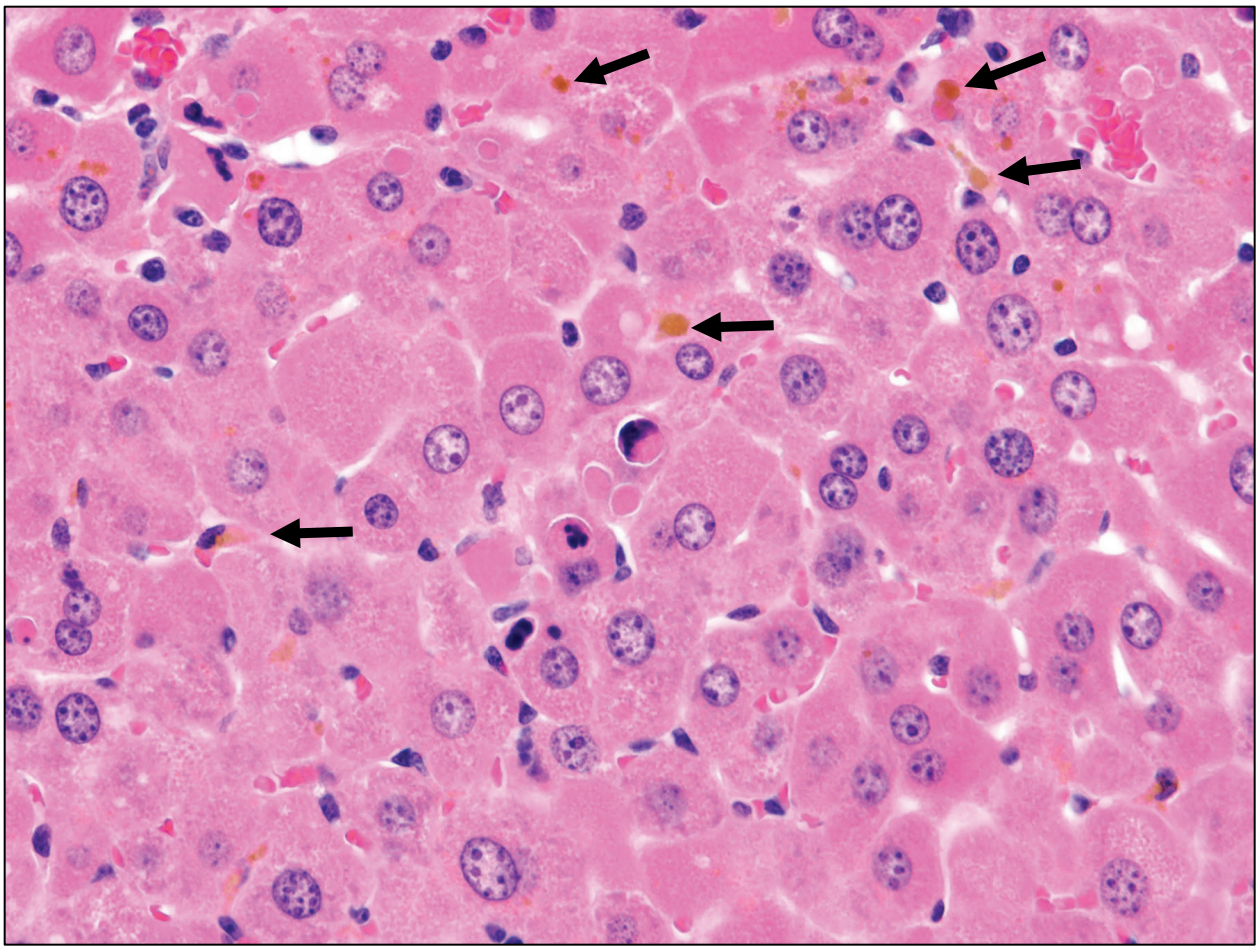

Figure 13. Pigment (arrows) in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. Special stains were not performed to identify the pigment, but the appearance and location were consistent with either inspissated bile in canaliculi (arrows) or byproducts from cell breakdown in Kupffer cells (see figure 14).

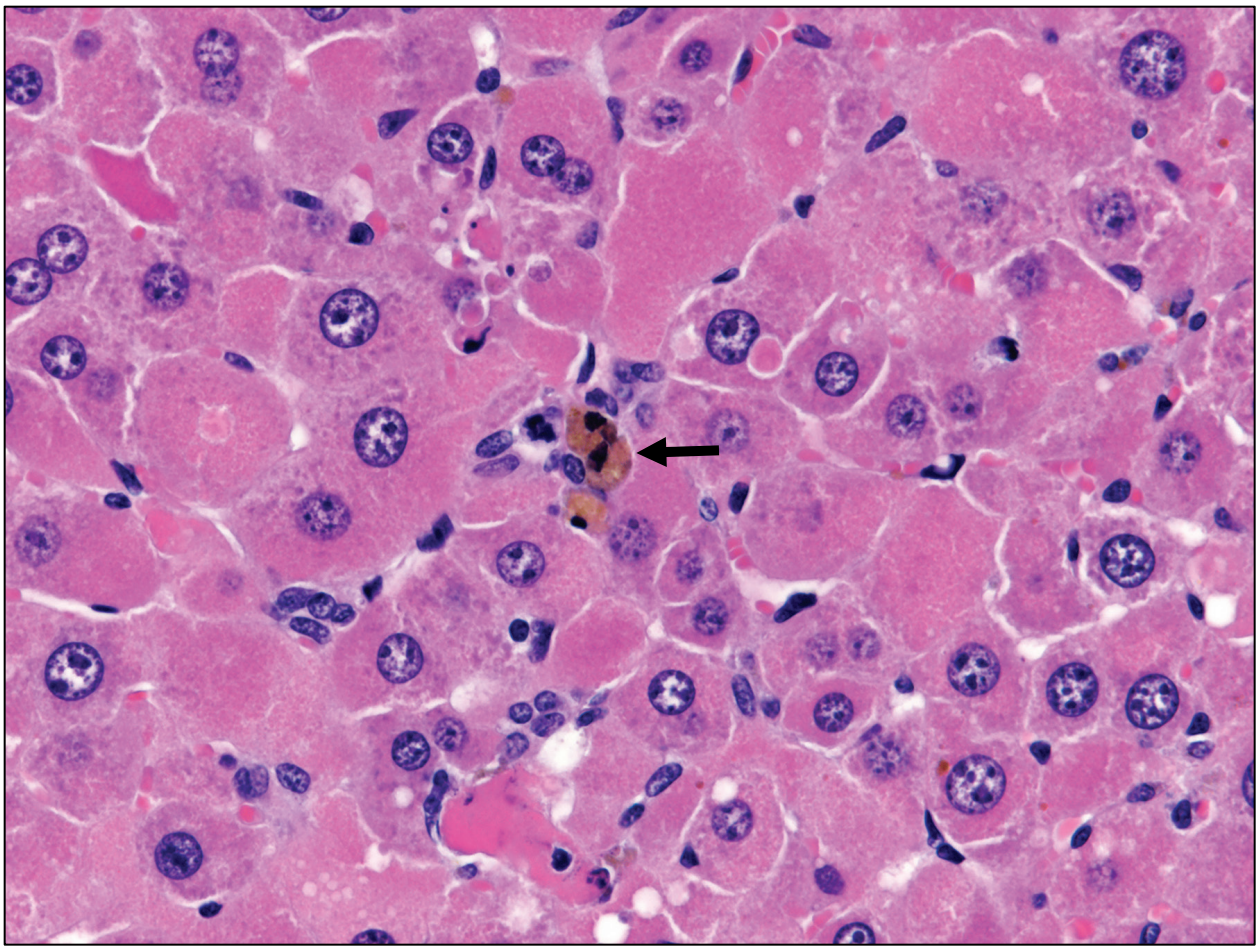

Figure 14. Pigment in the liver of a Group 4 male mouse (animal 410) from the 18405-1307 subchronic study. The pigment was found primarily in bile canaliculi or macrophages (Kupffer cells) but also in hepatocytes on occasion and was consistent with either inspissated bile in canaliculi or byproducts from cell breakdown (e.g. hemosiderin) (arrow).

Extramedullary hematopoiesis was recorded in one animal (a Group 4 male) in the 18405-1307 subchronic, and in 4 animals (a Group 3 male; two Group 4 males; & a Group 4 female) in the 18405-1037 reproduction/developmental study. It consisted of tight clusters of hematopoietic cells in varying degrees of maturity; most of the cells appeared to be myeloid cells.

Cytoplasmic vacuolation was recorded in several male mice in Group 3 and several female mice in Group 4 in the 18405-1037 reproduction/developmental study. It was characterized by very small vacuoles within the cytoplasm of hepatocytes, consistent with microvesicular fatty change. In most of the animals, it primarily involved the centrilobular hepatocytes, although in some animals the change was also present in midzonal hepatocytes.

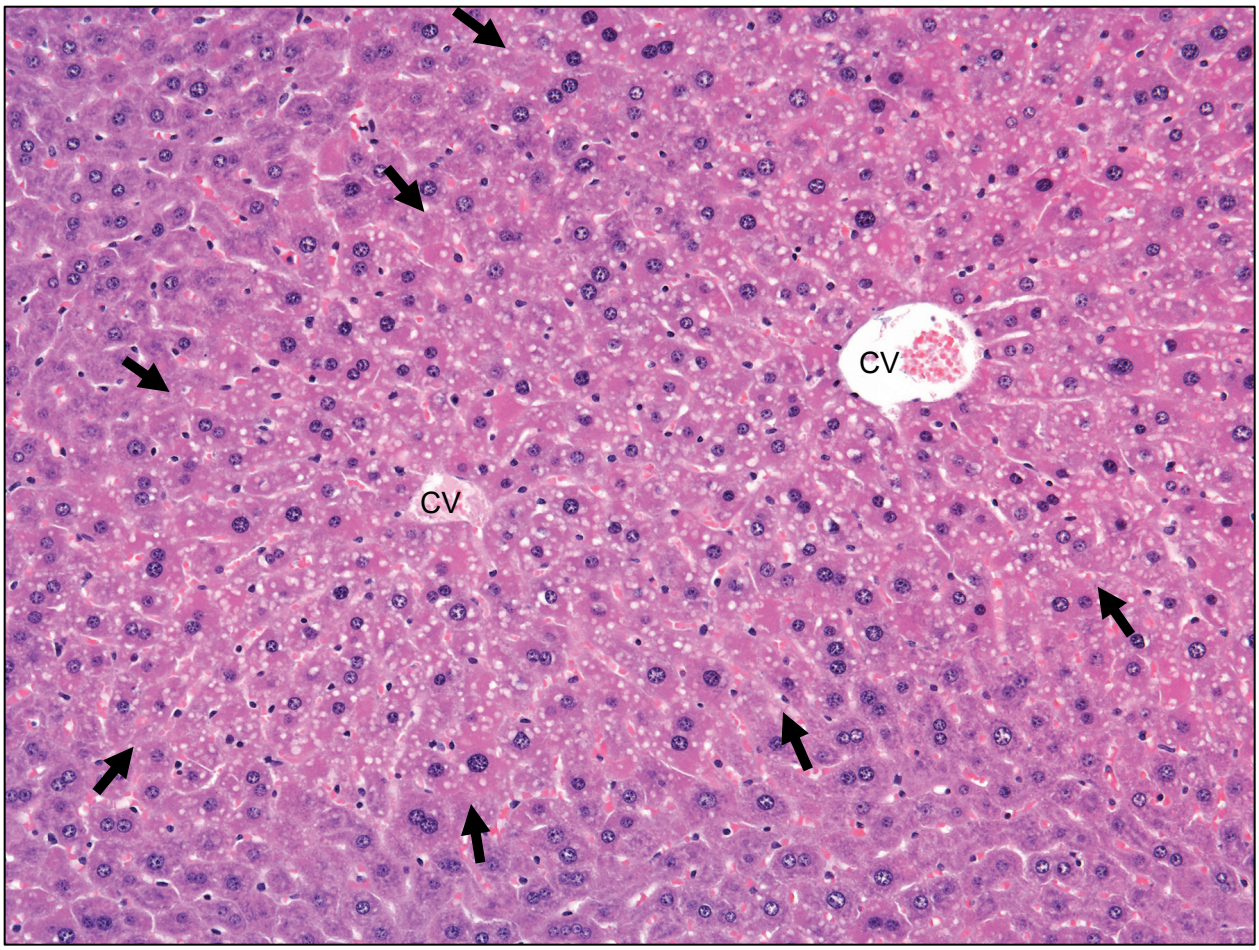

Figure 15. Cytoplasmic vacuolation in the liver of a Group 4 female mouse (animal 5073) from the 18405-1037 reproduction/developmental study. The vacuoles were small and slightly less regular than those observed in macrovesicular fatty change, and found primarily in the centrilobular region (area delineated by arrows), around the central vein. CV=central vein.

Oval cell hyperplasia was recorded in six Group 4 male mice in the 18405-1037 reproduction/developmental study. Only minimal oval cell hyperplasia was recorded, and it was characterized by an increase in oval cells in several periportal regions, with some oval cells present in the surrounding hepatic parenchyma.

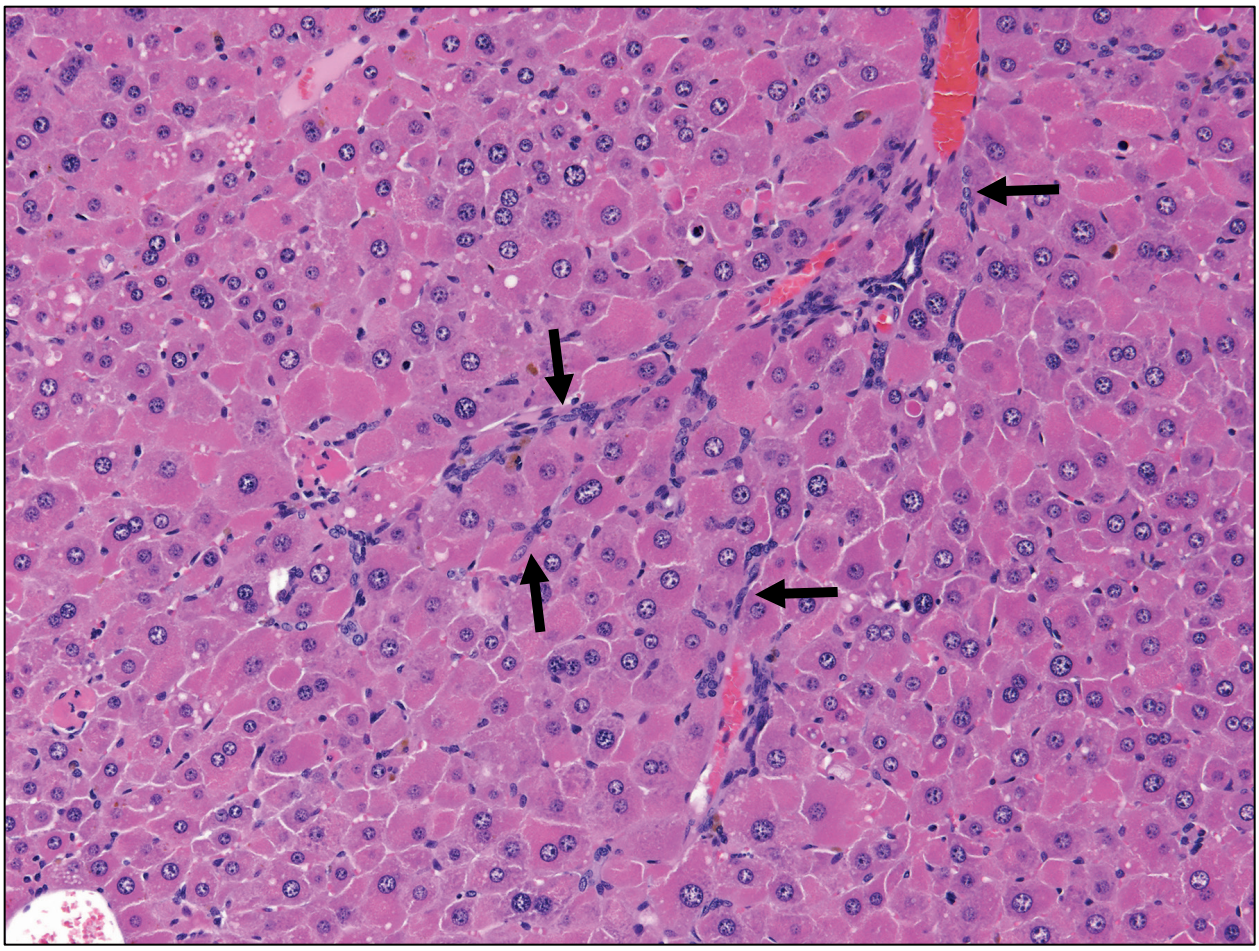

Figure 16. Oval cell hyperplasia in the liver of a Group 4 male mouse (animal 7730) from the 18405-1037 reproduction/developmental study. Oval cells (arrows), small cells with round to oval nuclei, appear to originate in the portal region and branch out from there.

Bile duct hyperplasia was recorded in one Group 4 male mouse in the 18405-1307 subchronic study, and was characterized by increased profiles of bile ducts in the periportal region; only minimal bile duct hyperplasia was recorded.

Table 1. Study 18405-1307 Subchronic Toxicity 90-Day Gavage Study
Summary incidences of lesions observed in the liver during slide review

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Within normal limits	4	4	0	0	4	6	7	0
Mixed cell infiltrate	6	6	4	6	5	3	3	7
Single cell necrosis; hepatocellular	0	1	0	9	0	0	0	3

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**10**	**10**	**10**	**10**	**10**	**9**	**10**	**10**
Cytoplasmic alteration	0	0	10	10	0	0	0	10
Focal necrosis	0	0	0	1	1	0	2	4
Cytoplasmic vacuolation	0	0	0	0	0	0	0	0
Extramedullary hematopoiesis,	0	0	0	1	0	0	0	0
Pigment, increased	0	0	0	10	0	0	0	4
Apoptosis; hepatocellular	0	0	0	10	0	0	0	3
Mitotic figures increased	0	0	0	7	0	0	0	0
Bile duct hyperplasia	0	0	0	1	0	0	0	0

Table 2. Study 18405-1037 Reproduction/developmental Toxicity Screening Study Summary incidences of lesions observed in the liver during slide review

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Within normal limits	18	21	5	0	11	14	3	0
Mixed cell infiltrate	6	3	11	8	12	7	17	15
Single cell necrosis; hepatocellular	1	1	2	24	0	2	3	19
Cytoplasmic alteration	0	0	10	25	0	1	16	25
Focal necrosis	0	0	4	3	2	2	4	5
Cytoplasmic vacuolation	0	0	3	0	0	0	0	1
Extramedullary hematopoiesis	0	0	1	2	0	0	0	1
Pigment, increased	0	0	0	21	0	0	0	3

Group	Group 1 Male	Group 2 Male	Group 3 Male	Group 4 Male	Group 1 Female	Group 2 Female	Group 3 Female	Group 4 Female
Number evaluated	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**
Apoptosis; hepatocellular	0	0	0	22	0	0	0	10
Mitotic figures increased	0	0	0	17	0	0	0	2
Oval cell hyperplasia	0	0	0	4	0	0	0	0
Inflammation, granulomatous	0	0	0	0	0	0	1	0
Polyarteritis nodosa	0	0	0	0	0	0	0	1

PATHOLOGY WORKING GROUP

A PWG was convened on October 15, 2019 at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park (RTP), NC to histologically evaluate selected tissues from this study. The participants were Drs. Susan A. Elmore, MS, DVM, DACVP, DABT (NTP/NIEHS - PWG Coordinator), Amy Brix, DVM, PhD, DACVP (EPL - Reviewing Pathologist), David Malarkey, DVM, PhD, DACVP (NTP/NIEHS), Arun Pandiri, BVSc&AH, PhD, DACVP, DABT (NTP/NIEHS), Robert Sills, DVM, PhD, DACVP (NTP/NIEHS), Brian Berridge, DVM, PhD, DACVP (NTP/NIEHS), Robert Maronpot, DVM, MS, MPH (Maronpot Consulting, LLC) and Michael Elwell, DVM, PhD (Apex ToxPath, LLC).

The PWG Coordinator selected slides for review by the PWG that included 3 examples each of normal liver, hepatocellular apoptosis, hepatocellular single cell necrosis and hepatocellular cytoplasmic alteration, as well as 2 examples each of focal necrosis, pigment, increased mitoses, mixed cell infiltrates, cytoplasmic vacuolation and 1 example of oval cell hyperplasia. There was a majority consensus for all reviewed lesions. The PWG consensus opinion for each slide, including any additional diagnoses made by the PWG panel, was recorded on the slide review worksheet attached to the end of this report.

After review of all lesions, there was discussion about potential adversity. Adversity is a term indicating “harm” to the test animal within the constraints of a given study design (dose, duration, etc.). Assessment of adversity should represent empirical measurements (i.e. objective data) integrated with well-informed subjective judgements to determine whether or not a response is considered harmful to an organism (Kerlin et al. 2016). After discussion, the PWG members agreed that the dose response and constellation of lesions (i.e. cytoplasmic alteration, apoptosis, single cell necrosis, and focal necrosis) rather than one lesion by itself, represents adversity within the confines of this study.

SUMMARY

This review generally supported the study pathologist's findings. When appropriate, the diagnosis of apoptosis, hepatocellular was added to distinguish cells with morphological characteristics of apoptosis from those with morphologic characteristics of single cell necrosis. The diagnostic term of "cytoplasmic alteration" was used to indicate hepatocyte hypertrophy, frequently coupled with a brightly eosinophilic, often granular appearance of the cytoplasm of hepatocytes. Other changes were recommended based upon using terminology preferred by the NTP. The dose response and constellation of lesions were together considered to be indicators of adversity within the confines of this study.

CONFLICT OF INTEREST STATEMENT

This statement is to certify that the reviewing pathologist, Dr. Brix, an on-site NTP Pathologist employed by Experimental Pathology Laboratories, Inc. (EPL®), participated in the pathology peer review of the liver of the Subchronic Toxicity 90 Day Gavage Study In Mice (Project ID: DuPont-18405-1307) and An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 In Mice (Study Number WIL-180225) (Project ID: DuPont-18405-1037). She has not been involved in any aspect of the study for any organization other than NTP which conducted the study nor the generation and/or evaluation of materials or data which were reviewed prior to the receipt of materials from the study lab. Hence, her participation in the review poses no apparent or actual conflict of interest.

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PWG SLIDE REVIEW WORKSHEET

Flat/#	Animal #	Study	Lesion in question	Other/ Comments
1-1	101	1307	Within normal limits	7 agreed, 0 disagreed
1-2	7722	1037	Within normal limits	7 agreed, 0 disagreed
1-3	7718	1037	Within normal limits	7 agreed, 0 disagreed
1-4	410	1307	Apoptosis	7 agreed, 0 disagreed
1-5	401	1307	Apoptosis	7 agreed, 0 disagreed
1-6	7770	1037	Apoptosis	7 agreed, 0 disagreed
1-7	405	1307	Single cell necrosis	7 agreed, 0 disagreed
1-8	7730	1037	Single cell necrosis	7 agreed, 0 disagreed
1-9	7804	1037	Single cell necrosis	7 agreed, 0 disagreed
1-10	406	1307	Cytoplasmic alteration	7 agreed, 0 disagreed
1-11	404	1307	Cytoplasmic alteration	7 agreed, 0 disagreed
1-12	7759	1037	Cytoplasmic alteration	7 agreed, 0 disagreed
1-13	7744	1037	Focal necrosis	7 agreed, 0 disagreed
1-14	456	1307	Focal necrosis	7 agreed, 0 disagreed
1-15	403	1307	Pigment	7 agreed, 0 disagreed
1-16	7780	1037	Pigment	7 agreed, 0 disagreed
1-17	408	1307	Increased mitoses	7 agreed, 0 disagreed
1-18	409	1307	Increased mitoses	7 agreed, 0 disagreed
1-19	407	1307	Mixed cell infiltrates	5 agreed, 2 voted for “inflammation”
1-20	7723	1037	Mixed cell infiltrates	5 agreed, 2 voted for “inflammation”
2-1	5073	1037	Cytoplasmic Vacuolation	7 agreed, 0 disagreed
2-2	7799	1037	Cytoplasmic Vacuolation	7 agreed, 0 disagreed
2-3	7778	1037	Oval cell hyperplasia	6 agreed, 1 voted for biliary hyperplasia

Appendix A. Slide Review Worksheets

Project 18405-1307 Males

Animal #	Organ	SP Diagnosis	NTP Diagnosis
101	Liver	Within normal limits	Agree with SP
102	Liver	Within normal limits	Agree with SP
103	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
104	Liver	Within normal limits	Mixed cell infiltrate; minimal
105	Liver	Within normal limits	Agree with SP
106	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
107	Liver	Within normal limits	Agree with SP
108	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
109	Liver	Within normal limits	Mixed cell infiltrate; minimal
110	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
201	Liver	Within normal limits	Agree with SP
202	Liver	Within normal limits	Agree with SP
203	Liver	Within normal limits	Agree with SP
204	Liver	Within normal limits	Agree with SP
205	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal
206	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
207	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
208	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
209	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
210	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
301	Liver	Within normal limits	Cytoplasmic alteration; mild
302	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild
303	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
304	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
305	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
306	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
307	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal
308	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
309	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
310	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
401	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Not present in section Apoptosis; hepatocellular; minimal
402	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hyperplasia; bile duct; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal	Agree with SP Pigment, increased; minimal Agree with SP Cytoplasmic alteration; marked Agree with SP Apoptosis; hepatocellular; minimal
403	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
404	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
405	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Apoptosis; hepatocellular; minimal
406	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Not present in section Pigment, increased; minimal Cytoplasmic alteration; marked Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
407	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Not present in section Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
408	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Mitotic figures; mild Mixed cell infiltrate; minimal Apoptosis; hepatocellular; minimal
409	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; mild Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Not present in section Apoptosis; hepatocellular; minimal Extramedullary hematopoiesis; minimal
410	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mitotic figures; minimal Mononuclear cell infiltrate; minimal	Agree with SP Pigment, increased; minimal Cytoplasmic alteration; marked Agree with SP Mixed cell infiltrate; minimal Apoptosis; hepatocellular; mild

Project 18405-1307 Females

Animal #	Organ	SP Diagnosis	NTP Diagnosis
151	Liver	Within normal limits	Mixed cell infiltrate; minimal
152	Liver	Within normal limits	Mixed cell infiltrate; minimal
153	Liver	Within normal limits	Agree with SP
154	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
155	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
156	Liver	Within normal limits	Agree with SP
157	Liver	Focal necrosis, moderate: diffuse and restricted to one lobe (likely due to lobular torsion)	Agree with SP
158	Liver	Within normal limits	Agree with SP
159	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
160	Liver	Within normal limits	Agree with SP
251	Liver	Within normal limits	Slide missing
252	Liver	Within normal limits	Agree with SP
253	Liver	Within normal limits	Agree with SP
254	Liver	Within normal limits	Agree with SP
255	Liver	Within normal limits	Agree with SP
256	Liver	Within normal limits	Mixed cell infiltrate; minimal
257	Liver	Within normal limits	Agree with SP
258	Liver	Within normal limits	Agree with SP
259	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
260	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
351	Liver	Mononuclear cell infiltrate; minimal	Mixed cell infiltrate; minimal
352	Liver	Within normal limits	Agree with SP
353	Liver	Within normal limits	Agree with SP
354	Liver	Within normal limits	Agree with SP
355	Liver	Within normal limits	Agree with SP
356	Liver	Within normal limits	Agree with SP

Animal #	Organ	SP Diagnosis	NTP Diagnosis
357	Liver	Focal necrosis; minimal Mononuclear cell infiltrate; minimal	Agree with SP Mixed cell infiltrate; minimal
358	Liver	Focal necrosis; minimal Mononuclear cell infiltrate; minimal	Agree with SP Mixed cell infiltrate; minimal
359	Liver	Within normal limits	Agree with SP
360	Liver	Within normal limits	Agree with SP
451	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild
452	Liver	Pigment increased; Kupffer cells; minimal Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; minimal	Pigment, increased; minimal Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal
453	Liver	Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
454	Liver	Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; minimal	Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal Focal necrosis; minimal Singe cell necrosis; minimal Pigment, increased; minimal Apoptosis; hepatocellular; minimal
455	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; minimal Pigment, increased; minimal
456	Liver	Focal necrosis; mild: sub-capsular Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Focal necrosis; mild Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
457	Liver	Hepatocellular hypertrophy; mild	Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal
458	Liver	Hepatocellular hypertrophy; minimal	Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
459	Liver	Single cell necrosis; hepatocellular; minimal Pigment increased; Kupffer cells; minimal Focal necrosis; mild Hepatocellular hypertrophy; mild Mononuclear cell infiltrate; mild	Agree with SP Pigment, increased; minimal Focal necrosis; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; mild Apoptosis; hepatocellular; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
460	Liver	Focal necrosis; mild Hepatocellular hypertrophy; minimal Mononuclear cell infiltrate; minimal	Focal necrosis; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal

Appendix B. Slide Review Worksheets

Project 18405-1037 Males

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7714	Liver	Within normal limits	Agree with SP
7717	Liver	Within normal limits	Agree with SP
7718	Liver	Within normal limits	Agree with SP
7722	Liver	Within normal limits	Agree with SP
7723	Liver	Hematopoiesis, extramedullary; minimal	Mixed cell infiltrate; mild
7732	Liver	Within normal limits	Agree with SP
7734	Liver	Within normal limits	Agree with SP
7742	Liver	Within normal limits	Agree with SP
7750	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
7752	Liver	Within normal limits	Mixed cell infiltrate; minimal
7758	Liver	Within normal limits	Agree with SP
7763	Liver	Within normal limits	Agree with SP
7765	Liver	Within normal limits	Agree with SP
7769	Liver	Within normal limits	Agree with SP
7772	Liver	Within normal limits	Agree with SP
7775	Liver	Within normal limits	Agree with SP
7788	Liver	Within normal limits	Agree with SP
7792	Liver	Within normal limits	Agree with SP
7798	Liver	Within normal limits	Mixed cell infiltrate; minimal
7800	Liver	Within normal limits	Mixed cell infiltrate; minimal
7803	Liver	Within normal limits	Mixed cell infiltrate; minimal
7810	Liver	Within normal limits	Agree with SP
7813	Liver	Within normal limits	Mixed cell infiltrate; minimal
7823	Liver	Within normal limits	Agree with SP
7825	Liver	Within normal limits	Agree with SP
7710	Liver	Within normal limits	Agree with SP

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7728	Liver	Within normal limits	Agree with SP
7731	Liver	Within normal limits	Mixed cell infiltrate; minimal
7737	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
7743	Liver	Within normal limits	Agree with SP
7748	Liver	Within normal limits	Agree with SP
7749	Liver	Within normal limits	Agree with SP
7754	Liver	Within normal limits	Agree with SP
7768	Liver	Within normal limits	Agree with SP
7776	Liver	Within normal limits	Mixed cell infiltrate; minimal
7777	Liver	Within normal limits	Agree with SP
7779	Liver	Within normal limits	Agree with SP
7783	Liver	Within normal limits	Agree with SP
7784	Liver	Within normal limits	Agree with SP
7786	Liver	Within normal limits	Agree with SP
7787	Liver	Within normal limits	Mixed cell infiltrate; minimal
7794	Liver	Within normal limits	Agree with SP
7797	Liver	Within normal limits	Agree with SP
7805	Liver	Within normal limits	Agree with SP
7807	Liver	Within normal limits	Agree with SP
7808	Liver	Within normal limits	Agree with SP
7809	Liver	Within normal limits	Agree with SP
7811	Liver	Within normal limits	Agree with SP
7817	Liver	Within normal limits	Agree with SP
7826	Liver	Within normal limits	Agree with SP
7711	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
7720	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7721	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
7729	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal
7740	Liver	Within normal limits	Agree with SP
7741	Liver	Within normal limits	Agree with SP
7745	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal	No remarkable lesion Focal necrosis; minimal Hepatocyte; cytoplasmic vacuolation; minimal
7746	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7756	Liver	Within normal limits	Mixed cell infiltrate; minimal Hepatocyte; cytoplasmic vacuolation; minimal
7760	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hematopoiesis, extramedullary; mild	Mixed cell infiltrate; minimal Agree with SP
7761	Liver	Within normal limits	Agree with SP
7762	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
7767	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
7774	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
7789	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; mild
7790	Liver	Necrosis, single cell; minimal	Agree with SP
7793	Liver	Within normal limits	Mixed cell infiltrate; minimal
7796	Liver	Within normal limits	Agree with SP
7799	Liver	Fatty change, centrilobular; minimal	Hepatocyte; cytoplasmic vacuolation; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7802	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; minimal
7814	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Not present in section (within normal limits)
7820	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal	Mixed cell infiltrate; minimal Focal necrosis; minimal
7822	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section
7827	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7828	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
7709	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7712	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal No remarkable lesions Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
7715	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7716	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
7724	Liver	Pigment, increased; minimal Hematopoiesis, extramedullary; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
7726	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7730	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal Oval cell hyperplasia; minimal
7735	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Extramedullary hematopoiesis; minimal
7736	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7738	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7739	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7744	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal Focal necrosis; minimal
7747	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7751	Liver	Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
7759	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Oval cell hyperplasia; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7764	Liver	Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
7770	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; moderate
7778	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Oval cell hyperplasia; minimal
7780	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Oval cell hyperplasia; minimal
7781	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7782	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
7785	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; moderate Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7801	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
7804	Liver	Pigment, increased; minimal Mitotic figures increased; mild Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Mitotic figures increased; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; mild Focal necrosis; minimal
7815	Liver	Pigment, increased; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Not present in section Focal necrosis; minimal Apoptosis; hepatocellular; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal

Project 18405-1037 Females

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4956	Liver	Within normal limits	Mixed cell infiltrate; minimal
4958	Liver	Within normal limits	Mixed cell infiltrate; minimal
4962	Liver	Within normal limits	Agree with SP
4966	Liver	Within normal limits	Mixed cell infiltrate; minimal
4967	Liver	Within normal limits	Mixed cell infiltrate; minimal
4968	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4978	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4985	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4986	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4987	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4991	Liver	Within normal limits	Agree with SP
4999	Liver	Within normal limits	Agree with SP
5001	Liver	Within normal limits	Agree with SP
5003	Liver	Within normal limits	Agree with SP
5013	Liver	Within normal limits	Agree with SP
5018	Liver	Within normal limits	Agree with SP
5021	Liver	Within normal limits	Agree with SP
5030	Liver	Necrosis, single cell; minimal	Focal necrosis; minimal
5045	Liver	Within normal limits	Agree with SP
5058	Liver	Necrosis, focal/multifocal; minimal	Focal necrosis; minimal
5059	Liver	Within normal limits	Agree with SP
5060	Liver	Within normal limits	Agree with SP
5064	Liver	Within normal limits	Mixed cell infiltrate; minimal
5066	Liver	Within normal limits	Mixed cell infiltrate; minimal
5071	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4954	Liver	Within normal limits	Agree with SP
4957	Liver	Within normal limits	Agree with SP
4961	Liver	Within normal limits	Agree with SP
4973	Liver	Within normal limits	Agree with SP
4979	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal
4981	Liver	Within normal limits	Agree with SP
4988	Liver	Within normal limits	Focal necrosis; minimal
4989	Liver	Infiltrate, neutrophil, focal/multifocal; minimal	Mixed cell infiltrate; minimal
4990	Liver	Within normal limits	Mixed cell infiltrate; minimal
4997	Liver	Within normal limits	Mixed cell infiltrate; minimal
5000	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal	Mixed cell infiltrate; minimal
5004	Liver	Within normal limits	Agree with SP
5005	Liver	Within normal limits	Agree with SP
5010	Liver	Within normal limits	Agree with SP
5015	Liver	Within normal limits	Agree with SP
5025	Liver	Within normal limits	Agree with SP
5036	Liver	Within normal limits	Mixed cell infiltrate; minimal
5040	Liver	Within normal limits	Agree with SP
5041	Liver	Within normal limits	Agree with SP
5046	Liver	Necrosis, single cell; minimal	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
5047	Liver	Within normal limits	Mixed cell infiltrate; minimal
5049	Liver	Within normal limits	Agree with SP
5061	Liver	Within normal limits	Agree with SP
5063	Liver	Within normal limits	Mixed cell infiltrate; minimal
5072	Liver	Necrosis, single cell; minimal	Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4960	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4963	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Cytoplasmic alteration; minimal
4969	Liver	Within normal limits	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
4974	Liver	Within normal limits	Single cell necrosis; hepatocellular; minimal
4975	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal
4976	Liver	Within normal limits	Mixed cell infiltrate; minimal
4977	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4980	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
4993	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Cytoplasmic alteration; minimal
5007	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5011	Liver	Within normal limits	Agree with SP
5014	Liver	Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal	Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal
5022	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5023	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5031	Liver	Within normal limits	Mixed cell infiltrate; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5034	Liver	Hypertrophy, hepatocellular, diffuse; minimal	Within normal limits
5037	Liver	Within normal limits	Mixed cell infiltrate; minimal
5043	Liver	Within normal limits	Mixed cell infiltrate; minimal
5048	Liver	Within normal limits	Agree with SP
5050	Liver	Necrosis, focal/multifocal; minimal	Focal necrosis; minimal Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5052	Liver	Within normal limits	Mixed cell infiltrate; minimal
5056	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5057	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Focal necrosis; minimal Cytoplasmic alteration; minimal Inflammation; granulomatous; focal; minimal
5065	Liver	Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
5070	Liver	Within normal limits	Cytoplasmic alteration; minimal Mixed cell infiltrate; minimal
4955	Liver	Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Not present in section Apoptosis; hepatocellular; minimal Cytoplasmic alteration; mild Polyarteritis nodosa; moderate
4959	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
4972	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, focal/multifocal; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
4982	Liver	Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
4984	Liver	Pigment, increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Agree with SP Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
4998	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; minimal Apoptosis; hepatocellular; minimal
5002	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Mitotic figures increased; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Not present in section Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
5006	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
5008	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5009	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
5017	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Hematopoiesis, extramedullary; minimal Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Agree with SP Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
5020	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; mild Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; mild Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
5027	Liver	Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
5028	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Mixed cell infiltrate; minimal
5029	Liver	Necrosis, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Focal necrosis; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Mixed cell infiltrate; minimal
5033	Liver	Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Cytoplasmic alteration; mild
5035	Liver	Hypertrophy, hepatocellular, diffuse; mild	Cytoplasmic alteration; mild
5051	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; minimal (with eosinophilic cytoplasmic stippling)	Not present in section Cytoplasmic alteration; minimal Mitotic figures increased; minimal Mixed cell infiltrate; minimal Focal necrosis; minimal

Animal #	Organ	SP Diagnosis	NTP Diagnosis
5062	Liver	Pigment, increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Agree with SP Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal Mixed cell infiltrate; minimal
5068	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate (with eosinophilic cytoplasmic stippling)	Mixed cell infiltrate; minimal Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate
5069	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Apoptosis; hepatocellular; minimal
5073	Liver	Fatty change, centrilobular; mild Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Hepatocyte; cytoplasmic vacuolation; mild Cytoplasmic alteration; mild
5074	Liver	Pigment, increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild (with eosinophilic cytoplasmic stippling)	Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild
5075	Liver	Infiltrate, mononuclear cell, focal/multifocal; minimal Pigment, increased; minimal Mitotic figures increased; minimal Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; moderate	Mixed cell infiltrate; minimal Not present in section Not present in section Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; moderate Apoptosis; hepatocellular; minimal
5077	Liver	Necrosis, single cell; minimal Hypertrophy, hepatocellular, diffuse; mild	Single cell necrosis; hepatocellular; minimal Cytoplasmic alteration; mild Focal necrosis; minimal Mixed cell infiltrate; minimal

Appendix E: Benchmark Dose Modeling

E.1 Oral Reproduction/Developmental Toxicity Study in Mice (DuPont-18405-1037 2010)

U.S. Environmental Protection Agency (EPA) Center for Public Health and Environmental Assessment conducted dose response modeling of this study using the Benchmark Dose Software (BMDS) 3.1.2. program. This work used data from the reevaluation of the DuPont oral reproductive/ developmental toxicity study slides by the National Toxicology Program (NTP) Pathology Working Group (see section 4.5 for a description) and addresses the constellation of liver lesions the NTP defined as adverse (i.e., cytoplasmic alteration, single-cell and focal necrosis, and apoptosis) in parental male and parental female mice.

E.1.1 Constellation of Lesions (Cytoplasmic Alteration, Apoptosis, Single-Cell Necrosis, and Focal Necrosis) in the Liver, Parental Males

Increased incidence of a constellation of lesions in the liver was observed in the parental males. Dichotomous models were used to fit dose-response data (DuPont-18405-1037, 2010). A benchmark response (BMR) of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (EPA, 2012). The doses and response data used for the modeling are listed in Table E-1.

Table E-1. Constellation of Lesions in the Male Liver Selected for Dose-Response Modeling

Dose (mg/kg/day)	Number of mice (males)	Constellation of Liver Lesions
0	25	1
0.1	24	1
0.5	24	13
5	24	24

Note: mg/kg/day = milligrams per kilogram per day.

The benchmark dose (BMD) modeling results for the constellation of lesions are summarized in Table E-2 and Figure E-1. The best fitting model was the Probit model based on adequate *p*-values (greater than 0.1), the benchmark dose lower limits (BMDLs) were sufficiently close (less than threefold difference) among adequately fitted models, and the Probit model had the lowest Akaike information criterion (AIC). The lower bound on the dose level corresponding to the 95% lower confidence limit for a 10% response level ($BMDL_{10}$) from the selected Probit model is 0.14 milligram per kilogram per day (mg/kg/day).

Table E-2. Summary of BMD Modeling Results for Constellation of Lesions in Male Mice

Model[a]	Goodness of fit		Scaled residual for:		BMD_{10Pct} (mg/kg/day)	$BMDL_{10Pct}$ (mg/kg/day)	Basis for model selection
	p-value	AIC	Dose group near BMD	Dose group near BMDL			
Dichotomous Hill	N/A[b]	57.818	0.007	-0.007	0.29	0.11	EPA ORD selected the Weibull model. All models, except Dichotomous Hill, had adequate fit (*p*-values > 0.1), the BMDLs were sufficiently close (<3-fold difference), and the Probit model had the lowest AIC.
Gamma	0.994	55.815	-0.005	0.005	0.26	0.09	
Log-Logistic	0.977	55.816	0.000	-0.020	0.34	0.11	
Multistage Degree 3	0.997	53.820	-0.053	0.047	0.26	0.08	
Multistage Degree 2	0.905	54.026	-0.368	0.248	0.19	0.08	
Multistage Degree 1	0.279	57.026	-1.402	0.452	0.08	0.05	
Weibull	0.937	53.951	-0.290	0.205	0.20	0.08	
Logistic	0.888	54.048	-0.327	0.359	0.22	0.15	
Log-Probit	0.990	55.816	0.001	-0.001	0.24	0.10	
Probit	**0.907**	**52.444**	**-0.635**	**0.093**	**0.19**	**0.14**	

Notes: ORD = Office of Research and Development.
[a] Selected model in bold.
[b] degrees of freedom=0, saturated model (Goodness of fit test cannot be calculated).

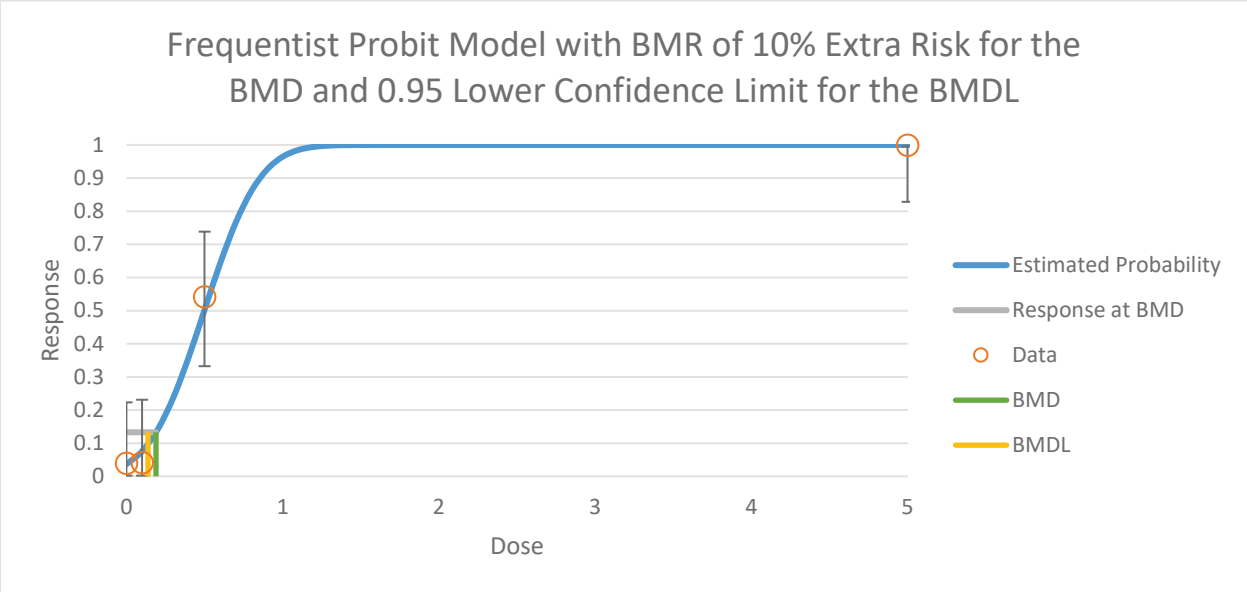

Figure E-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Constellation of Lesions in Male Mice (dose shown in mg/kg/day)

E.1.2 Constellation of Lesions (Cytoplasmic Alteration, Apoptosis, Single-Cell Necrosis, and Focal Necrosis) in the Liver, Parental Females

Increased incidence of the constellation of lesions in the liver was observed in the parental females. Dichotomous models were used to fit dose-response data (DuPont-18405-1037, 2010). A BMR of 10% extra risk was chosen per EPA's *Benchmark Dose Technical Guidance* (EPA, 2012). The doses and response data used for the modeling are listed in Table E-3.

Table E-3. Constellation of Lesions in the Female Liver Selected for Dose-Response Modeling

Dose (mg/kg/day)	Number of mice (females)	Constellation of Liver Lesions
0	24	2
0.1	22	3
0.5	24	17
5	24	24

The BMD modeling results for constellation of lesions are summarized in Table E-4 and Figure E-2. The best fitting model was the Probit model based on adequate *p*-values greater than 0.1), the BMDLs were sufficiently close (less than threefold difference) among adequately fitted models, and the Probit model had the lowest AIC. The $BMDL_{10}$ from the selected Probit model is 0.09 mg/kg/day.

Table E-4. Summary of BMD Modeling Results for Constellation of Lesions in Female Mice

Model[a]	Goodness of fit		Scaled residual for:		BMD_{10Pct} (mg/kg/day)	$BMDL_{10Pct}$ (mg/kg/day)	Basis for model selection
	p-value	AIC	Dose group near BMD	Dose group near BMDL			
Dichotomous Hill	N/A[b]	68.371	0.108	-0.077	0.14	0.05	EPA ORD selected the Probit model. All models, except Dichotomous Hill and Multistage Degree 3, had adequate fit (*p*-values > 0.1), the BMDLs were sufficiently close (<3-fold difference), and the Probit model had the lowest AIC.
Gamma	1.000	66.268	0.000	0.000	0.13	0.04	
Log-Logistic	0.804	66.371	0.108	-0.077	0.14	0.05	
Multistage Degree 3	N/A[b]	68.268	0.003	-0.002	0.15	0.04	
Multistage Degree 2	0.998	66.268	-0.002	0.001	0.14	0.04	
Multistage Degree 1	0.448	66.021	-1.087	0.393	0.05	0.04	
Weibull	1.000	66.268	0.000	0.000	0.14	0.04	
Logistic	0.993	64.283	-0.086	0.085	0.13	0.09	
Log-Probit	0.932	66.282	0.024	-0.015	0.13	0.05	
Probit	**0.971**	**62.514**	**-0.328**	**-0.101**	**0.12**	**0.09**	

Notes: ORD = Office of Research and Development.
[a] Selected model in bold.
[b] degrees of freedom=0, saturated model (Goodness of fit test cannot be calculated).

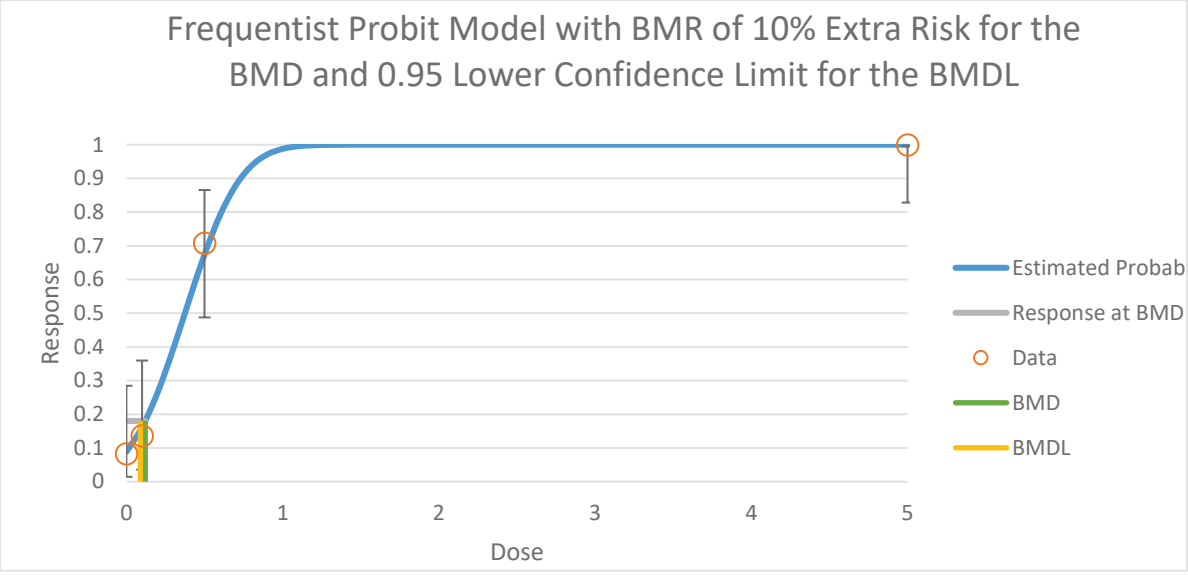

Figure E-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Probit Model for Constellation of Lesions in Female Mice; (dose shown in mg/kg/day)