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Public Comment Draft

Human Health Toxicity Values for

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

Also Known as "GenX Chemicals"

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Acronyms and Abbreviations

A/G	Albumin to globulin
AIC	Akaike information criterion
ALD	Approximate lethal dose
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BAF	Bioaccumulation factor
BCF	Bioconcentration factor
BBDR	Biologically based dose-response
BMD	Benchmark dose
BMDL	Benchmark dose lower limit
BMR	Benchmark response
BOD	Biochemical oxygen demand
BUN	Blood urea nitrogen
BW	Body weight
CASRN	Chemical Abstracts Service Registry Number
CFR	Code of Federal Regulations
CoA	Coenzyme A
COV	Coefficient of variation
DAF	Dosimetric adjustment factor
DWEL	Drinking water equivalent level
DWTP	Drinking water treatment plant
E1	Heptafluoropropyl 1,2,2,2-tetrafluorethyl ether
EPA	U.S. Environmental Protection Agency
FABP	Fatty acid-binding protein
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
GD	Gestation day
GLP	Good Laboratory Practices
H_3O+	Hydronium ion
HERO	Health & Environmental Research Online
HED	Human equivalent dose
HFPO	Hexafluoropropylene oxide
hL-FABP	Human liver fatty acid-binding protein
HPLC	High-performance liquid chromatography
ICR	Institute of Cancer Research
LC_{50}	Median lethal concentration
LD	Lactation day
LD_{50}	Median lethal dose
LLNA	Local lymph node assay
LOAEL	Lowest-observed-adverse-effect level

LOQ	Limit of quantification
µg/L	Microgram per liter
mg/L	Milligram per liter
mg/kg	Milligram per kilogram
mg/kg/day	Milligram per kilogram per day
MOA	Mode of action
NC DHHS	North Carolina Department of Health and Human Services
ng/g	nanograms per gram
ng/mL	Nanograms per milliliter
NHANES	National Health and Nutrition Examination Survey
NOAEL	No-observed-adverse-effect level
OECD	Organization for Economic Cooperation and Development
OPPT	Office of Pollution Prevention and Toxics
PBPK	Physiologically based pharmacokinetic
PFAS	Per- and polyfluoroalkyl substances
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonate
PMN	Premanufacture notice
PND	Postnatal day
POD	Point of departure
POD_{HED}	Point of departure human equivalent dose
PPARα	Peroxisome proliferator-activated receptor alpha
RBC	Red blood cell
RfD	Reference dose
RIVM	National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu)
SDH	Sorbitol dehydrogenase
$T_{1/2}$	Half-life
TDAR	T cell-dependent antibody response
TG	Test guideline
TSCA	Toxic Substances Control Act
UF	Uncertainty factor(s)
UF_A	Interspecies uncertainty factor
UF_D	Database uncertainty factor
UF_H	Intraspecies uncertainty factor
UF_L	LOAEL to NOAEL extrapolation uncertainty factor
UF_S	Extrapolation from subchronic to a chronic exposure duration
WOS	Web of Science

Executive Summary

The U.S. Environmental Protection Agency (EPA) is issuing draft subchronic and chronic oral toxicity values (i.e., reference doses, or RfDs) for 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6)—or hexafluoropropylene oxide (HFPO) dimer acid—and 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt for public comment. 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 includes toxicity values associated with potential noncancer health effects following oral exposure (in this case, oral reference doses (RfDs). This assessment evaluates human health hazards. The toxicity assessment and the values contained within 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. When final, 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 fluorinated organic chemicals that are part of a larger group of chemicals referred to as "per- and polyfluoroalkyl substances." In 2006, the PA 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. 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, oral animal toxicity studies of acute, short-term, subchronic, and chronic duration are available in rats and mice. Limited information identifying health effects from inhalation or dermal exposures to GenX chemicals in animals 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. One oral reproductive and developmental toxicity study in mice and one prenatal developmental toxicity study in rats are available. These studies report liver toxicity (increased relative liver weight, hepatocellular hypertrophy, and single-cell necrosis), kidney toxicity (increased relative kidney weight), immune effects (antibody suppression), developmental effects (increased early deliveries and delays in genital development), and cancer (liver and pancreatic tumors). Overall, the available toxicity studies demonstrate that the liver is particularly sensitive to HFPO dimer acid- and HFPO dimer acid ammonium salt-induced toxicity.

The EPA followed the general guidelines for risk assessment set forth by the National Research Council (1983) and the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (2014a) in determining the point of departure (POD) for the derivation of the RfDs for these chemicals. Consistent with the recommendations presented in the EPA's *A Review of the Reference Dose and Reference Concentration Processes* (USEPA 2002), the

Agency applied uncertainty factors (UF) 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 is the oral reproductive/developmental toxicity study in mice with a no-observed-adverse-effect level of 0.1 milligrams per kilogram per day (mg/kg/day) based on liver effects (single-cell necrosis in males) (DuPont-18405-1037, 2010). Using the EPA's *Benchmark Dose Technical Guidance Document* (2012), benchmark dose modeling was used to empirically model the dose-response relationship in the range of observed data. Additionally, the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (2011b) was used to allometrically scale a toxicologically equivalent dose of orally administered agents from adult laboratory animals to adult humans. The use of allometric scaling addresses some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes (i.e., interspecies uncertainty factor). The resulting POD human equivalent dose is 0.023 mg/kg/day. UF applied include a 10 for intraspecies variability, 3 for interspecies differences, and 3 for database deficiencies, including immune effects and additional developmental studies, to yield a subchronic RfD of 0.0002 mg/kg/day. In addition to those above, a UF of 3 was also applied for extrapolation from a subchronic to a chronic duration in the derivation of the chronic RfD of 0.00008 mg/kg/day.

1.0 Introduction and Background

1.1 History of Assessment of GenX Chemicals

In 2008, DuPont submitted two 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-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoic acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6)—or hexafluoropropylene oxide (HFPO) dimer acid—and 2,3,3,3-tetrafluoro-2-(1,1,2,2,3,3,3-heptafluoropropoxy)propanoate (CASRN 62037-80-3)—or HFPO dimer acid ammonium salt—that are part of the GenX processing aid technology they developed. It should be noted that in July 2015, DuPont announced it had separated its Performance Chemicals segment through the creation of The Chemours Company. 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, most of the studies are referenced with DuPont identifiers.

Upon receipt, the 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 for public comment (USEPA, 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.

The 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 the EPA's hazard assessment at that time. The 28-day toxicity study in mice, from which the 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 and followed Good Laboratory Practices (GLP) (DuPont-24459, 2008; OECD, 2008). The submitted studies were also used, in concert with information on other PFAS chemicals, to inform the decision for further testing included in the Consent Order that concluded the PMN review (USEPA, 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. The studies included in the Consent Order relevant to human health and this assessment are listed below:

- 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)

- 90-Day Oral (Gavage) Toxicity Study (OECD TG 408) (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 TG 453) (Dupont-18405-1238, 2013)

It is noted that 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, 2018).

Specifically, for the required oral reproductive/developmental toxicity test, the EPA OPPT included requirements for specific modifications to the test to increase robustness of the study for this class of chemicals (DuPont-18405-1037, 2010; OECD, 2016). These modifications are stated in the Consent Order (USEPA, 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, the 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 the 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, additional OECD TG studies were conducted and submitted for Agency review (e.g., the prenatal and developmental toxicity study in rats (OECD TG 414) (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 (i.e., six or more carbons)[1]) has been phased out in the United States and has been generally replaced by production of shorter chain PFAS, the EPA is aware of ongoing uses by companies that did not participate in the PFOA Stewardship Program and ongoing uses of long-chain PFAS that are available in existing stocks or are 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. Applications of

[1] Long-chain PFASs comprise two subcategories: (1) long-chain perfluoroalkyl carboxylic acids (PFCAs) with eight or more carbons, including PFOA, and (2) perfluoroalkane sulfonates (PFSAs) with six or more carbons, including perfluorohexane sulfonic acid (PFHxS) and perfluorooctane sulfonic acid (PFOS). https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-pfass#tab-3.

fluoropolymers include architecture, fabrics, automotive uses, cabling materials, food processing, pharmaceutical and biotech manufacturing, and semiconductor manufacturing (DuPont, 2013).

One of the PMNs the 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 the EPA under PMN P-08-0509, and it 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 the HFPO dimer acid, HFPO dimer acid ammonium salt, other HFPO dimer acid derivatives, fluoropolymers (including polyethers), and other specialty agrochemical and pharmaceutical applications. 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. There are other PFAS chemicals that 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 micrograms 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, ground water, 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. 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).

GenX chemicals have been identified in other media, including rainwater and air emissions. Estimates from the Chemours Fayetteville Works plant (in the North Carolina Cape Fear watershed) indicate that Chemours' annual emissions of GenX chemicals could exceed 2,700 pounds per year (NCDEQ, 2018a). Additional details on air emissions of GenX chemicals at the Fayetteville Works plant can be found here. 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, West Virginia. The 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 can be found here 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 µg/L in the private drinking water wells. All samples were less than 0.010 µg/L after treatment (USEPA, 2018a).

Finally, 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).

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).

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. 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 no-observed-adverse-effect level (NOAEL) of 0.1 mg/kg/day for liver effects (single-cell necrosis) in mice (DuPont-24459, 2008; DuPont-18405-1037, 2010). The total uncertainty factor (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. Additional details are available at NC DHHS.

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

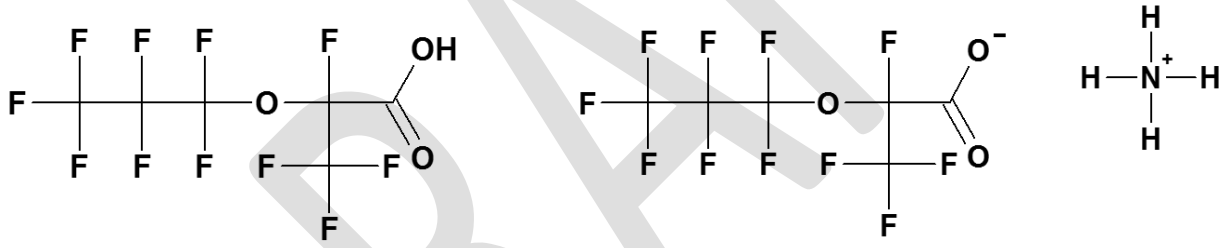
The National Institute for Public Health and the Environment (RIVM) in the Netherlands evaluated the data for GenX chemicals to set a safe limit for air. RIVM's assessment focused on the precursor 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoic acid (FRD-903 (a synonym

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-tetrafluorethyl 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. UF 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. Additional details are available in the RIVM assessment.

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 < 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 portion of the 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
Chemical Abstracts Service Registry Number (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			
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	DuPont.
Chemical Formula	$C_6HF_{11}O_3$	$C_6H_4F_{11}NO_3$	
Molecular Weight	330.06 g/mol	347.08 g/mol	
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	< 2.5×10^{-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.

Property	HFPO Dimer Acid	HFPO Dimer Acid Ammonium Salt	Source
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).
Half-life ($T_{1/2}$) in Water (25 ˚C)	Stable	Stable	Measured hydrolysis values for salt. No degradation in 5 days at 50 ˚C, 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). The EPA concurs with DuPont's assessment that the salt is assumed to be similar. Measured $OH^{\cdot}$ 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 bioconcentration factor (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 bioaccumulation factor (BAF))	< 10	< 10	Pan et al., 2017.[c]

[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 bioconcentration factor (BCF) study so the values are limits based on the limit of quantification (LOQ) 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 bioaccumulation factor (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

The 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., 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 associate with particulate matter. They are not expected to be removed during conventional wastewater treatment or conventional drinking water treatment. They have low potential to bioaccumulate in fish (Table 1).

When released to the fresh water environment, the 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 ($NH4^+$). 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 the HFPO dimer acid and/or ammonium salt show that they are highly soluble in water (Table 1). The measured base dissociation constants (pKb) 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 greater 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 indicates 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 long-range transport could occur. Removal from air is expected to occur through scavenging by water droplets and attachment to particulates followed by precipitation and settling. Studies regarding long-range transport or air removal rates are not available.

2.2.3 Sediments and Soils

Organic carbon normalized sorption coefficients were measured by high-performance liquid chromatography (HPLC) (following OECD TG 121). The sorption of the HFPO dimer acid ammonium salt to soil and sludge were 12.0 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

The 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).[2] The HFPO dimer acid ammonium salt showed no inhibition of activated sludge respiration (OECD TG 209) at up to 1,000 milligrams per liter (mg/L) (DuPont-25938 RV1, 2008).

2.2.5 Incineration

A preliminary study submitted to the 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 the HFPO dimer acid and/or its ammonium salt. The acid $T_{1/2}$ was reported to be about 2,500 seconds at 150 ºC and about 1,900 seconds at 200 ºC. The salt $T_{1/2}$ was 500 seconds at 150 ºC and 200 seconds at 200 ºC (DuPont-PMN Attachment 119, 2008).

2.2.6 Bioaccumulation

Measured bioconcentration factors (BCFs) and bioaccumulation factors (BAFs) suggest that the HFPO dimer acid and its ammonium salt have low potential to bioaccumulate in biota. 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 replicated—BCFs of less than 3 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).

[2] HFPO dimer acid aerobic aquatic biodegradation $T_{1/2}$ = 0% by biochemical oxygen demand [BOD] and 1.5% by ultra-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).

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 transport to the fetus during development and to the neonate during lactation was noted in one study (DuPont-18405-1037, 2010). Urine is the primary pathway for excretion. Based on data from studies of acute, single-dose, gavage, or 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 the male and female monkeys, whereas the female rats' $T_{1/2}$s are shorter.

The HFPO dimer acid is a strong acid (pKa = 2.84) and will be predominantly ionize in aqueous solutions with pHs higher than 4 and in both plasma and serum (DuPont-26349, 2008). Once in solution, the cation that counter balances 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 pHs higher than 4 (e.g., 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 below is the HFPO dimer acid anion concentration regardless of whether animals are dosed with the HFPO dimer acid or its ammonium salt.

2.3.1 Absorption

Oral. Sprague Dawley rats (five of each sex (5/sex)) were administered (via gavage) a single oral dose of 30 milligram 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 was collected and pooled for the first 12 hours and again for 12 to 168 hours after dosing (blood/serum was not sampled). The 12-hour urine collections accounted for a mean of 95% to 97% of the dose, supporting a conclusion that these Gen-X 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. In the 12-hour pooled urine, 31% (mean) of the substance was found for the males and 39% (mean) for the females. By 168 hours postdosing, the mean 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 HFPO dimer acid ammonium salt. This study additionally shows mice either absorb HFPO dimer acid ammonium salt slower or eliminate it in urine slower than rats. In mice, the HFPO dimer in urine was found as both the protonated HFPO dimer acid and its sodium salt. The authors report the sodium salt as being a product of the analytical method. Thus, the authors considered the recovered compound to be the dosed ionized HFPO acid (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 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 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 concentrations 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, 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 animals/sex/group were included for quantitation of the test substance concentration 2 hours after dosing on day 0 (the first day of dosing) (5/sex/dose), providing a measure of postdosing absorption (Table 2). Overall, concentrations increased in a dose-related manner, with broad standard deviations indicative of considerable interanimal variability in the absorption. The doses evaluated differ from those used by Rushing et al. (2017), limiting comparisons of the postexposure 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-hour postdosing 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[a]

Dose mg/kg/day	Males		Females	
	ng/mL	SD	ng/mL	SD
0	0	0	0	0
0.1	736	99	824	72
0.5	3,806	1,175	3,608	1,308
5	42,580	5,214	35,340	9,362

Notes: ng/mL = nanograms per milliliter; SD = standard deviation.
[a] Adapted from Dupont-18405-1307 (2010)

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), groups of three young male and female Crl:CD(SD) rats were exposed to aerosols containing 0, 13, and 100 milligrams per cubic meter (mg/m^3) of HFPO dimer acid ammonium salt (84% purity) for 4 hours per day for 2 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).

Penetration rates were 70.3 ± 5.3 and 6.2 ± 5.3 micrograms per square centimeter per hour, respectively, and these have dermal permeability coefficients (K_p) of 5.71E-4 ± 4.3E-5 and 5.02E-5 ± 4.3E-5 centimeters per hour for rats and 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%) 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 (Pharmacokinetic Clearance and Half-life Data). Liver and fat samples were collected for analysis after sacrifice. 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); however, this ratio equilibrated at the high dose (30 mg/kg) for the ammonium salt (0.78) and the acid (0.71). At both doses, females had a lower accumulation of HFPO dimer acid and its ammonium salt in the liver. In females dosed with HFPO dimer acid ammonium salt at the low dose (10 mg/kg), liver HFPO dimer acid anion concentrations above the limit of quantification (LOQ) (20 nanograms per gram (ng/g)) were observed in two of three animals (20.6 and 54.1 ng/g), while none were observed at the high dose (30 mg/kg). Females dosed with HFPO dimer acid did not have liver anion concentrations above the LOQ (20 ng/g). No HFPO dimer acid anion was detected in the fat tissue samples of any of the rats given HFPO dimer acid or HFPO dimer acid ammonium salt (LOQ 20 ng/g) (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, the 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 (Pharmacokinetic Clearance and Half-life Data). Liver and fat samples as well as plasma were collected for analysis after sacrifice. In males, the mean concentration of HFPO dimer acid anion in the liver was 384 ng/g (ranging from 90.7 to 929 ng/g) for the low dose (10 mg/kg) and 457 ng/g (ranging from 87.9 to 750 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 males the mean concentration in plasma was 756 nanograms per milliliter (ng/mL) (ranging from 139 to 1330 ng/mL) for the high dose (30 mg/kg) and 830 ng/g (ranging from 174 to 1850 ng/mL) for the low dose (10 mg/kg). In females only one of three mice in each dose group had a plasma concentration above the LOQ, which was 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 liver-to-plasma ratio was 0.50 to 0.53, and at the high dose (30 mg/kg), it was 0.52 to 0.63.

To the extent that the perfluorinated ether portion of the HFPO dimer acid is comparable to 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). No studies investigating albumin binding to HFPO dimer acid or its ammonium salt were identified. Although experimental data demonstrating that the HFPO dimer acid anion interacts with albumin are lacking, albumin is the serum protein likely to provide the highest number of primary binding sites for perfluorinated compounds because it accounts for about 60% of total serum protein. Additionally, albumin contains about 15% positively charged amino acids (Spahr and Edsall, 1964), which are likely to be at the hydrophilic surface in contact with serum, where they would electrochemically attract the HFPO dimer acid anion. Some support for the albumin-binding hypothesis comes from the increased albumin and A/G ratio observed in the subchronic and chronic mice and rat studies (DuPont-24459, 2008; DuPont-24447, 2008; DuPont-17751-1026, 2009; DuPont-18405-1238, 2013).

A study by Sheng et al. (2018) recently identified that the HFPO dimer acid anion binds to fatty acid-binding protein (FABP). FABPs are intracellular lipid carrier proteins that reversibly bind 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 might 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 during gestation, as demonstrated in an OECD TG 421 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. Blood was collected from the dams 2 hours after dosing on LD 21 (scheduled termination) and pooled. Trunk blood was collected from the culled pups on postnatal day (PND) 4 and pooled. HFPO dimer acid anion was present in the pooled plasma of PND 4 pups at concentrations approximately two to four times lower than the concentrations in the dams on LD 21. These results indicate that there is transfer of HFPO dimer acid anion from maternal serum. It cannot be determined from this study, however, whether transfer occurs during gestation, during lactation, or both (DuPont-18405-1037, 2010).

Dosing of five dams per dose group continued following pup delivery (PND 1) until LD 20 to enable quantification of uptake from maternal milk by the pups in the DuPont-18405-1037 (2010) study. Blood samples were collected from the dams and pups on LD 21. The plasma levels in the pups were 10 to 32 times lower than the concentrations in pups on PND 4 and were 40 to 60 times lower than those measured in the dams on LD 21. The declining plasma concentrations from PND 4 to LD 21 suggest that there is little-to-no lactational transfer via maternal milk. The F1 pups were then dosed daily from PND 21 to PND 40 by gavage with 0, 0.1, 0.5, or 5 mg/kg/day HFPO dimer acid ammonium salt. The plasma levels in the pups following the 20 days of direct dosing were comparable to those of the dams. Overall, pup plasma serum concentrations increased in a dose-related manner from PND 21 to PND 40, after which time they were comparable to those of the dams. Sex differences in HFPO dimer acid anion concentrations were not observed in the offspring (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 GD 6 to GD 20 (Dupont-18405-849 RV1, 2011). On GD 20, blood was collected from individual dams 2 hours after dosing and trunk blood was collected from the fetuses and pooled for analysis. The plasma concentration in the dam blood samples was three times higher than 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.

In the studies of rats dosed during pregnancy in which plasma concentrations in both the dams and fetuses were measured at GD 20 (Dupont-18405-849 RV1, 2011) the HFPO dimer acid anion plasma concentration ratio for dams to fetuses is approximately three for the rat. In the study of mice dosed during pregnancy (Dupont 18405-1037, 2010), plasma concentrations were measured in dams on LD 21 and in pups on PNDs 4, 21, and 40. If the plasma concentrations in dams on LD 21 are assumed to be representative of those on LD 4, the comparison to pup plasma concentrations on PND 4 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 an *in vitro* study, hepatocytes (1 x 10^6 cells/mL) from male and female Sprague Dawley rats were incubated with 5 micrometers of HFPO dimer acid ammonium salt for a total of 120 minutes (DuPont-23460, 2007). Samples were analyzed for HFPO dimer acid and suspected metabolites at 0, 30, 45, 60, 90, and 120 minutes. Heat inactivated hepatocytes were used as negative controls. 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. No metabolites were detected. 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 postdosing in the urine collections accounted for 103% $\pm$ 2.73% and 99.8% $\pm$ 6.41% of the administered dose for males and females, respectively, and there was no detection of metabolites (DuPont-18405-1017 RV1, 2011).

2.3.5 Excretion

Urine. Studies in rats, mice, and monkeys indicate that urine is the primary excretory pathway for these Gen-X chemicals. In the DuPont-18405-1017 RV1 study (2011), Sprague Dawley rats (5/sex) administered a single oral (gavage) dose of 10 mg/kg HFPO dimer acid ammonium salt excreted 95% to 97% of the dose in urine within 12 hours and by 168 hours, the pooled urine collections accounted for virtually all of the substance administered with no evidence of metabolic alteration. 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 was possibly 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 males and females, respectively. At 168 hours postdosing, 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). Based on the amounts in urine and the clearance from blood, mice appear to have a lower ability to clear the HFPO dimer acid anion by transferring it to urine in the early postexposure period than rats. 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, exposure duration, and excretion observed in serum measurements from the Rushing et al. study (2017) 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 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 in males while females declined more slowly than at the lower doses.

Feces. Fecal elimination of HFPO dimer acid appears to be minor in rats and mice after acute, subacute, subchronic, and chronic exposures. The data for combined fecal matter and cage-wash (dried fecal matter) 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 (DuPont-18405-1017 RV1, 2011; DuPont-18647-1017 RV1, 2011).

2.3.6 Pharmacokinetic 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 per sex per dose, received a single oral dose of 0, 10, or 30 mg/kg/day HFPO dimer acid ammonium salt (84.6% 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. In females, the plasma levels peaked at 1 hour for the low dose and had usually declined to the LOQ (20 ng/mL) by 24 hours. At the 30-mg/kg dose, the plasma levels peaked at one-half to1-hour post-dosing and declined to the LOQ (20 ng/mL) by 24 or 28 hours for males and females, respectively. In male rats, the authors identified 12 hours as the clearance time at the low dose and 22 hours at the high dose (Table 3). In female rats, the clearance values were 4 hours and 8 hours for the low dose and high dose, respectively.

Table 3. Clearance Times in 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	ND
	30 mg/kg			
HFPO dimer acid ammonium salt	22 hr	139 hr	8 hr	62 hr
HFPO dimer acid	22 hr	ND	4 hr	ND

Notes: hr = hour; ND = no data.
[a] Adapted from Dupont-24281 (2008), Dupont-24286 (2008), and Dupont-25300 (2008) where 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 the HFPO dimer acid (98% purity) (Dupont-24286, 2008). At the low dose, plasma concentrations peaked at 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 3).

The protocol outlined above was also followed with mice with a total of 12 Crl:CD(ICR) mice, 3 per sex per 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 postdosing. 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 3).

In the oral toxicokinetic studies, the clearance times were more rapid in rats than in mice and were most rapid in female rats compared to male rats for both anions from the HFPO dimer acid and its ammonium salt. In rats at the 10-mg/kg dose, the HFPO dimer acid took longer to clear than its ammonium salt in both male and female rats. At 30 mg/kg dose, however, both the 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, Sprague Dawley rats (3/sex) and Cynomolgus monkeys (3/sex) were administered a single intravenous dose 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 postdosing 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 the females by approximately one to two orders of magnitude, consistent with the indication that female rats have more rapid elimination. In the monkeys, the plasma levels were nearly identical for the males and females over the first 24 hours. Beyond that point, the plasma concentrations in the males were slightly higher than in the females. The levels of the anion from HFPO dimer acid ammonium salt were very low at 168 hours in male (4 ng/mL) and female (1 ng/mL) monkeys. For 408 hours and beyond, concentrations were below the LOQ, which was 1 ng/mL.

Additionally, in this study, another group of Sprague Dawley rats (3/sex) received a 50-mg/kg intravenous dose of HFPO dimer acid ammonium salt. Over the first 72 hours after the intravenous injection, the HFPO dimer acid anion plasma levels in females were consistently lower than in males by approximately one to two orders of magnitude (6703 ng/mL for males versus 269 ng/mL for females at 12 hours and 776 ng/mL for males versus 7 ng/mL for females at 24 hours). The standard deviations on each serum mean were broad, indicative of wide differences across the three males and three females evaluated at that dose (Dupont-17751-1579 RV1, 2009).

Half-lives. In Gannon et al. (2016), the goodness of fit was calculated for the plasma concentrations after oral and intravenous dosing 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 4 and for the oral studies in Table 5. The alpha phase $T_{1/2}$ represents the plasma concentration in the early postinjection 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 to 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 4. $T_{1/2}$ Estimates from the Intravenous Injection in Sprague Dawley Rats and Cynomolgus Monkeys[a]

$T_{1/2}$	Intravenous Exposures			
	Male Rat	Male Monkey	Female Rat	Female Monkey
Alpha (distribution) Phase (hours)	3.6	2.3	0.4	1.9
Beta (elimination) Phase (hours)	89.1	64.1	22.6	79.6

Note: $T_{1/2}$ = half-life.
[a] Adapted from 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. 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, while 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). The crude metric ratio of the beta (elimination) phase $T_{1/2}$ from intravenous dosing in female rats (approximately 1 day) to female monkeys (3.3 days) is approximately 3, which suggests a smaller magnitude of concern for rodent-to-primate interspecies differences in toxicokinetics than is associated with other perfluorinated compounds (e.g., PFOA).

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. 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 more rapid in females than in males and the beta (elimination) phase $T_{1/2}$ is comparable for both sexes (Table 5). In mice, the $T_{1/2}$ estimates for both the alpha and beta phases are similar for both sexes and the clearance times are shorter for females than for males (Table 5). 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. While further study would be needed to better understand the differences in toxicokinetics between males and females as well as between species, these differences are generally threefold or less (e.g., the ratio of beta phase $T_{1/2}$s in female rats and monkeys described above).

Table 5. $T_{1/2}$ Estimates in Sprague Dawley Rats and Crl/CD1(ICR) Mice Exposed to a Single Oral Dose of HFPO Dimer Acid Ammonium Salt[a]

$T_{1/2}$	Oral Exposures			
	Male Rat	Female Rat	Male Mouse	Female Mouse
Alpha (distribution) Phase (hours)	2.8	0.2	5.8	4.6
Beta (elimination) Phase (hours)	72.2	67.4	36.9	24.2

Note: $T_{1/2}$ = half-life.
[a] Adapted from Gannon et al. (2016).

The time that 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 (more than 90%) is a function of the multiple $T_{1/2}$s for the intercompartmental distribution and elimination; however, at later times the elimination $T_{½}$ 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. This was calculated by multiplying the beta phase $T_{1/2}$ (36.9 hours) 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, the 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 made at 2 hours, 28 days, and 95 days (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 6). 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. study (2017) 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 6. Mean Plasma Concentrations with Standard Deviations (SD) of Dosing with HFPO Dimer Acid Ammonium Salt for at Least 90 Days[a]

Dose mg/kg/day	Day 0			Day 28			Day 95		
	ng/mL	SD	COV	ng/mL	SD	COV	ng/mL	SD	COV
	Males								
0	ND	NA	NA	ND	NA	NA	ND	NA	NA
0.1	736	99	13%	1,124	238	21%	1,276	309	24%
0.5	3,806	1,197	31%	7,192	3,055	42%	7,068	2,398	34%
5	42,580	5,214	12%	52,240	16,725	32%	67,980	13,717	20%
	Females								
0	ND	NA	NA	ND	NA	NA	ND	NA	NA
0.1	824	72	9%	704	350	50%	740	282	38%
0.5	3,606	1,308	36%	4,198	1,239	30%	5,438	1, 696	31%
5	35,340	9,262	26%	46,580	16,842	36%	45,580	5741	13%

Note: COV = coefficient of variation (SD / mean); ND = not detected; NA = not applicable.
[a] Adapted from Dupont 18405-1307 (2010).

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 study (2010) (Table 6). At the 0.5-mg/kg/day dose, plasma concentrations are relatively constant from day 28 to 95 days for the males, but the females' plasma concentrations increased from 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 due to interanimal 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 (46,580 and 45,580 ng/mL, respectively) (Table 6). In the males, the plasma levels at 28 days increased from 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 due to variability in these measurements as a result of interanimal 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 at the bottom right of Figure 2, the green boxes indicate where there are 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 white boxes indicate that there are no data publicly available to allow for determining if these GenX chemicals are found in certain sources and that no human toxicity data exist.

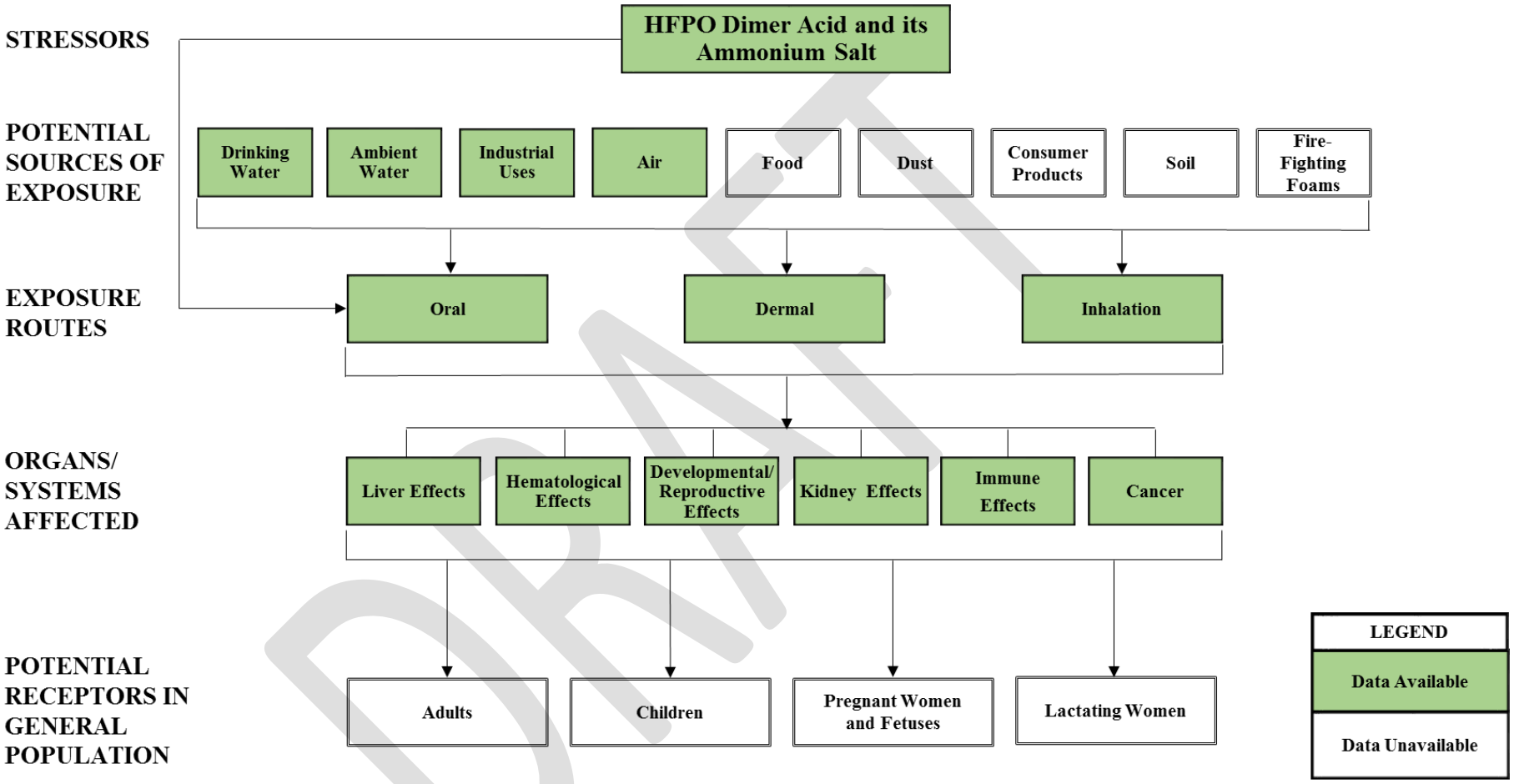


Figure 2. Conceptual Diagram for HFPO Dimer Acid and/or Its Ammonium Salt

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 the EPA considers in the process of developing a POD (depicted in Figure 2).

Stressors: 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). 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, there are only two acute dermal toxicity tests: one dermal irritation study in rabbits, and one acute inhalation toxicity test in rats. Repeated-dose toxicity data are available for oral exposure, but not for the other exposure routes (inhalation and dermal exposures). The only quantitative data available for HFPO dimer acid and its ammonium salt are for oral routes of exposure. Thus, this assessment applies only to the oral route of exposure.

Receptors: The receptors are those in the general population (i.e., adults, the elderly, women of childbearing age, pregnant women, and fetuses, infants, and children) who could be exposed to GenX chemicals in tap water through ingestion, dermal contact, or inhalation at their homes, workplaces, schools, and daycare centers. In the conceptual model in Figure 2, the box for adults also includes sensitive life stages (e.g., women of childbearing age and the elderly).

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* (USEPA, 2002). By 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, peroxisome proliferation, and increased liver weight relative to BW), hematological effects (e.g., decreased red blood cell (RBC) count, hemoglobin, and hematocrit), kidney toxicity (e.g., increased kidney weight, necrosis, and hyperplasia), developmental effects (e.g., BW changes), immune effects (e.g., T cell-dependent antibody response (TDAR) suppression and lymphocyte increases), and suggestive evidence of tumor formation (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. The EPA evaluated liver effects resulting from exposure to GenX chemicals in the context of the Hall criteria (Hall et al., 2012), where liver effects can be considered adverse when changes in liver weight or hepatocellular hypertrophy are accompanied with necrosis, inflammation, and/or fibrosis. The observance of liver necrosis indicates that cytotoxicity also could be a mode of action (MOA) for liver damage.

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

To derive the RfD, the EPA assembled 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 to the EPA by DuPont/Chemours, the manufacturer of GenX chemicals, under TSCA, including with PMNs, as required pursuant to a consent order (USEPA, 2009) or as required under TSCA reporting requirements (e.g., section 8(e)).

To identify public literature available for HFPO dimer acid and its ammonium salt, a comprehensive contractor-led search was conducted of four databases (PubMed, Toxline, Web of Science (WOS), and TSCATS) using CASRN, synonyms, and additional relevant search

strings (see appendix A for a full list). Because the results of this core search were so limited, additional databases were searched for physicochemical property information, health effects, toxicokinetics, and mechanistic information. A list of the additional databases searched is provided in appendix A. Combined, these database searches returned 27 studies for HFPO dimer acid and its ammonium salt, after accounting for duplicates. The submitted studies and literature identified by the search of publicly available sources are available through the EPA's Health & Environmental Research Online (HERO) website at https://hero.epa.gov/hero/index.cfm/project/page/project_id/2627.

3.3.2 Study Screening Process and Study Evaluation

For the publicly available literature, inclusion and exclusion criteria (outlined in appendix A) reduced the database to potentially relevant studies. Potential relevance was based primarily on a title and abstract screen.

For HFPO dimer acid and its ammonium salt, however, most of the available data were submitted to the EPA under TSCA. 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." All available studies were considered for inclusion (see appendices A and B). The majority of the studies selected for this assessment, however, 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.

In addition to these study quality considerations, the EPA OPPT evaluated all studies considered for the derivation of the RfDs using quality criteria for various metrics published in the recent *Application of Systematic Review in TSCA Risk Evaluations* (USEPA, 2018b). Studies were evaluated according to the following domains—test substance, test design, exposure characterization, test model, outcome assessment, confounding/variable control, and data presentation and analysis. As discussed in appendix B, data evaluation is a qualitative assessment of confidence in a study or dataset. A scoring system is applied to ascertain a qualitative rating in order to provide consistency and transparency to the evaluation process. Applying the scoring system results in assigning a confidence level rating of high, medium, low, or unacceptable. Any score falling within the range of the confidence level is associated with the high, medium, or low confidence levels. The system is not intended to imply precision and/or accuracy of the scoring results. For example, any score between 1 and 1.7 is considered as a high confidence level, and

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

differences within that range should be interpreted with caution, as they might not necessarily reflect a relative difference across studies (i.e., a study with score of 1.1 might not be different in terms of quality than a study with a score of 1.3; both studies are judged to have a high confidence level).

The GenX study reviews were conducted by EPA staff with expertise in toxicology, biology, pharmacokinetics/modeling, and related fields with understanding of OECD TGs and risk assessment. Each expert reviewed 1–2 studies. Following individual study reviews, a scientist performed a final review of all reviewers' evaluations to provide a level of consistency across the evaluations. The results of data evaluation for these individual studies are provided in appendix B.

3.3.3 Approach for Derivation of 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 put forth by the National Research Council (1983) and the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (USEPA, 2014a). Additional EPA guidelines and other Agency reports used in the development of this assessment include the following:

- *Guidelines for Developmental Toxicity Risk Assessment* (USEPA, 1991)
- *Guidelines for Reproductive Toxicity Risk Assessment* (USEPA, 1996)
- *Guidelines for Neurotoxicity Risk Assessment* (USEPA, 1998)
- *A Review of the Reference Dose and Reference Concentration Processes* (USEPA, 2002)
- *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005a)
- *Supplemental Guidance for Assessing Susceptibility from Early-Life Exposure to Carcinogens* (USEPA, 2005b)
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (USEPA, 2006)
- *Exposure Factors Handbook* (USEPA, 2011a)
- *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (USEPA, 2011b)
- *Benchmark Dose Technical Guidance Document* (USEPA, 2012)
- *Child-Specific Exposure Scenarios Examples* (USEPA, 2014b)
- *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* (USEPA, 2014c)
- *Application of Systematic Review in TSCA Risk Evaluations* (USEPA, 2018b)

The EPA's *A Review of the Reference Dose and Reference Concentration* document describes a multistep approach to dose-response assessment, including analysis in the range of observation followed by extrapolation to lower levels (USEPA, 2002). The 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, the EPA used benchmark dose (BMD) modeling to refine the 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, endpoints are selected that the risk assessor judges 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. The OPPT evaluated all toxicokinetic, repeated-dose toxicity studies of 28 days and longer and potentially relevant published *in vitro* studies using the approach describe in *Application of Systematic Review in TSCA Risk Evaluations* (USEPA, 2018b). Results are provided in appendix B.

Step 2: Conduct BMD Modeling. Using the EPA's *Benchmark Dose Technical Guidance Document* (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 describes 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 using the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (USEPA, 2011b).

Step 4: Provide rationale for selecting UF. UF 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 using a $BW^{3/4}$ allometric scaling approach consistent with EPA guidance (USEPA, 2011b).

UF = Total UF established 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.

3.3.4 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, BW, and fetal development are adversely impacted by exposure to

GenX chemicals. 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 to derive an inhalation reference concentration was not conducted due to 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 portal of entry effects, albeit at a high dose. Tumors were also observed following oral exposure to GenX chemicals; however, the tumor data failed to demonstrate a direct response to dose and thus were not considered quantitatively.

4.0 Study Summaries

4.1 Acute Toxicity Studies

There are over 10 studies available detailing the acute toxicity and irritation studies of HFPO dimer acid and its ammonium salt. This section summarizes the available acute oral, dermal, and inhalation toxicity studies for HFPO dimer acid and its ammonium salt as well as dermal and eye irritation studies. Detailed study summaries are available in appendix C.

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) 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 the 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-770-95, 1996; DuPont-2-63, 1963).

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-22932,

2007; DuPont-24126, 2007; Dupont-25438 RV1, 2008; DuPont-25875, 2008; DuPont-770-95, 1996). 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) 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 greater than 5,000 mg/kg (both sexes). Erythema was observed only in females, whereas hyperkeratosis and ulceration were observed in some 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 greater than 5,000 mg/kg. In this study, erythema persisted for 13 days postapplication and was accompanied by scaling and sloughing of skin. One of the rabbits also exhibited necrosis for 2–6 days postapplication (DuPont-839-95, 1996).

Inhalation Toxicity. One study (conducted using 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 greater 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) 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). 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 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, HFPO dimer acid ammonium salt (purity 88%) was administered on 28 consecutive days via gavage (vehicle was deionized water) (OECD, 2008; 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 due to 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 some 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 P-450 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 P-450 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 P-450 content remained unaltered (DuPont-24447, 2008). The 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, 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.

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% compared to controls, respectively. 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 ADH (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 P-450 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 P-450 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 P-450 content. The 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). The 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)
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 animals/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.

A small decrease compared to control in mean corpuscular hemoglobin concentration (3%) in males and increased bilirubin (14%) in females were 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).

The EPA concluded that the NOAEL in this study is 0.5 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) at a LOAEL of 5 mg/kg/day.

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 (DuPont-18405-1238, 2013), 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; 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.

Mean survival was unaffected by treatment. All females were sacrificed before study termination at 101 weeks, however, because of decreased survival across all groups, including the control. There were no statistically significant differences in survival across groups. The females in the high-dose group were observed to have papillary necrosis and inflammation of the kidneys that were 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). The 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 animals, the EPA recommended that the submitter (a) reexamine their test data, (b) identify the animals that died without 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, the EPA agreed 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, 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 tumor types 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/mg/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/d 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.

Based upon the 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 the EPA accepted the results of the reanalysis that excluded the early deaths.

4.5 Reproductive and Developmental Toxicity Studies

DuPont-18405-1037 (2010)

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, 2016). 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 LD 20 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 PND 21 through PND 40.

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. At the high dose, mean BW gains were increased (5.1%–14.0%) compared to controls throughout lactation; the differences were significant on LDs 1, 4, and 21. 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 administered 5 mg/kg/day, respectively.

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 5 mg/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, male and female F_1 pups exhibited lower mean BWs at PNDs 4, 7, 14, 21, and 28. Male F_1 pups continued to exhibit lower mean BWs at PNDs 35 and 40. Although values for the attainment of balanopreputial separation and vaginal patency 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 may 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.

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 GD 6 through GD 20 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 GD 6. Lack of dosing for males and females prior to and during mating and failure to dose the dams during the GD 0 to GD 6 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. 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 (GD 6–GD 21) at the highest dose. Early delivery on GD 21 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 in the available historical controls data for early deliveries in this rat strain (17 datasets), no females showed early deliveries (i.e., before GD 21).

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 tissues for the dams. Of note is that a 1,000 mg/kg/day dam that died on GD 20 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.

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 were 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 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

Overall, there are limited data providing mechanistic insight into the effects of HFPO dimer acid and/or its ammonium salt.

Wang et al., 2016
In one study investigating changes in gene expression, male ICR mice (n=12/group) were administered 1 mg/kg/day HFPO dimer acid ammonium salt via oral gavage for 28 days (Wang

et al., 2016). 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. At termination, blood was collected and liver weights were determined. Liver samples were processed for histopathological analysis or frozen for high-throughput RNA-sequencing analysis (3/group).

Statistically significant treatment-related findings reported include increased absolute liver weight (31%) and relative liver weight (28%), ALP (150%), low-density lipoprotein cholesterol (50%), decreased total bilirubin (37%), and decreased direct bilirubin (45%) compared to control. Qualitative histopathological findings were also reported in the liver and included lipid droplet accumulation, hepatocellular hypertrophy, mild steatosis, and karyolysis. To potentially gain mechanistic insight into the causes of these liver effects, high-throughput RNA-sequencing was conducted and 146 hepatic transcripts (101 upregulated and 45 downregulated) were statistically significantly changed from control following treatment with HFPO dimer acid ammonium salt. These changes in hepatic transcripts indicated differential gene expression of four cell signaling pathways associated with lipid metabolism: the PPARα signaling pathway, and the pathways for retinol metabolism and fatty acid degradation, as well as the pathway for the catabolism of the polyunsaturated fatty acid arachidonic acid.

Sheng et al., 2018
Sheng et al. (2018) used *in vitro* experiments to investigate perfluoroalkyl cytotoxicity and binding to proteins. The study tested multiple perfluoroalkyl substances, including the HFPO dimer acid ammonium salt (CASN 62037-80-3). The study authors state that they used the HFPO dimer acid; however, the CASN listed in the study is for the ammonium salt. The study authors used a cell viability assay in a human liver cell line (HL-7702) to determine the cytotoxicity of the various perfluoralkyl substances and used flow cytometry to investigate effects on cell proliferation. The authors noted, however, that the effects of HFPO dimer acid ammonium salt on cytotoxicity and cell proliferation were undeterminable through these assays because of the chemical's low boiling point and high volatility. Therefore, the only data on HFPO dimer acid ammonium salt generated by this study pertain to its ability to bind to human liver fatty acid-binding protein (hL-FABP). This binding affinity was explored because previous PFAS have exhibited effective binding to hL-FABP and this binding might explain how PFAS can enter into hepatocytes—a target cell for the HFPO dimer acid and/or its ammonium salt. Ultimately, this study found that HFPO dimer acid ammonium salt exhibited a weaker binding affinity and bound differently to hL-FABP than PFOA and perfluorooctane sulfonate (PFOS) (Sheng et al., 2018). These results were replicated using a predictive model of binding affinity to hL-FABP (Cheng and Ng, 2018).

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 D.

4.7 Mode of Action

The MOA of HFPO dimer acid and/or ammonium salt toxicity is not clearly understood. Additionally, the mode of carcinogenic action of HFPO dimer acid and/or ammonium salt is not clearly understood. For some PFAS (e.g., PFOA), PPARα agonism has been proposed as a potential MOA for the liver tumors (Klaunig et al., 2003, 2012; Maloney and Waxman, 1999). In this MOA, binding of PFOA to the PPARα receptor results in increased peroxisome proliferation and cell replication. PPARα is primarily expressed in the liver, but also is present in the kidney, intestines, heart, and brown adipose tissue (Hall et al., 2012). There are four key events in the PPARα-agonist MOA for liver tumors (Klaunig et al., 2003, 2012). The first key event is activation of PPARα. Increased palmitoyl-CoA oxidase activity is used in many studies as a biomarker for PPARα activation. Other associated indicators are hepatocellular hypertrophy and increased liver weight. These indicators alone, however, are not sufficient to establish a PPARα MOA because they also are caused by chemicals that have no influence on PPARα. Additional key events outlined by Klaunig et al. (2003, 2012) include cell proliferation and decreased apoptosis, development of preneoplastic foci, and their clonal expansion.

For HFPO dimer acid and/or ammonium salt, there are some data that demonstrate activation of peroxisome proliferation. Activation of peroxisome proliferation was demonstrated in multiple 28-day studies (DuPont-24447, 2008; DuPont-24459, 2008; Rushing et al., 2017; Wang et al., 2016). 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. There were no significant increases in acyl-CoA oxidase activity at 1 mg/kg/day. Rushing et al. (2017) concluded that the HFPO dimer acid appears to be less potent than PFOA in inducing hepatic peroxisome proliferation. The DuPont studies used β-oxidation activity and total cytochrome P-450 content as markers of peroxisome proliferation. 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 HFPO dimer acid ammonium salt by 57%, 744%, and 648%, respectively, and total cytochrome P-450 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 P-450 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 P-450 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 P-450 content remained unaltered (DuPont-24447, 2008). Finally, Wang et al. (2016) demonstrates significant increases in hepatic mRNA levels of many PPAR targets (e.g., CD36 antigen, acyl-

CoA oxidase 1, cytochrome P450 family members) after administration of 1 mg/kg/day HFPO dimer acid ammonium salt for 28 days.

Although findings consistent with PPARα agonists were observed (e.g., increases in liver weight, hepatocellular hypertrophy, and increased β-oxidation activity), data gaps exist for key events, like apoptosis. Other indicators such as steatosis were not assessed in any of the DuPont/Chemours studies. Wang et al. (2016) is the only publicly available study to qualitatively mention observing steatosis in mouse liver samples, but does not provide quantitative measurements. Additionally, liver necrosis was consistently observed in rodent toxicity studies with HFPO dimer acid ammonium salt, which suggests that cytotoxicity is a possible MOA for the observed liver tumors. Overall, the findings are not adequate to definitively conclude that a PPARα MOA is operative for HFPO dimer acid and/or ammonium salt. Additionally, no data support identification of a potential MOA for the pancreatic and testicular tumors as being related to PPARα or any of the proposed alternative MOAs for the tumor development in either organ.

5.0 Summary of Hazard

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 for PPARα activation. The increased relative liver weight and hepatocellular hypertrophy are only considered adverse when they are accompanied by effects such as necrosis, fibrosis, inflammation, steatosis, and significantly increased serum levels for enzymes indicative of liver tissue damage (Hall et al., 2012).

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 up to 28 days or up to 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., 2016). 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). 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%) as compared to male mice administered the same dose. 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.

Finally, liver damage was confirmed microscopically in male and female mice and rats in several less than chronic studies (15–90 day) 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., 2016). The most prevalent liver effects following both subchronic and chronic exposure to HFPO dimer acid and/or ammonium salt were hepatocellular hypertrophy and single-cell and/or focal necrosis. In both sexes of mice exposed subchronically, hepatocellular hypertrophy was observed at 0.5 mg/kg/day, while male and female rats showed these effects 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 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%). Hepatocellular necrosis was detected in nearly all the available studies and at the lowest doses tested. In the oral/reproductive subchronic study, male and female mice presented single-cell necrosis in doses as low as 0.5 mg/kg/day (21% and 8%, respectively), which significantly increased at 5 mg/kg/day (100% and 88%, respectively). Male and female rats exhibited subchronic hepatocellular necrosis at much higher doses, with males showing 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%). This finding indicates that mice are more sensitive to liver necrosis than rats in 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.

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 HFPO dimer acid ammonium salt. 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 alone significantly decreased by 4% when female Crl:CD(SD) rats were administered 1 mg/kg/day 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 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 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 Developmental/Reproductive

Evidence suggests HFPO dimer acid and/or ammonium salt could target the reproductive system and the developing fetus/child. Currently, there are two available studies investigating the developmental and reproductive effects of HFPO dimer acid and/or ammonium salt (DuPont-18405-841, 2010; DuPont-18405-1037, 2010). In both studies, there was a decrease in rodent pup weight that ranged from 9%–24% when the pups were exposed to 5 mg/kg/day–1,000 mg/kg/day in utero. 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 (a finding that might 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 also 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). In the mouse study, F_0 males exhibited a decrease of 12% in the relative epididymis weight (DuPont-18405-1037, 2010). Overall, no treatment-related effects were identified for many reproductive parameters in the mouse study (mating, fertility, and 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). In the rat developmental toxicity study, however, early delivery on GD 21 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 were significantly altered from control at 1,000 mg/kg/day (DuPont-18405-841, 2010). Moreover, in this 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.

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 (USFDA, 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 some 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. Given uncertainties, 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 the EPA's *Guidelines for Carcinogen Risk Assessment* (USEPA, 2005), 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 Dose Response Assessment

6.1 Study and Endpoint Selection

Multiple animal studies were available for consideration for the development of the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt. These studies included short-term, subchronic, and chronic exposures, including developmental and reproductive toxicity studies. These studies and their NOAELs and/or LOAELs are presented in Table 7.

Table 7. Summary of Study NOAELS/LOAELS

Study	Overall Study Quality (See Appendix B)	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
28-Day Oral (Gavage) Toxicity Study in Rats (OECD TG 407) DuPont-24447 (2008)	High (≥ 1 and < 1.7)	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 TG 407) DuPont-24459 (2008)	High (≥ 1 and < 1.7)	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)	High (≥ 1 and < 1.7)	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 TG 408) DuPont-17751-1026 (2009)	High (≥ 1 and < 1.7)	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)
90-Day Oral (Gavage) Toxicity Study in Mice (OECD TG 408) DuPont-18405-1307 (2010)	High (≥ 1 and < 1.7)	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 ↑ hepatocellular hypertrophy and single-cell necrosis in males)

Study	Overall Study Quality (See Appendix B)	Doses (mg/kg/day)	NOAEL or LOAEL (mg/kg/day)	Effects at the LOAEL
Combined Chronic Toxicity/ Oncogenicity Study in Rats (OECD TG 453) DuPont-18405-1238 (2013)	High (≥ 1 and < 1.7)	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 TG 421; modified according to the Consent Order) DuPont-18405-1037 (2010)	High (≥ 1 and < 1.7)	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 in males, and ↑ relative liver weight in both sexes) Developmental effects (↓ pup weights, and delays in the attainment of balanopreputial separation and vaginal patency)
Prenatal and Developmental Toxicity Study in Rats (OECD TG 414) DuPont-18405-841 (2010)	High (≥ 1 and < 1.7)	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, and ↓ gravid uterine weight)

As summarized in section 5 and in Table 7, adverse effects, including liver toxicity, BW changes, and hematological and immune effects were observed in the range of 0.5–1,000 mg/kg/day. These studies were evaluated further based on duration of exposure, use of a control and two or more doses, and provision of NOAEL and/or LOAEL values.

Data from these available studies indicate that the liver is the most sensitive target of GenX chemicals toxicity. 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 of exposure to GenX chemicals. As highlighted above, immune and hematological effects were also observed at low doses; however, these endpoints are not as consistently observed compared to 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 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 on the potential for GenX chemicals to impact aspects of immune function beyond the immunosuppression (e.g., allergic responses and autoimmunity) exist. 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).

Therefore, the EPA considered studies that observed adverse liver effects at the lowest dose tested in the selection of the critical study for derivation of the RfDs. Because liver effects such as increases in liver weight and hypertrophy can be associated with activation of cellular PPARα receptors, the 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. Only the doses associated with the effects classified as adverse were used for the quantification. With these criteria in mind, it can be concluded that some of the observed liver effects indicate toxicity of relevance to humans as opposed to PPARα activation unique to rodents.

For the GenX chemicals database, the studies that identified adverse liver effects at the lowest doses are the 28-day oral (gavage) toxicity study in mice (DuPont-24459, 2008) 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 and single-cell necrosis at doses as low as 0.5 mg/kg/day. These studies were

completed according to OECD TGs and followed GLP. Both studies used a sufficient number of mice per dose group, 10/dose group in the 28-day oral (gavage) toxicity study and 22 -25/ dose group in the oral (gavage) reproduction/developmental toxicity study. Additionally, both studies were completed on the mouse, which the data indicate is more sensitive than the rat to liver effects. Thus, the liver effects noted in these studies (DuPont-24459, 2008 and DuPont-18405-1037, 2010), specifically single-cell necrosis in males, were selected for BMD modeling.

Longer term studies identifying liver effects were also considered, but not selected for BMD modeling. Liver effects observed in the 90-day study in mice (DuPont-18405-1307, 2010) were observed at higher doses (greater than or equal to 5 mg/kg/day) than in the oral reproductive/developmental toxicity study in mice (0.5 mg/kg/day). Although these studies used the same strain of mice (Crl:CD1(ICR)), the modified developmental/reproductive study (DuPont-18405-1037, 2010) evaluated 22–25 mice/dose group while the 90-day study in mice only used 10 mice/dose group for liver endpoints. The difference in the number of mice per dosing group might have an impact on observed effect levels in these studies. For example, in the 90-day study, statistically significant adverse effects in the liver were not observed until 5 mg/kg/day, yet there are indications of liver damage at 0.5 mg/kg/day, although these effects did not reach a statistically significant difference from the control group. Specifically, absolute and relative liver weight increased in males by 12% and 11%, respectively, relative to control mice at 0.5 mg/kg/day. In males dosed with 0.5 mg/kg/day, 4/10 livers were observed to be discolored, compared to 0/10 for control mice. There were also increases in serum liver proteins at 0.5 mg/kg/day in males, although they did not differ significantly from control. AST, ALP, and ALT increased 35%, 40%, and 35%, respectively, compared to control. Finally, these increases in serum liver protein levels were associated with hepatocellular hypertrophy in males at 0.5 mg/kg/day (8/10), compared to 0 in control. Thus, the difference in the NOAEL for liver effects between the 90-day study and the reproductive/developmental study might reflect on the difference in animal number per dose group.

Additionally, the chronic 2-year cancer bioassay was not selected as the critical study in the derivation of the RfD for several reasons. Effects observed at low doses in this study include changes in serum albumin levels and the A/G ratio in male rats. 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 inconsistencies. 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 only evaluated rats, and data indicate that mice appear to be more sensitive. For example, male mice presented with single-cell necrosis in doses as low as 0.5 mg/kg/day (5/24 mice compared to 1/25 in the control), which significantly increased at 5 mg/kg/day (24/24 mice compared to 1/25 in the control). Female mice also had a significant increase in incidence compared to control at 5 mg/kg/day for both focal/multifocal (5/24 mice compared to 1/24 in the control) and single-cell necrosis (21/24 mice compared to 1/24 in the control). Conversely, male and female rats exhibited no subchronic hepatocellular necrosis in the 90-day study (DuPont-17751-1026, 2009), yet hepatocellular necrosis is observed in the chronic study at much higher doses (DuPont-18405-1238, 2013). Specifically, rats have significant increases in incidence compared to control in focal liver degeneration and centrilobular necrosis at 50 mg/kg/day (male) and 500 mg/kg/day (female), respectively. While typically a chronic

study is the preferred duration for development of lifetime RfD, in this case, the oral reproductive/developmental toxicity study indicates that adverse effects on the liver are observed in the parental mice at lower doses than those reported in the chronic study in rats.

6.2 BMD Modeling

There are no biologically based dose-response (BBDR) models available for HFPO dimer acid and its ammonium salt. Thus, using its Benchmark Dose Software (version 2.6), the 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.

Consistent with the EPA's *Benchmark Dose Technical Guidance* (USEPA, 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. Candidate PODs were estimated from all three doses (plus control) for DuPont-18405-1037 (2010) and DuPont-24459 (2008). Results of the analyses are available in Table 8. Further details, including the modeling output and graphical results for the selected models, are provided in appendix E of this report.

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

EPA guidance was followed to calculate a candidate POD_{HED} from the $BMDL_{10}$ using a $BW^{3/4}$ allometric scaling approach (USEPA, 2011b). The allometric scaling approach 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. The HFPO dimer acid ammonium salt $BMDL_{10}$s from the experimental animal studies (DuPont-18405-1037, 2010; DuPont-24459, 2008) 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). For the short-term 28-day oral study in mice (DuPont-24459, 2008), a BW_a value of 0.0347 kg was identified as the mean BW of the male mouse controls on study day 28 (the final day of animal dosing). A BW_h of 80 kg for humans was selected based on National Health and Nutrition Examination Survey (NHANES) sampling data (USEPA, 2011a). For adults (more than 21 years of age), the 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 the EPA's *Exposure Factors Handbook* (USEPA, 2011a). The updated BW represents the mean weight for

adults ages 21 and older. The resulting DAF for the allometric scaling of doses from mice to humans is 0.15 for DuPont-18405-1037 (2010) and 0.14 for DuPont-24459 (2008). Applying the DAF to the two $BMDL_{10}$s identified for liver effects in adult mice yields a POD_{HED} as follows:

$$POD_{HED} = BMDL_{10} \text{ animal dose (mg/kg/day)} \times DAF$$

Using the $BMDL_{10}$ of 0.15 mg/kg/day to complete the calculation results in a POD_{HED} for single-cell necrosis of the liver from DuPont-18405-1037 (2010) of 0.023 mg/kg/day. Using the $BMDL_{10}$ of 0.3 mg/kg/day to complete the calculation results in a POD_{HED} for single-cell necrosis of the liver from DuPont-24459 (2008) of 0.042 mg/kg/day. Table 8 presents a summary of the determination of the POD_{HED}s.

Table 8. Summary of Determination of POD_{HED}

Endpoint	Species/ Sex	NOAEL (mg/kg/day)	Model	BMR	BMD_{10} (mg/kg/day)	$BMDL_{10}$ (mg/kg/day)	DAF	POD_{HED}[a] (mg/kg/day)
Liver single-cell necrosis in parental males (DuPont-18405-1037 2010)	Crl:CD1(ICR) mice F_0 parental male	0.1	Benchmark Dose Multistage 2	10%	0.37	0.15	0.15	0.023
Liver single-cell necrosis in males (DuPont-24459 2008)	Crl:CD1(ICR) male mice	0.1	Benchmark Dose Multistage 2 and Quantal-Linear	10%	0.60 (Multistage 2) and 1.4 (Quantal-Linear)	0.3	0.14	0.042

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

6.4 Derivation of the RfD

6.4.1 Critical Study and Effect

The oral reproductive/developmental toxicity study in mice and liver effects (single-cell necrosis in males) were selected as the critical study and the effect for deriving the subchronic and chronic RfDs for HFPO dimer acid and its ammonium salt (DuPont-18405-1037, 2010). This study used a larger sample size (n=24 / dose versus n=10 / dose in 28 day study) to provide the most health-protective POD_{HED}. Additionally, this study was of a longer duration (84/85 days versus 28 days) in the mouse, which is the more sensitive species.

Several of the other studies provide support for the selection of this study as the critical study and liver necrosis as the critical effect (DuPont-24459, 2008; DuPont-24447, 2008; DuPont-18405-1307, 2010; DuPont-18405-1238, 2013; DuPont-18405-841, 2010). 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).

6.4.2 Uncertainty Factors

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 the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (USEPA, 2011b). This guidance describes approaches for deriving POD_{HED}s from data from laboratory animals, with the preferred approach being physiologically based pharmacokinetic (PBPK) modeling. For HFPO dimer acid and ammonium salt, no PBPK models have been developed and 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 some aspects of cross-species extrapolation of toxicokinetic and toxicodynamic processes, some residual uncertainty remains (i.e., MOA) (USEPA, 2011b). Thus, in the absence of chemical-specific data to quantify this uncertainty, the EPA's guidance recommends use of a UF of 3.

An intraspecies uncertainty factor (UF_H) of 10 is assigned 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 (life style) 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 (single-cell necrosis in male mice) 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 3 ($10^{1/2} = 3.16$, rounded to 3) was applied for the derivation of the chronic RfD, but not of the subchronic RfD. The study selected as the critical study was the oral reproductive/developmental toxicity study of HFPO dimer acid ammonium salt in mice (DuPont-18405-1037, 2010). The critical effect selected is single-cell necrosis in parental (F_0) male mice. The duration of dosing of these F_0 males was 84–85 days, a study duration that falls short of a standard subchronic study and below the duration of a chronic study. Chronic studies typically employ repeated dosing for longer than 90 days or more than 10% of the human life span (USEPA, 2002). Chronic data in another species are available to inform whether effects would be expected to occur with longer exposure durations. A 2-year combined chronic toxicity/oncogenicity study is available in rats (DuPont-18405-1238, 2013), which identified a NOAEL of 1 mg/kg/day for liver effects (increased liver enzyme levels and centrilobular hepatocellular hypertrophy and cystic focal degeneration in males and centrilobular necrosis in both sexes). These effects are consistent with those observed

in the oral reproductive/developmental toxicity screen in mice. The NOAELs for the oral reproductive/developmental toxicity study and the chronic study are within one order of magnitude of each other, suggesting consistency in dose-response relationships between these studies. The combined chronic toxicity/oncogenicity study was conducted, however, in rats that appear to be less sensitive than mice. For these reasons, a UF of 3 was used to account for extrapolation from subchronic to chronic exposure duration for the chronic RfD. 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 3 ($10^{1/2} = 3.16$, rounded to 3) was applied to account for database deficiencies. There are no human data from epidemiological studies in the general population or worker cohorts evaluating the effects of exposure to these GenX chemicals. The database for the HFPO dimer acid and its ammonium salt includes data submitted to the EPA under TSCA assessing 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. Many of the studies were conducted according to OECD TGs and principles of GLP, and full study reports were submitted for Agency review. For the HFPO dimer acid ammonium salt specifically, there are two short-term and two subchronic studies in both rats and mice (28 and 90 days) and a chronic carcinogenicity study (2 years) in rats. One reproductive and developmental toxicity study in mice and one prenatal and developmental toxicity study in rats are also available. In addition to studies submitted under TSCA, a small number of studies presenting other health data are available in the public literature, including a single immunotoxicity study. In some cases, the identified publications in the open literature represent the studies previously submitted to the EPA and do not constitute additional information.

There are several deficiencies in the database for HFPO dimer acid and its ammonium salt, however, including limited testing of developmental toxicity and immunological responses. Other PFAS, particularly PFOA and PFOS, have been shown to lead to developmental effects, including skeletal variations, decreased neonatal survival, altered fetal BW, and developmental alterations such as delayed vaginal opening, accelerated preputial separation, and delayed mammary gland development (USEPA, 2016a, 2016b). For HFPO dimer acid and/or ammonium salt, there is evidence of developmental toxicity, although at higher doses than effects on the liver (DuPont-18405-1037, 2010; DuPont-18405-841, 2010). Significant adverse effects observed following exposure to HFPO dimer acid ammonium salt include early delivery of pups, decreased pup BW, and delays in the attainment of balanopreputial separation and vaginal patency. The lack of a full two-generation reproductive toxicity study evaluating exposures during early organogenesis (i.e., GD 0 to GD 6) and studies evaluating additional developmental endpoints that have been observed following exposure to other PFAS (i.e., skeletal development in mice and altered puberty in mice (USEPA, 2016a, 2016b)) is a database deficiency.

PFAS chemicals, including PFOS and PFOA, interact with the immune system in studies of both humans and animals (USEPA, 2016a, 2016b). The GenX chemicals database includes two LLNAs 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, 2010). The LLNAs were conducted with HFPO dimer

acid ammonium salt preparations of varied purity and yielded equivocal results (one positive and one negative). Evaluation of additional immune function assays, histopathology, and immune endpoints such as antibody levels are not available. The combined dataset was found to be weak as it did not include sufficient measures of immunopathology, humoral immunity, cell-mediated immunity, nonspecific immunity, or host resistance. 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, there are some additional research needs that factor into the database uncertainty. First, the lack of a chronic study in the mouse, which appears to be more sensitive than the rat to GenX chemicals exposure, is a data gap. This uncertainty, however, is also addressed in the subchronic-to-chronic UF. Second, additional research is needed to help determine if the inconsistent hematological effects observed in many of the studies are adverse and should be considered critical.

6.5 Subchronic RfD

The subchronic RfD is calculated as follows:

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

$$= \frac{0.023 \frac{mg}{kg}/day}{100}$$

= 0.0002 mg/kg/day or 0.2 µg/kg/day

where:

POD_{HED} = 0.023 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (single-cell necrosis) in parental male mice exposed to HFPO dimer acid ammonium salt by gavage for 84–85 days (DuPont-18405-1037, 2010).

Total UF = 100, including a 10 for UF_H, a 3 for UF_A, and a 3 for UF_D.

6.6 Chronic RfD

The chronic RfD is calculated as follows:

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

$$= \frac{0.023 \frac{mg}{kg}/day}{300}$$

= 0.00008 mg/kg/day or 0.08 µg/kg/day

where:

POD_{HED} = 0.023 mg/kg/day, the HED based on the $BMDL_{10}$ for liver effects (single-cell necrosis) in parental male mice exposed to HFPO dimer acid ammonium salt by gavage for 84–85 days (DuPont-18405-1037, 2010).

Total UF = 300, including a 10 for UF_H, a 3 for UF_A, a 3 for UF_S, and a 3 for UF_D.

7.0 Discussion of Uncertainties

7.1 Uncertainty and Variability

The variability and uncertainty in an RfD is a function of both intrinsic and extrinsic factors. The EPA has identified eight short-term, subchronic, and chronic studies that provided 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 EPA selected studies with the lowest NOAELs for BMD modeling and determination of the POD. The Agency believes the uncertainty in the chosen POD is minimized because of the available data following various durations of exposure that support the liver as the primary target of toxicity.

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 genetically, relatively 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, are minimized using the modeled outcomes and use of allometric scaling to help inform the application of the UF.

While the 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 (USEPA, 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. $BW^{3/4}$ scaling is appropriate in this scenario because GenX chemicals are not metabolized and have relatively short clearance times, especially compared to other longer chain PFAS chemicals such as PFOA (USEPA, 2011b; DuPont-18405-1017 RV1, 2011; 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 (USEPA, 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-18405-1307 (2010), DuPont-18405-1238 (2013), and DuPont-18405-1037 (2010).

The chronic RfD is based on the POD_{HED} derived from the parental males from the oral reproductive/developmental toxicity study in mice with application of a total UF of 300 to account for variability in the human population, database uncertainties, and possible differences in the ways in which humans and rodents respond to the HFPO dimer acid and/or its ammonium salt that reaches their tissues (DuPont-18405-1037, 2010). The selected RfD is based on the adverse liver effects observed in the parental male animals. Selection of this endpoint is expected to provide protection to both the sensitive life stages and the general population. The RfD is supported by the outcomes from other studies (DuPont-17751-1026, 2009; DuPont-18405-1037, 2010; DuPont-24459, 2008) based on different endpoints, including hematological, immune, and developmental effects. These supporting data from the HFPO dimer acid and its ammonium salt database increase confidence in the RfD.

7.2 Composition of Test Substance

Most of the available data for HFPO dimer acid and/or its ammonium salt were submitted to the EPA by DuPont/Chemours, the manufacturer of GenX chemicals, under TSCA, including with PMNs, as required pursuant to a consent order for these chemicals (USEPA, 2009) or as required under TSCA reporting requirements (e.g., section 8(e)). In these submissions, DuPont/Chemours 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/Chemours 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/Chemours 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/Chemours 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 to 30 mg/kg-d have been identified for effects including developmental, liver, and immune endpoints (US EPA, 2016a). Despite trace amounts of PFOA that might be present as an impurity, the 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, the 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-18405-1307, 2010; DuPont-18405-841, 2010; DuPont-18405-1238, 2013). Additionally, the same test substance (Lot H-28397) was used in both the mouse and rat 28-day studies (DuPont-24459, 2008; DuPont-24447, 2008). Despite differences in test substance purity, adverse effects were observed consistently across the DuPont/Chemours studies. The available published literature did not report purity in their methods and formulations of HFPO dimer acid and ammonium salt (Rushing et al., 2017; Sheng et al., 2018; Wang et al., 2016).

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, the EPA concluded that the DuPont/Chemours 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.

7.3 Use of Data-Derived Extrapolation Factors

For HFPO dimer acid and/or ammonium salt, there are no human data and no BBDR or PBPK models available to evaluate toxicokinetic and toxicodynamic differences between humans and animals. Additionally, only a few studies are available in 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. The 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 (USEPA, 2014c). Using the decision process described in Figure 2, the 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 (USEPA, 2011b). These approaches are described further below.

7.4 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. The available data for HFPO dimer acid ammonium salt suggest that mice might be more sensitive to exposure to these GenX chemicals than rats. Given the evidence that the liver is the target organ for toxicity and the primary organ for tumor development, there is a need for additional research using chronic duration exposures in mice. This uncertainty is not considered in the application of the UF_D given that a noncancer toxicity value was developed for this assessment.

7.5 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-18405-1238, 2013; DuPont-18405-1307, 2010; Wang et al., 2016). This finding was surprising given that increased serum bilirubin levels rather than decreased levels are typically indicative of liver damage, and multiple studies outlined above have confirmed microscopic liver damage (DuPont-18405-841, 2010; DuPont-18405-1238, 2013; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; 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., 2016); this finding was not replicated in the other 28-day studies (DuPont-24459, 2008; DuPont-24447, 2008). The

biological or mechanistic significance of this effect is unknown, yet the consistency of this effect across multiple studies is noteworthy.

7.6 Susceptible Populations and Life Stages

Data for the elucidation of differential susceptibility dependent on life stage (e.g., developing 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 exposures and/or unique windows of developmental susceptibility during the prenatal and postnatal periods for both pregnant mothers and the developing fetus. No human toxicity or epidemiological studies are available in the literature that address early developmental or reproductive life stages. DuPont submitted data examining reproductive and developmental endpoints in both mice and rats (DuPont-18405-1037, 2010; DuPont-18405-841, 2010), and summaries of these studies can be found in section 5.4 (Developmental/Reproductive). HFPO dimer acid ammonium salt can be transferred from a pregnant animal to the fetus, although with the available data, it cannot be determined if this transfer occurs during gestation or during lactation (DuPont-18405-1037, 2010). When present, developmental and reproductive effects were found at doses higher than those associated with the selected critical effect: single-cell necrosis in the liver of male mice. The UF_H of 10 accounts for variability in the responses within human populations because of both intrinsic (including life stage) and extrinsic (life style) 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 6.5 and 6.6 (Subchronic RfD and Chronic RfD) are applicable to all life stages. While this document is not itself an assessment of risk, when reviewing data pertinent to the hazard potential of GenX chemicals, the EPA adhered to the requirements of its 2013 reaffirmation of the Policy on Evaluating Health Risks to Children (USEPA, 2013).

Sex-specific variation in the toxicokinetics of these two GenX chemicals is pronounced in rodents. Toxicokinetic data show the HFPO dimer acid and its ammonium salt clearance times to be considerably faster for females than for males (see the summary in section 2.3 (Toxicokinetics). For example, DuPont-24281 (2008) identified 12 hours as the clearance time for HFPO dimer acid ammonium salt in male rats at the low dose and 22 hours for the high dose. In female rats, the clearance values were 4 and 8 hours for the low dose and the high dose, respectively. A difference in toxicokinetics was not observed in primates where beta phase $T_{1/2}$s for male and female monkeys were 64.1 and 79.6 hours, respectively. The observed sex-specific toxicokinetic differences in rodents likely contribute to the observed sex-specific differences in toxic response.

Toxicity also varied by sex. Most of the statistically significant changes in clinical chemistries of rats were observed in the males. Decreases in total globulin and increases in the A/G ratio were observed in males and female rats; however, male rats exhibited a total globulin decrease at the 3- and 30-mg/kg/day doses while females responded only at 300 mg/kg/day (DuPont-24447, 2008). The most prevalent liver effects following both subchronic and chronic exposure to HFPO dimer ammonium salt were hepatocellular hypertrophy and single-cell and/or focal necrosis. In both sexes of mice exposed subchronically, hepatocellular hypertrophy was observed at 0.5 mg/kg/day, while male and female rats showed these effects at 3 mg/kg/day and 30 mg/kg/day, respectively (DuPont-17751-1026, 2009; DuPont-18405-841, 2010; DuPont-

18405-1238, 2013; DuPont-18405-1037, 2010; DuPont-18405-1307, 2010; DuPont-24447, 2008; DuPont-24459, 2008; Wang et al., 2016). In the oral/reproductive subchronic study, male and female mice presented single-cell necrosis in doses as low as 0.5 mg/kg/day (5/24 mice, or 21% in males and 2/24 mice, or 8% in females (not a significant increase from control in females), which significantly increased at 5 mg/kg/day (24/24 male mice and 21/24 female mice). While these findings indicate that mice are more sensitive to liver necrosis than rats in subchronic exposure scenarios, they also show that adverse impacts on the clinical chemistry and liver toxicity manifest at lower doses in males than females. The critical effect of liver single-cell necrosis in male mice was found at the lowest identified NOAEL. Its use in the derivation of the subchronic and chronic RfDs is assumed to be protective of females as well.

8.0 References

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Appendix A: Literature Search Strategy

This appendix presents the details of the literature search strategy used to identify primary, peer-reviewed literature pertaining to HFPO dimer acid (Chemical Abstracts Service Registry Number (CASRN) 13252-13-6) and/or its ammonium salt (CASRN 62037-80-3). The literature search was conducted using the databases listed in Table A-1. The literature searches for these GenX chemicals were conducted in July 2017 (acid) and January/February 2018 (ammonium salt). 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 searched for physiochemical property information, health effects, toxicokinetics, and mechanistic information. A list of these additional databases is provided in Table A-3 and Table A-4. Combined, these database searches returned 27 studies for HFPO dimer acid and HFPO dimer acid ammonium salt. The available data for GenX chemicals comes primarily from studies submitted under Toxic Substances Control Act (TSCA). These studies were combined with the results of the search of the publicly available peer-reviewed literature for evaluation for relevance to the assessment. Potential relevance was based primarily on a title and abstract screen. The inclusion/exclusion criteria applied to the literature searches conducted are presented in Table A-5.

Table A-1. Summary of Core Database Search Results

Search Date	PubMed	WOS	Toxline	TSCATS via Toxline/NLM
HFPO dimer acid (13252-13-6) (HERO project id: 2627)				
7/24/17	3	12	0	0
HFPO dimer acid ammonium salt (62037-80-3) (HERO project id: 2683)				
1/18 and 2/18	9	12	0	0

Table A-2. Database Search Strings

	HFPO Dimer Acid (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] OR "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] OR "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]))

	HFPO Dimer Acid (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.

	HFPO Dimer Acid (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.

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

System Used	Selected Key Reference(s) or Sources	References Identified
TSCATS[a]	TSCA Test Submissions 2.0; website now retired (https://yosemite.epa.gov/oppts/epatscat8.nsf/ReportSearch?OpenForm)	0
	Chemical Data Access Tool (CDAT); website now retired (https://java.epa.gov/oppt_chemical_search/)	0
	ChemView (https://java.epa.gov/chemview)	0
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) USEPA ACToR (https://actor.epa.gov/actor/home.xhtml) USEPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) USEPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) USEPA ChemView (https://java.epa.gov/chemview) USEPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents	3

System Used	Selected Key Reference(s) or Sources	References Identified
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) USEPA NSCEP (https://www.epa.gov/nscep) USFDA (http://www.fda.gov/) WHO (http://www.who.int/ipcs/assessment/en/)	0

[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, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, EPA-IRIS, and EPA-SRS.

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

System Used	Selected Key Reference(s) or Sources	References Identified
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) USEPA ACToR (https://actor.epa.gov/actor/home.xhtml) USEPA CDAT; website now retired (https://java.epa.gov/oppt_chemical_search/) USEPA Chemistry Dashboard (https://comptox.epa.gov/dashboard/) USEPA ChemView (https://java.epa.gov/chemview) USEPA Substance Registry Services (SRS) (https://ofmpub.epa.gov/sor_internet/registry/substreg/searchandretrieve/substancesearch/search.do) Web-based search for chemical manufacturer documents	1

System Used	Selected Key Reference(s) or Sources	References Identified
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) USFDA (http://www.fda.gov) WHO (http://www.who.int/ipcs/assessment/en/)	2

Notes:
[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, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD-HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, EPA-IRIS, and EPA-SRS.

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

	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, 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	

	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; PFAS = pre- and polyfluoroalkyl substances.

[a] Although these criteria were used for the peer-reviewed literature, the current document describes data on 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 described in the current document to give context to the toxicity values.

Appendix B: Systematic Review of DuPont/Chemours Submissions

Background

As mentioned in Section 3.3.2, most of the available data were submitted to the U.S. Environmental Protection Agency (EPA) under the Toxic Substances Control Act (TSCA) for hexafluoropropylene oxide (HFPO) dimer acid and/or ammonium salt. Submitted test data on HFPO dimer acid and/or ammonium salt were available for numerous endpoints such as acute toxicity, metabolism and toxicokinetics, genotoxicity, and systemic toxicity in mice and rats with dosing durations of up to 2 years. Most of these submitted studies were conducted according to Organization for Economic Cooperation and Development (OECD) Test Guidelines (TGs) and/or EPA Health Effects Test Guidelines 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." The majority of the studies adhered to the Good Laboratory Practices (GLP) principles, and full study reports were submitted for Agency review either with the Pre-manufacture Notice under TSCA or subsequently as the result of a Consent Order associated with that TSCA new chemical review.

The EPA Office of Pollution Prevention and Toxics (OPPT) reviewed studies for quality and adherence to the guidelines when they were received. However, for the purpose of developing the toxicity values, EPA OPPT evaluated the human health TSCA guideline studies, including toxicokinetic and repeated-dose studies of 28 days or longer and other studies identified in the literature search (see section 3.3.1) according to the evaluation strategy described in this appendix. The data quality criteria and scoring tables are provided in the subsequent pages for the studies supporting the development of toxicity values for HFPO dimer acid and dimer acid ammonium salt. The data quality criteria used in this document may slightly differ from those later published in the Applications of Systematic Review in TSCA Risk Evaluations (2018; https://www.epa.gov/sites/production/files/2018-06/documents/final_application_of_sr_in_tsca_05-31-18.pdf). These slight modifications are not expected to change the overall confidence of the studies.

Strategy for Assessing the Quality of Data/Information

The strategy for assessing the quality of data/information sources is based on a structured framework with predefined criteria for each type of data/information source. EPA OPPT developed a numerical scoring system to inform the characterization of the data/information sources during the data integration phase. The goal is to provide transparency and consistency to the evaluation process.

The general structure of the evaluation strategy is composed of evaluation domains, metrics, and criteria. Evaluation domains represent general categories of attributes that are evaluated in each data/information source (e.g., test substance, test design). Each domain contains a unique set of metrics, or sub-categories of attributes, intended to assess an aspect of the methodological conduct of the data/information source. Each metric specifies criteria expressing the relevant elements or conditions for assessing confidence that, along with professional judgement, will guide the identification of study strengths and limitations/deficiencies.

Reporting quality is an important aspect of a study that needs to be considered in the evaluation process. The challenge, in many cases, is to distinguish a deficit in reporting from a problem in the underlying methodological quality of the data/information source. The evaluation strategy incorporates reporting criteria within the existing domains, rather than adding a separate reporting domain as recommended in some evaluation tools/frameworks. Since reporting contributes to the evaluation of each facet of the data source, EPA OPPT assesses reporting and methodological quality simultaneously with the idea of untangling reporting from study conduct while the reviewer is assessing a particular metric for each domain. Developing a reporting checklist, guidance document, or a separate reporting quality domain may be possible in the near future as EPA OPPT uses and optimizes the evaluation strategy for animal toxicity and *in vitro* studies.

Data/information sources should also be evaluated for their relevance or appropriateness to support the risk assessment. Specifically, data/information sources should support the assessment questions, analytical approaches, methods, models, and considerations that are laid out during problem formulation. EPA OPPT uses a tiered approach to check for relevance starting at the data search stage and continuing during the title/abstract and full text screening and evaluation and integration stages. By design, the systematic review process uses a fit-for-purpose literature search and relevance-driven eligibility criteria to end up evaluating the most relevant data/information sources for the risk assessment. The reviewers also check for relevance while assessing the quality of the data/information source and are asked to document[4] any relevancy issues during the evaluation process.

The evaluation strategy in some cases refers to study guidelines along with professional judgement as a helpful guidance in determining the adequacy or appropriateness of certain study designs or analytical methods. This should not be construed to imply that non-guideline studies have lower confidence than guideline or GLP studies. EPA OPPT will consider any and all available, relevant data and information irrespective of whether they were collected in accordance with standardized methods (e.g., OECD TGs or GLP standards).

EPA OPPT will consider data and information from alternative test methods and strategies (or new approach methodologies or NAMs), as applicable and available. This is consistent with EPA OPPT's *Strategic Plan to Promote the Development and Implementation of Alternative Test Methods* to reduce, refine, or replace vertebrate animal testing (USEPA, 2018). The data/information quality criteria may need to be optimized or new criteria may need to be developed as part of evaluating and integrating NAMs in risk assessments.

B.1.1. Evaluation Method

Based on the strengths, limitations, and deficiencies of each data/information source, the reviewer assigns a confidence level score of 1 (high confidence), 2 (medium confidence), 3 (low confidence), or 4 (unacceptable) for each individual metric that is used to evaluate a particular aspect of the methodological conduct of the data/information source. Although many metrics have criteria for all four bins (i.e., *High, Medium, Low, and Unacceptable*), there are some metrics with dichotomous or trichotomous criteria to fit better the nature of the criteria.

[4] Relevancy issues will be documented in the reviewer's comments, when pertinent.

The confidence levels and corresponding scores at the metric level are defined as follows:

- **High:** No notable deficiencies or concerns are identified in the domain metric that are likely to influence results (score of 1).
- **Medium:** Minor uncertainties or limitations are noted in the domain metric that are unlikely to have a substantial impact on results (score of 2).
- **Low:** Deficiencies or concerns are noted in the domain metric that are likely to have a substantial impact on results (score of 3).
- **Unacceptable:** Serious flaws are noted in the domain metric that consequently make the data/information source unusable (score of 4).
- **Not rated/applicable:** Rating of this metric is not applicable to the data/information source being evaluated (no score). *Not rated/applicable* will also be used in cases in which studies cite a literature source for their test methodology instead of providing detailed descriptions. In these circumstances, EPA will score the metric as *Not rated/not applicable* and capture the score in the reviewer's notes. If the data/information source is not classified as "unacceptable" in the initial review, the cited literature source will be reviewed during a subsequent evaluation step, and the metric will be rated at that time.

A numerical scoring method is used to convert the confidence level for each metric into the overall quality level for the data/information source. The overall study score is equated to an overall quality level (*High*, *Medium*, or *Low*) using the level definitions and scoring scale shown in Table B-1. The scoring scale was obtained by calculating the difference between the highest possible score of 3 and the lowest possible score of 1 (i.e., $3 - 1 = 2$) and dividing into three equal parts ($2 \div 3 = 0.67$). This results in a range of approximately 0.7 for each overall data quality level, and this range was used to estimate the transition points (cut-off values) in the scale between *High* and *Medium* scores and *Medium* and *Low* scores. These transition points between the ranges of 1 and 3 were calculated as follows:

- Cut-off values between *High* and *Medium*: $1 + 0.67 = 1.67$, rounded up to 1.7 (scores lower than 1.7 will be assigned an overall quality level of *High*)
- Cut-off values between *Medium* and *Low*: $1.67 + 0.67 = 2.34$, rounded up to 2.3 (scores between 1.7 and lower than 2.3 will be assigned an overall quality level of *Medium)*

A study is disqualified from further consideration if the confidence level of one or more metrics is rated as *Unacceptable* (score of 4). EPA OPPT plans to use data with an overall quality level of *High, Medium*, or *Low* confidence, but does not plan to use data rated as *Unacceptable*. Data or information from *Unacceptable* studies might be useful qualitatively and such use of unacceptable studies may be done on a case-by-case basis.

Table B-1. Definition of Overall Quality Levels and Corresponding Quality Scores

Overall Quality Level	Definition	Overall Quality Score
High	No notable deficiencies or concerns are identified and the data therefore could be used in the assessment with a high degree of confidence.	≥ 1 and < 1.7
Medium	Possible deficiencies or concerns are noted and the data therefore could be used in the assessment with a medium degree of confidence.	≥ 1.7 and < 2.3

Overall Quality Level	Definition	Overall Quality Score
Low	Deficiencies or concerns are noted and the data therefore could be used in the assessment with a low degree of confidence.	≥ 2.3 and ≤ 3
Unacceptable	Serious flaw(s) are identified and therefore, the data cannot be used for the assessment.	4

After the overall score is applied to determine an overall quality level, professional judgment may be used to adjust the quality level obtained by the weighted score calculation. The reviewer must have a compelling reason to invoke the adjustment of the overall score and written justification must be provided. This approach has been used in other established tools such as the ToxRTool (Toxicological data Reliability Assessment Tool) developed by the European Commission (https://eurl-ecvam.jrc.ec.europa.eu/about-ecvam/archive-publications/toxrtool).

B.1.2. Documentation and Instructions for Reviewers

Data evaluation is conducted in Excel to track and record the evaluation for each data/information source. Refer to Section B.5 for the data evaluation and scoring tables documenting the evaluation.

A confidence level is assigned for each relevant metric within each domain by following the confidence level specifications provided in section B.2.2, along with professional judgment to identify study strengths and limitations. The assigned confidence level is indicated by placing a score between 1 and 4 in the column labeled *Metric Score*. In some cases, reference to study guidelines (in addition to professional judgement) might be helpful in determining the adequacy or appropriateness of certain study designs or analytical methods. This should not be construed to imply that non-guideline studies necessarily have lower confidence than guideline studies. If a publication reports more than one study/data type or endpoint, each study/data type or endpoint will be evaluated separately and assigned a separate overall quality level.

Some metrics might not be applicable to all study types. If a metric is not applicable to the study under review, zero, which is equivalent to *NR* (not rated), is placed in the *Metric Score* column for this metric.

After scoring of the individual metrics within each domain, the overall study *Weighted Score* is calculated and assigned to the corresponding bin (*High*, *Medium*, *Low*, or *Unacceptable*).

In the *Comments* column, the reviewer documents concerns, uncertainties, strengths, limitations, deficiencies, and any additional comments observed for each metric, when necessary. For instance, EPA might not always provide a comment for a metric that has been categorized as *High*. However, a reviewer is strongly encouraged to provide a comment for metrics categorized as *Medium* or *Low* to improve transparency. The reviewer also records any relevance issues with the data/information source (e.g., study is not useful to answer assessment questions).

B.1.3. Important Caveats about the Evaluation Method

The following is a discussion of important caveats for the data quality evaluation method:

- Although specifications for the data quality evaluation metrics have been developed, professional judgment is required to assess the metrics.
- Data evaluation is a qualitative assessment of confidence in a study or dataset. In order to provide consistency and transparency to the evaluation process, a scoring system is being applied to ascertain a qualitative rating. Scores will be used for the purpose of assigning the confidence level rating of *High, Medium, Low, or Unacceptable*, and inform the characterization of data/information sources during the data integration phase. The system is not intended to imply precision and/or accuracy of the scoring results.
- Every study or dataset is unique and therefore the individual metrics and domains may have various degrees of importance (e.g., more or less important). The weighting approach might need to be adjusted as EPA OPPT tests the evaluation method with different types of studies.
- The metrics developed are intended to be indicators of data quality. They were selected because they are generally considered common and important for a broad range of studies. Other metrics not listed may also be important and added if necessary. Also, there is the possibility of deviating from the calculated overall confidence level score in case the metric criteria are unable to capture professional judgement. A reviewer must provide a justification for the score adjustment to ensure transparency for the decision.

Data Quality Criteria for Studies on Animal and *In Vitro* Toxicity

B.1.4. Types of Data Sources

The data quality will be evaluated for a variety of animal and *in vitro* toxicity studies. Table B-2 provides examples of types of studies falling into these two broad categories. EPA OPPT may tailor the criteria to capture the inherent characteristics of particular studies that are not captured in the current criteria (e.g., optimization of criteria to evaluate the quality of new approach methodologies or NAMs).

Table B-2. Types of Animal and *In Vitro* Toxicity Data

Data Category	Type of Data Sources
Animal Toxicity	Oral, dermal, and inhalation routes: lethality, irritation, sensitization, reproduction, fertility, developmental, neurotoxicity, carcinogenicity, systemic toxicity, metabolism, pharmacokinetics, absorption, immunotoxicity, genotoxicity, mutagenicity, endocrine disruption
In Vitro Toxicity Studies	Irritation, corrosion, sensitization, genotoxicity, dermal absorption, phototoxicity, ligand binding, steroidogenesis, developmental, organ toxicity, mechanisms, high throughput, immunotoxicity

Mechanistic evidence is highly heterogeneous and may come from human, animal, or *in vitro* toxicity studies. Mechanistic evidence may provide support for biological plausibility and help explain differences in tissue sensitivity, species, gender, life-stage, or other factors (USEPA, 2006). Although highly preferred, the availability of a fully elucidated mode of action (MOA) or

adverse outcome pathway (AOP) is not required to conduct the human health hazard assessment for a given chemical.

EPA OPPT plans to prioritize the evaluation of mechanistic evidence instead of evaluating all of the identified evidence upfront. This approach has the advantage of allowing for a focused review of those mechanistic studies that are most relevant to the hazards under evaluation.

B.1.5. Data Quality Evaluation Domains

The methods for evaluation of study quality were developed after review of selected references describing existing study quality and risk of bias evaluation tools for toxicity studies (EC, 2018; Cooper et al., 2016; Lynch et al., 2016; Moermond et al., 2016b; Samuel et al., 2016; NTP, 2015a; Hooijmans et al., 2014; Koustas et al., 2014; Kushman et al., 2013; Hartling et al., 2012; Hooijmans et al., 2010). These publications, coupled with professional judgment and experience, informed the identification of domains and metrics for consideration in the evaluation and scoring of study quality.

The data quality of animal toxicity studies and *in vitro* toxicity studies are evaluated by assessing the following seven domains: Test Substance, Test Design, Exposure Characterization, Test Organisms/Test Model, Outcome Assessment, Confounding/Variable Control, and Data Presentation and Analysis. The data quality within each domain is evaluated by assessing unique metrics that pertain to each domain. The domains are defined in Table B-3 and further information on evaluation metrics is provided in section B.3.3.

Table B-3. Data Evaluation Domains and Definitions

Evaluation Domain	Definition
Test Substance	Metrics in this domain are used to evaluate whether the information provided in the study provides a reliable[a] confirmation that the test substance used in a study has the same (or sufficiently similar) identity, purity, and properties as the substance of interest.
Test Design	Metrics in this domain are used to evaluate whether the experimental design enables the study to distinguish the effect of exposure from other factors. This domain includes metrics related to the use of control groups and randomization in allocation to ensure that the effect of exposure is isolated.
Exposure Characterization	Metrics in this domain are used to assess the validity and reliability of methods used to measure or characterize exposure. These metrics evaluate whether exposure to the test substance was characterized using a method(s) that provides valid and reliable results, whether the exposure remained consistent over the duration of the experiment, and whether the exposure levels were appropriate to the outcome of interest.
Test Organisms/Test Model	These metrics are used to assess the appropriateness of the population or organism(s), group sizes used in the study (i.e., number of organisms and/or number of replicates per exposure group), and the organism conditions in order to determine the outcome of interest associated with the exposure of interest.
Outcome Assessment	Metrics in this domain are used to assess the validity and reliability of methods, including sensitivity of methods, that are used to measure or otherwise characterize the outcome(s) of interest.

Evaluation Domain	Definition
Confounding/Variable Control	Metrics in this domain are used to assess the potential impact of factors other than exposure that might affect the risk of outcome. The metrics evaluate whether studies identify and account for factors that are related to exposure and independently related to outcome (confounding factors) and whether appropriate experimental or analytical (statistical) methods are used to control for factors unrelated to exposure that might affect the risk of outcome (variable control).
Data Presentation and Analysis	Metrics in this domain are used to assess whether appropriate statistical methods were used and whether data for all outcomes are presented.
Other	Metrics in this domain are added as needed to incorporate chemical- or study-specific evaluations.

Note:
[a] Reliability is defined as "the inherent property of a study or data, which includes the use of well-founded scientific approaches, the avoidance of bias within the study or data collection design and faithful study or data collection conduct and documentation" (ECHA, 2011a).

B.1.6. Data Quality Evaluation Metrics

The data quality evaluation domains are evaluated by assessing unique metrics that have been developed for animal and *in vitro* studies. Each metric is binned into a confidence level of *High*, *Medium*, *Low*, or *Unacceptable*. Each confidence level is assigned a numerical score (i.e., 1 through 4) that is used in the method of assessing the overall quality of the study.

Table B-4 lists the data evaluation domains and metrics for animal toxicity studies, including metrics that inform risk of bias and types of bias, and Table B-5 lists the data evaluation domains and metrics for *in vitro* toxicity studies. Each domain has between 2 and 6 metrics; however, some metrics might not apply to all study types. A general domain for other considerations is available for metrics that are specific to a given test substance or study type.

EPA may modify the metrics used for animal toxicity and *in vitro* toxicity studies as the Agency acquires experience with the evaluation tool. Any modifications will be documented.

Table B-4. Data Evaluation Domains and Metrics for Animal Toxicity Studies

Evaluation Domain	Number of Metrics Overall	Metrics (Metric Number and Description, Type of Bias)
Test Substance	3	• Metric 1: Test Substance Identity • Metric 2: Test Substance Source • Metric 3: Test Substance Purity (*information bias[a]) (*detection bias[b])
Test Design	3	• Metric 4: Negative and Vehicle Controls (*performance bias[b]) • Metric 5: Positive Controls (*information bias[a]) • Metric 6: Randomized Allocation of Animals (*selection bias[a,b])
Exposure Characterization	6	• Metric 7: Preparation and Storage of Test Substance • Metric 8: Consistency of Exposure Administration • Metric 9: Reporting of Doses/Concentrations • Metric 10: Exposure Frequency and Duration • Metric 11: Number of Exposure Groups and Dose/Concentration Spacing • Metric 12: Exposure Route and Method

Evaluation Domain	Number of Metrics Overall	Metrics (Metric Number and Description, Type of Bias)
Test Organisms	3	• Metric 13: Test Animal Characteristics • Metric 14: Adequacy and Consistency of Animal Husbandry Conditions • Metric 15: Number of Animals per Group (*missing data bias[a])
Outcome Assessment	5	• Metric 16: Outcome Assessment Methodology (*information bias[a]) (*detection bias[b]) • Metric 17: Consistency of Outcome Assessment • Metric 18: Sampling Adequacy • Metric 19: Blinding of Assessors (*selection bias[a]) (*performance bias[b]) • Metric 20: Negative Control Responses
Confounding/ Variable Control	2	• Metric 21: Confounding Variables in Test Design and Procedures (*other bias[b]) • Metric 22: Health Outcomes Unrelated to Exposure (*attrition/exclusion bias[b])
Data Presentation and Analysis	2	• Metric 23: Statistical Methods (*information bias[a]) (*other bias[b]) • Metric 24: Reporting of Data (*selective reporting bias[b])

Notes:
Items marked with an asterisk (*) are examples of metrics that can be used to assess internal validity/risk of bias.
[a]National Academies of Sciences, Engineering, and Medicine, 2017.
[b]NTP, 2015b.

Table B-5. Data Evaluation Domains and Metrics for In Vitro Toxicity Studies

Evaluation Domain	Number of Metrics Overall	Metrics (Metric Number and Description, Type of Bias)
Test Substance	3	• Metric 1: Test Substance Identity • Metric 2: Test Substance Source • Metric 3: Test Substance Purity
Test Design	4	• Metric 4: Negative Controls[a] • Metric 5: Positive Controls[a] • Metric 6: Assay Procedures • Metric 7: Standards for Test
Exposure Characterization	6	• Metric 8: Preparation and Storage of Test Substance • Metric 9: Consistency of Exposure Administration • Metric 10: Reporting of Doses/Concentrations • Metric 11: Exposure Duration • Metric 12: Number of Exposure Groups and Dose Spacing • Metric 13: Metabolic Activation
Test Model	2	• Metric 14: Test Model • Metric 15: Number per Group
Outcome Assessment	4	• Metric 16: Outcome Assessment Methodology • Metric 17: Consistency of Outcome Assessment • Metric 18: Sampling Adequacy • Metric 19: Blinding of Assessors

Evaluation Domain	Number of Metrics Overall	Metrics (Metric Number and Description, Type of Bias)
Confounding/ Variable Control	2	• Metric 20: Confounding Variables in Test Design and Procedures • Metric 21: Confounding Variables in Outcomes Unrelated to Exposure
Data Presentation and Analysis	4	• Metric 22: Data Analysis • Metric 23: Data Interpretation • Metric 24: Cytotoxicity Data • Metric 25: Reporting of Data

Note:
[a]These are for the assay performance, not necessarily for the "validation" of extrapolating to a particular apical outcome (i.e., assay performance versus assay validation).

B.1.7. Scoring Method and Determination of Overall Data Quality Level

Section B.2.2 provides information about the evaluation method that is used to assess the quality of the data/information. This section provides details about the scoring system that is applied to animal and *in vitro* toxicity studies, including the weighting factors assigned to each metric score of each domain.

Some metrics are given greater weights than others, if they are regarded as key or critical metrics. Thus, EPA OPPT uses a weighting approach to reflect that some metrics are more important than others when assessing the overall quality of the data.

B.1.7.1. Weighting Factors

Each metric is assigned a weighting factor of 1 or 2, with the higher weighting factor (2) given to metrics deemed critical for the evaluation. The critical metrics are identified based on professional judgment in conjunction with consideration of the factors that are most frequently included in other study quality/risk of bias tools for animal toxicity studies (reviewed by Lynch et al. (2016); Samuel et al. (2016)). In selecting critical metrics, EPA OPPT recognized that the relevance of an individual study to the risk analysis for a given substance is determined by its ability to inform hazard identification and/or dose-response assessment. Thus, the critical metrics are those that can be used to determine how well a study answers these key questions:

- Is a change in health outcome demonstrated in the study?
- Is the observed change more likely than not attributable to the substance exposure?
- At what substance dose(s) does the change occur?

EPA OPPT assigns a weighting factor of 2 to each metric considered critical to answering these questions. Remaining metrics are assigned a weighting factor of 1. Tables B-6 and B-7 identify the critical metrics (i.e., those assigned a weighting factor of 2) for animal toxicity and *in vitro* toxicity studies, respectively, and provide a rationale for selection of each metric. Tables B-8 and B-9 identify the weighting factors assigned to each metric for animal toxicity and *in vitro* toxicity studies, respectively.

Table B-6. Animal Toxicity Metrics with Greater Importance in the Evaluation and Rationale for Selection

Domain	Critical Metrics with Weighting Factor of 2 (Metric Number)[a]	Rationale
Test Substance	Test Substance Identity (Metric 1)	The test substance must be identified and characterized definitively to ensure that the study is relevant to the substance of interest.
Test Design	Negative and Vehicle Controls (Metric 4)	A concurrent negative control and vehicle control (when indicated) are required to ensure that any observed effects are attributable to substance exposure. Note that more than one negative control might be necessary in some studies.
Exposure Characterization	Reporting of Doses/Concentrations (Metric 9)	Dose levels must be defined without ambiguity to allow for determination of the dose-response relationship and to enable valid comparisons across studies.
Test Organisms	Test Animal Characteristics (Metric 13)	The test animal characteristics must be reported to enable assessment of (a) whether they are suitable for the endpoint of interest; (b) whether there are species, strain, sex, or age/lifestage differences within or between different studies; and (c) to enable consideration of approaches for extrapolation to humans.
Outcome Assessment	Outcome Assessment Methodology (Metric 16)	The methods used for outcome assessment must be fully described, valid, and sensitive to ensure that effects are detected, that observed effects are true, and to enable valid comparisons across studies.
Confounding/ Variable Control	Confounding Variables in Test Design and Procedures (Metric 21)	Control for confounding variables in test design and procedures is necessary to ensure that any observed effects are attributable to substance exposure and not to other factors.
Data Presentation and Analysis	Reporting of Data (Metric 24)	Detailed results are necessary to determine whether the study authors' conclusions are valid and to enable dose-response modeling.

Note:
[a]A weighting factor of 1 is assigned for the remaining metrics.

Table B-7. *In Vitro* Toxicity Metrics with Greater Importance in the Evaluation and Rationale for Selection

Domain	Critical Metrics with Weighting Factor of 2 (Metric Number)[a]	Rationale
Test Substance	Test Substance Identity (Metric 1)	The test substance must be identified and characterized definitively to ensure that the study is relevant to the substance of interest.
Test Design	Negative and Vehicle Controls (Metric 4)	A concurrent negative control and vehicle control (when indicated) are required for comparison of results between exposed and unexposed models to allow determination of treatment-related effects.
	Positive Controls (Metric 5)	A concurrent positive control or proficiency control (when applicable) is required to determine whether the chemical of interest produces the intended outcome for the study type.
Exposure Characterization	Reporting of doses/concentrations (Metric 10)	Dose levels must be defined without ambiguity to allow for determination of an accurate dose-response relationship or and to ensure valid comparisons across studies.
	Exposure Duration (Metric 11)	The exposure duration during the study must be defined to accurately assess potential risk.

Domain	Critical Metrics with Weighting Factor of 2 (Metric Number)[a]	Rationale
Test Model	Test Model (Metric 14)	The identity of the test model must be reported and suitable for the evaluation of outcome(s) of interest.
Outcome Assessment	Outcome Assessment Methodology (Metric 16)	The methods used for outcome assessment must be fully described, valid, and sensitive to ensure that effects are detected and that observed effects are true.
	Sampling Adequacy (Metric 18)	The number of samples evaluated must be sufficient to allow data interpretation and analysis.
Confounding/ Variable Control	Confounding Variables in Test Design and Procedures (Metric 20)	Control for confounding variables in test design and procedures are necessary to ensure that any observed effects are attributable to substance exposure and not to other factors.
Data Presentation and Analysis	Data Interpretation (Metric 23)	The criteria for scoring and/or evaluation criteria are necessary so that the correct categorization (e.g., positive, negative, equivocal) can be determined for the chemical of interest.
	Reporting of Data (Metric 25)	Detailed results are necessary to determine whether the study authors' conclusions are valid and to enable dose-response modeling.

Note:
[a]A weighting factor of 1 is assigned for the remaining metrics.

B.1.7.2. Calculation of Overall Study Score

A confidence level (1, 2, or 3 for *High, Medium*, or *Low* confidence, respectively) is assigned for each relevant metric within each domain. To determine the overall study score, the first step is to multiply the score for each metric (1, 2, or 3 for *High, Medium*, or *Low* confidence, respectively) by the appropriate weighting factor (as shown in Tables B-8 and B-9 for animal toxicity and *in vitro* studies, respectively) to obtain a weighted metric score. The weighted metric scores are then summed and divided by the sum of the weighting factors (for all metrics that are scored) to obtain an overall study score between 1 and 3. The equation for calculating the overall score is shown below:

$$\textit{Overall Score (range of 1 to 3)} = \sum(\textit{Metric Score x Weighting Factor})/\sum(\textit{Weighting Factors})$$

Some metrics might not be applicable to all study types. These metrics will not be included in the numerator or denominator of the equation above. The overall score will be calculated using only those metrics that receive a numerical score. Scoring examples for animal toxicity and *in vitro* toxicity studies are in tables B-10 through B-13.

Studies with any single metric scored as unacceptable (score = 4) will be automatically assigned an overall quality score of 4 (*Unacceptable*). An unacceptable score means that serious flaws are noted in the domain metric that consequently make the data unusable. If a metric is not applicable for a study type, the serious flaws would not be applicable for that metric and would not receive a score. EPA OPPT plans to use data with an overall quality level of High, Medium, or Low confidence to quantitatively or qualitatively support the risk evaluations, but it does not plan to use data rated as *Unacceptable.* An overall study score will not be calculated when a serious flaw is identified for any metric. If a publication reports more than one study or endpoint, each study or endpoint will be evaluated separately and given a separate overall quality rating.

Table B-8. Metric Weighting Factors and Range of Weighted Metric Scores for Animal Toxicity

Domain Number/ Description	Metric Number/Description	Range of Metric Scores[a]	Metric Weighting Factor	Range of Weighted Metric Scores[b]
1. Test Substance	1. Test Substance Identity	1 to 3	2	2 to 6
	2. Test Substance Source		1	1 to 3
	3. Test Substance Purity		1	1 to 3
2. Test Design	4. Negative and Vehicle Controls		2	2 to 6
	5. Positive Controls		1	1 to 3
	6. Randomized Allocation		1	1 to 3
3. Exposure Characterization	7. Preparation and Storage of Test Substance		1	1 to 3
	8. Consistency of Exposure Administration		1	1 to 3
	9. Reporting of Doses/Concentrations		2	2 to 6
	10. Exposure Frequency and Duration		1	1 to 3
	11. Number of Exposure Groups and Dose Spacing		1	1 to 3
	12. Exposure Route and Method		1	1 to 3
4. Test Organisms	13. Test Animal Characteristics		2	2 to 6
	14. Adequacy and Consistency of Animal Husbandry Conditions		1	1 to 3
	15. Number per Group		1	1 to 3
5. Outcome Assessment	16. Outcome Assessment Methodology		2	2 to 6
	17. Consistency of Outcome Assessment		1	1 to 3
	18. Sampling Adequacy		1	1 to 3
	19. Blinding of Assessors		1	1 to 3
	20. Negative Control Responses		1	1 to 3
6. Confounding/ Variable Control	21. Confounding Variables in Test Design and Procedures		2	2 to 6
	22. Health Outcomes Unrelated to Exposure		1	1 to 3
7. Data Presentation and Analysis	23. Statistical Methods		1	1 to 3
	24. Reporting of Data		2	2 to 6
	Sum (if all metrics scored)[c]		31	31 to 93
Range of Overall Scores, where Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factor				31/31 = 1; 93/31 = 3
	High: ≥1 and <1.7 / Medium: ≥1.7 and <2.3 / Low: ≥2.3 and ≤3			Range of overall score = 1 to 3[d]

Notes:
[a]For the purposes of calculating an overall study score, the range of possible metric scores is 1 to 3 for each metric, corresponding to High and Low confidence. No calculations will be conducted if a study receives an "Unacceptable" rating (score of "4") for any metric.
[b]The range of weighted scores for each metric is calculated by multiplying the range of metric scores (1 to 3) by the weighting factor for that metric.
[c]The sum of weighting factors and the sum of the weighted scores will differ if some metrics are not scored (not applicable).
[d]The range of possible overall scores is 1 to 3. If a study receives a score of 1 for every metric, then the overall study score will be 1. If a study receives a score of 3 for every metric, then the overall study score will be 3.

Table B-9. Metric Weighting Factors and Range of Weighted Metric Scores for *In Vitro* Toxicity Studies

Domain Number/ Description	Metric Number/Description	Range of Metric Scores[a]	Metric Weighting Factor	Range of Weighted Metric Scores[b]
1. Test Substance	1. Test Substance Identity	1 to 3	2	2 to 6
	2. Test Substance Source		1	1 to 3
	3. Test Substance Purity		1	1 to 3
2. Test Design	4. Negative and Vehicle Controls		2	2 to 6
	5. Positive Controls		2	2 to 6
	6. Assay Procedures		1	1 to 3
	7. Standards for Test		1	1 to 3
3. Exposure Characterization	8. Preparation and Storage of Test Substance		1	1 to 3
	9. Consistency of Exposure Administration		1	1 to 3
	10. Reporting of Doses/Concentrations		2	2 to 6
	11. Exposure Duration		2	2 to 6
	12. Number of Exposure Groups and Dose Spacing		1	1 to 3
	13. Metabolic Activation		1	1 to 3
4. Test Model	14. Test Model		2	2 to 6
	15. Number per Group		1	1 to 3
5. Outcome Assessment	16. Outcome Assessment Methodology		2	2 to 6
	17. Consistency of Outcome Assessment		1	1 to 3
	18. Sampling Adequacy		2	2 to 6
	19. Blinding of Assessors		1	1 to 3
6. Confounding/ Variable Control	20. Confounding Variables in Test Design and Procedures		2	2 to 6
	21. Outcomes Unrelated to Exposure		1	1 to 3
7. Data Presentation and Analysis	22. Data Analysis		1	1 to 3
	23. Data Interpretation		2	2 to 6
	24. Cytotoxicity Data		1	1 to 3
	25. Reporting of Data		2	2 to 6
	Sum (if all metrics scored)[c]		36	36 to 108
Range of Overall Scores, where Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factor				36/36 = 1; 108/36 = 3

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Range of overall score = 1 to 3[d]

Notes:
[a]For the purposes of calculating an overall study score, the range of possible metric scores is 1 to 3 for each metric, corresponding to High and Low confidence. No calculations will be conducted if a study receives an "Unacceptable" rating (score of "4") for any metric.
[b]The range of weighted scores for each metric is calculated by multiplying the range of metric scores (1 to 3) by the weighting factor for that metric.
[c]The sum of weighting factors and the sum of the weighted scores will differ if some metrics are not scored (not applicable).
[d]The range of possible overall scores is 1 to 3. If a study receives a score of 1 for every metric, then the overall study score will be 1. If a study receives a score of 3 for every metric, then the overall study score will be 3.

Table B-10. Scoring Example for Animal Toxicity Study with all Metrics Scored

Domain	Metric	Metric Score	Metric Weighting Factor	Weighted Score
Test Substance	1. Test Substance Identity	2	2	4
	2. Test Substance Source	3	1	3
	3. Test Substance Purity	2	1	2
Test Design	4. Negative and Vehicle Controls	1	2	2
	5. Positive Controls	2	1	2
	6. Randomized Allocation	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	2	1	2
	8. Consistency of Exposure Administration	2	1	2
	9. Reporting of Doses/Concentrations	1	2	2
	10. Exposure Frequency and Duration	2	1	2
	11. Number of Exposure Groups and Dose Spacing	1	1	1
	12. Exposure Route and Method	1	1	1
Test Organisms	13. Test Animal Characteristics	2	2	4
	14. Consistency of Animal Conditions	2	1	2
	15. Number per Group	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	2	2	4
	17. Consistency of Outcome Assessment	3	1	3
	18. Sampling Adequacy	2	1	2
	19. Blinding of Assessors	3	1	3
	20. Negative Control Responses	2	1	2
Confounding/Variable Control	21. Confounding Variables in Test Design and Procedures	2	2	4
	22. Health Outcomes Unrelated to Exposure	2	1	2
Data Presentation and Analysis	23. Statistical Methods	2	1	2
	24. Reporting of Data	2	2	4
NR= not rated/not applicable	Sum of scores		31	59
	Overall Study Score	**1.9**	**= Medium**	

Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Table B-11. Scoring Example for Animal Toxicity Study with Some Metrics Not Rated/Not Applicable

Domain	Metric	Metric Score	Metric Weighting Factor	Weighted Score
Test Substance	1. Test Substance Identity	2	2	4
	2. Test Substance Source	3	1	3
	3. Test Substance Purity	2	1	2
Test Design	4. Negative and Vehicle Controls	1	2	2
	5. Positive Controls	NR		
	6. Randomized Allocation	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	2	1	2
	8. Consistency of Exposure Administration	NR		
	9. Reporting of Doses/Concentrations	1	2	2
	10. Exposure Frequency and Duration	2	1	2
	11. Number of Exposure Groups and Dose Spacing	1	1	1
	12. Exposure Route and Method	1	1	1
Test Organisms	13. Test Animal Characteristics	2	2	4
	14. Consistency of Animal Conditions	2	1	2
	15. Number per Group	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	2	2	4
	17. Consistency of Outcome Assessment	NR		
	18. Sampling Adequacy	2	1	2
	19. Blinding of Assessors	NR		
	20. Negative Control Responses	2	1	2
Confounding/Variable Control	21. Confounding Variables in Test Design and Procedures	2	2	4
	22. Health Outcomes Unrelated to Exposure	2	1	2
Data Presentation and Analysis	23. Statistical Methods	2	1	2
	24. Reporting of Data	2	2	4
NR = not rated/not applicable	Sum		27	49
	Overall Study Score	**1.8**	**= Medium**	

Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factor

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Table B-12. Scoring Example for *In Vitro* Study with all Metrics Scored

Domain	Metric	Metric Score	Metric Weighting Factor	Weighted Score
Test Substance	1. Test Substance Identity	1	2	2
	2. Test Substance Source	2	1	2
	3. Test Substance Purity	2	1	2
Test Design	4. Negative Controls	1	2	2
	5. Positive Controls	1	2	2
	6. Assay Procedures	2	1	2
	7. Standards for Test	3	1	3
Exposure Characterization	8. Preparation and Storage of Test Substance	2	1	2
	9. Consistency of Exposure Administration	2	1	2
	10. Reporting of Doses/Concentrations	1	2	2
	11. Exposure Duration	1	2	2
	12. Number of Exposure Groups and Dose Spacing	1	1	1
	13. Metabolic Activation	3	1	3
Test Model	14. Test Model	2	2	4
	15. Number per Group	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	3	2	6
	17. Consistency of Outcome Assessment	2	1	2
	18. Sampling Adequacy	1	2	2
	19. Blinding of Assessors	2	1	2
Confounding/Variable Control	20. Confounding Variables in Test Design and Procedures	3	2	6
	21. Outcomes Unrelated to Exposure	2	1	2
Data Presentation and Analysis	22. Data Analysis	1	1	1
	23. Data Interpretation	2	2	4
	24. Cytotoxicity Data	2	1	2
	25. Reporting of Data	3	2	6
NR = not rated/not applicable	Sum		36	66
	Overall Study Score	**1.8**	**= Medium**	

Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factor

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Table B-13. Scoring Example for *In Vitro* Study with Some Metrics Not Rated/Not Applicable

Domain	Metric	Metric Score	Metric Weighting Factor	Weighted Score
Test Substance	1. Test Substance Identity	1	2	2
	2. Test Substance Source	2	1	2
	3. Test Substance Purity	2	1	2
Test Design	4. Negative Controls	1	2	2
	5. Positive Controls	1	2	2
	6. Assay Procedures	2	1	2
	7. Standards for Test	3	1	3
Exposure Characterization	8. Preparation and Storage of Test Substance	NR		
	9. Consistency of Exposure Administration	2	1	2
	10. Reporting of Doses/Concentrations	1	2	2
	11. Exposure Duration	1	2	2
	12. Number of Exposure Groups and Dose Spacing	1	1	1
	13. Metabolic Activation	NR		
Test Model	14. Test Model	2	2	4
	15. Number per Group	3	1	3
Outcome Assessment	16. Outcome Assessment Methodology	3	2	6
	17. Consistency of Outcome Assessment	2	1	2
	18. Sampling Adequacy	1	2	2
	19. Blinding of Assessors	NR		
Confounding/Variable Control	20. Confounding Variables in Test Design and Procedures	3	2	6
	21. Outcomes Unrelated to Exposure	2	1	2
Data Presentation and Analysis	22. Data Analysis	1	1	1
	23. Data Interpretation	2	2	4
	24. Cytotoxicity Data	NR		
	25. Reporting of Data	3	2	6
NR= not rated/not applicable	Sum		32	58
	Overall Study Score	**1.8**	**= Medium**	

Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factor

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

B.1.8. Data Quality Criteria

B.1.8.1. Animal Toxicity Studies

Detailed tables showing quality criteria for the metrics are provided in Tables B-14 through B-17 for animal toxicity and *in vitro* toxicity studies, including a table that summarizes the serious flaws that would make the data unacceptable for use in the environmental hazard assessment.

Table B-14. Serious Flaws that Would Make Animal Toxicity Studies Unacceptable

Domain	Metric	Description of Serious Flaw(s) in Data Source
Test Substance	Test Substance Identity	The test substance identity and form (the latter if applicable) cannot be determined from the information provided (e.g., nomenclature was unclear and Chemical Abstracts Service Registry Number (CASRN) or structure were not reported) **OR** for mixtures, the components and ratios were not characterized.
	Test Substance Source	The test substance was not obtained from a manufacturer **OR** if synthesized or extracted, analytical verification of the test substance was not conducted.
	Test Substance Purity	The nature and quantity of reported impurities were such that study results were likely to be due to one or more of the impurities.
Test Design	Negative and Vehicle Controls	A concurrent negative control group was not included or reported **OR** the reported negative control group was not appropriate (e.g., age/weight of animals differed between control and treated groups).
	Positive Controls	For study types that require a concurrent positive control group: When applicable, an appropriate concurrent positive control (i.e., inducing a positive response) was not used and its omission is a serious flaw that makes the study unusable.
	Randomized Allocation of Animals	The study reported using a biased method to allocate animals to study groups (e.g., judgement of investigator).
Exposure Characterization	Preparation and Storage of Test Substance	Information on preparation and storage was not reported **OR** serious flaws reported with test substance preparation and/or storage conditions will have critical impacts on dose/concentration estimates and make the study unusable (e.g., instability of test substance in exposure medium was reported, or there was heterogeneous distribution of test substance in exposure matrix (e.g., aerosol deposition in exposure chamber, insufficient mixing of dietary matrix)). For inhalation studies, there was no mention of the method and equipment used to generate the test substance, or the method used is atypical and inappropriate.
	Consistency of Exposure Administration	Critical exposure details (e.g., methods for generating atmosphere in inhalation studies) were not reported **OR** reported information indicated that exposures were not administered consistently across study groups (e.g., differing particle size), resulting in serious flaws that make the study unusable.

Domain	Metric	Description of Serious Flaw(s) in Data Source
Exposure Characterization (continued)	Reporting of Doses/Concentrations	The reported exposure levels could not be validated (e.g., lack of food or water intake data for dietary or water exposures in conjunction with evidence of palatability differences, lack of body weight (BW) data in conjunction with qualitative evidence for BW differences across groups, inconsistencies in reporting, etc.). For inhalation studies, actual concentrations not reported along with animal responses (or lack of responses) that indicate exposure problems due to faulty test substance generation. Animals were exposed to an aerosol but no particle size data were reported.
	Exposure Frequency and Duration	The exposure frequency or duration of exposure were not reported **OR** the reported exposure frequency and duration were not suited to the study type and/or outcome(s) of interest (e.g., study length inadequate to evaluate tumorigenicity).
	Number of Exposure Groups and Dose/Concentration Spacing	The number of exposure groups and spacing were not reported **OR** dose groups and spacing were not relevant for the assessment (e.g., all doses in a developmental toxicity study produced overt maternal toxicity).
	Exposure Route and Method	The route or method of exposure was not reported **OR** an inappropriate route or method (e.g., administration of a volatile organic compound via the diet) was used for the test substance without taking steps to correct the problem (e.g., mixing fresh diet, replacing air in static chambers). For inhalation studies, there is no description of the inhalation chamber used, or an atypical exposure method was used, such as allowing a container of test substance to evaporate in a room.
Test Organisms	Test Animal Characteristics	The test animal species was not reported **OR** the test animal (species, strain, sex, life-stage, source) was not appropriate for the evaluation of the specific outcome(s) of interest (e.g., genetically modified animals, strain was uniquely susceptible or resistant to one or more outcome of interest).
	Adequacy and Consistency of Animal Husbandry Conditions	There were significant differences in husbandry conditions between control and exposed groups (e.g., temperature, humidity, light-dark cycle) **OR** animal husbandry conditions deviated from customary practices in ways likely to impact study results (e.g., injuries and stress due to cage overcrowding).
	Number of Animals per Group	The number of animals per study group was not reported **OR** the number of animals per study group was insufficient to characterize toxicological effects (e.g., 1–2 animals in each group).

Domain	Metric	Description of Serious Flaw(s) in Data Source
Outcome Assessment	Outcome Assessment Methodology	The outcome assessment methodology was not reported **OR** the reported outcome assessment methodology was not sensitive for the outcome(s) of interest (e.g., evaluation of endpoints outside the critical window of development, a systemic toxicity study that evaluated only grossly observable endpoints, such as clinical signs and mortality).
	Consistency of Outcome Assessment	There were large inconsistencies in the execution of study protocols for outcome assessment across study groups **OR** outcome assessments were not adequately reported for meaningful interpretation of results.
	Sampling Adequacy	Sampling was not adequate for the outcome(s) of interest (e.g., histopathology was performed on exposed groups, but not controls).
	Blinding of Assessors	Information in the study report did not include whether assessors were blinded to treatment group for subjective outcomes and suggested that the assessment of subjective outcomes (e.g., functional observational battery, qualitative neurobehavioral endpoints, histopathological re-evaluations) was performed in a biased fashion (e.g., assessors of subjective outcomes were aware of study groups). This is a serious flaw that makes the study unusable.
	Negative Control Responses	The biological responses of the negative control groups were not reported **OR** there was unacceptable variation in biological responses between control replicates.
Confounding/ variable control	Confounding Variables in Test Design and Procedures	The study reported significant differences among the study groups with respect to initial BW, decreased drinking water/food intake due to palatability issues ($\geq$ 20% difference from control) that could lead to dehydration and/or malnourishment, or reflex bradypnea that could lead to decreased oxygenation of the blood.
	Health Outcomes Unrelated to Exposure	One or more study groups experienced serious animal attrition or health outcomes unrelated to exposure (e.g., infection).
Data Presentation and Analysis	Statistical Methods	Statistical methods used were not appropriate (e.g., parametric test for non-normally distributed data) **OR** statistical analysis was not conducted **AND** data were not provided preventing an independent statistical analysis.
	Reporting of Data	Data presentation was inadequate (e.g., the report does not differentiate among findings in multiple exposure groups) **OR** major inconsistencies were present in reporting of results.

Table B-15. Data Quality Criteria for Animal Toxicity Studies

Confidence Level (Score)	Description	Selected Score
Domain 1. Test Substance		
Metric 1. Test Substance Identity Was the test substance identified definitively (i.e., established nomenclature, CASRN, and/or structure reported, including information on the specific form tested (particle characteristics for solid-state materials, salt or base, valence state, hydration state, isomer, radiolabel, etc.) for materials that may vary in form)? If test substance is a mixture, were mixture components and ratios characterized?		
High (score = 1)	The test substance was identified definitively, and the specific form was characterized (where applicable). For mixtures, the components and ratios were characterized.	
Medium (score = 2)	The test substance and form (the latter if applicable) were identified, and components and ratios of mixtures were characterized, but there were minor uncertainties (e.g., minor characterization details were omitted) that are unlikely to have a substantial impact on results.	
Low (score = 3)	The test substance and form (the latter if applicable) were identified and components and ratios of mixtures were characterized, but there were uncertainties regarding test substance identification or characterization that are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The test substance identity and form (the latter if applicable) cannot be determined from the information provided (e.g., nomenclature was unclear and CASRN or structure were not reported) **OR** for mixtures, the components and ratios were not characterized. These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 2. Test Substance Source Was the source of the test substance reported, including manufacturer and batch/lot number for materials that may vary in composition? If synthesized or extracted, was test substance identity verified by analytical methods?		
High (score = 1)	The source of the test substance was reported, including manufacturer and batch/lot number for materials that may vary in composition, and its identity was certified by manufacturer and/or verified by analytical methods (melting point, chemical analysis, etc.).	
Medium (score = 2)	The source of the test substance and/or the analytical verification of a synthesized test substance was reported incompletely, but the omitted details are unlikely to have a substantial impact on results.	
Low (score = 3)	Omitted details on the source of the test substance and/or the analytical verification of a synthesized test substance are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The test substance was not obtained from a manufacturer **OR** if synthesized or extracted, analytical verification of the test substance was not conducted. These are serious flaws that makes the study unusable.	
Not rated/applicable		

Confidence Level (Score)	Description	Selected Score
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 3. Test Substance Purity Was the purity or grade (i.e., analytical, technical) of the test substance reported and adequate to identify its toxicological effects? Were impurities identified? Were impurities present in quantities that could influence the results?		
High (score = 1)	The test substance purity and composition were such that any observed effects were highly likely to be due to the nominal test substance itself (e.g., highly pure or analytical-grade test substance or a formulation comprising primarily inert ingredients with small amount of active ingredient).	
Medium (score = 2)	Minor uncertainties or limitations were identified regarding the test substance purity and composition; however, the purity and composition were such that observed effects were more likely than not due to the nominal test substance, and any identified impurities are unlikely to have a substantial impact on results. Alternately, purity was not reported but given other information purity was not expected to be of concern.	
Low (score = 3)	Purity and/or grade of test substance were not reported or were low enough to have a substantial impact on results (i.e., observed effects may not be due to the nominal test substance).	
Unacceptable (score = 4)	The nature and quantity of reported impurities were such that study results were likely to be due to one or more of the impurities. This is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 2. Test Design		
Metric 4. Negative and Vehicle Controls Was an appropriate concurrent negative control group included? If a vehicle was used, was the control group exposed to the vehicle? For inhalation and gavage studies, were controls sham-exposed?		
High (score = 1)	Study authors reported using an appropriate concurrent negative control group (i.e., all conditions equal except chemical exposure). If gavage or inhalation study, a vehicle and/or sham-treated control group was included.	
Medium (score = 2)	Study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups; however, the identified differences are considered to be minor limitations that are unlikely to have a substantial impact on results.	
Low (score = 3)	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported, and the lack of details is likely to have a substantial impact on results.	
Unacceptable (score = 4)	A concurrent negative control group was not included or reported **OR** the reported negative control group was not appropriate (e.g., age/weight of animals differed between control and treated groups). This is a serious flaw that makes the study unusable.	
Not rated/applicable		

Confidence Level (Score)	Description	Selected Score
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 5. Positive Controls Was an appropriate concurrent positive control group included if necessary based on study type (e.g., certain neurotoxicity studies)? This metric is not rated/applicable if positive control was not indicated by study type.		
High (score = 1)	When applicable, a concurrent positive control was used (if necessary for the study type) and a positive response was observed.	
Medium (score = 2)	When applicable, a concurrent positive control was used, but there were minor uncertainties (e.g., minor details regarding control exposure or response were omitted) that are unlikely to have a substantial impact on results.	
Low (score = 3)	When applicable, a concurrent positive control was used, but there were deficiencies regarding the control exposure or response that are likely to have a substantial impact on results (e.g., the control response was not described).	
Unacceptable (score = 4)	When applicable, an appropriate concurrent positive control (i.e., inducing a positive response) was not used and its omission is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 6. Randomized Allocation of Animals Did the study explicitly report randomized allocation of animals to study groups?		
High (score = 1)	The study reported that animals were randomly allocated into study groups (including the control group).	
Medium (score = 2)	The study reported methods of allocation of animals to study groups, but there were minor limitations in the allocation method (e.g., method with a nonrandom component like assignment to minimize differences in BW across groups) that are unlikely to have a substantial impact on results.	
Low (score = 3)	The study did not report how animals were allocated to study groups, or there were deficiencies regarding the allocation method that are likely to have a substantial impact on results (e.g., allocation by animal number).	
Unacceptable (score = 4)	The study reported using a biased method to allocate animals to study groups (e.g., judgement of investigator). This is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Domain 3. Exposure Characterization		
Metric 7. Preparation and Storage of Test Substance Did the study characterize the test substance preparation and storage conditions (e.g., test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, centrifugation/filtration)? Were the frequency of preparation and/or storage conditions appropriate to the test substance stability? For inhalation studies, was the aerosol/vapor generation method appropriate?		
High (score = 1)	The test substance preparation and storage conditions were reported and appropriate for the test substance (e.g., test substance well-mixed in diet). For inhalation studies, the method and equipment used to generate the test substance as a gas, vapor, or aerosol were reported and appropriate.	
Medium (score = 2)	The test substance preparation and storage conditions were reported, but there were only minor limitations in the test substance preparation and/or storage conditions (i.e., diet was not mixed fresh daily). Also, any omission of details regarding preparation and storage that are unlikely to have a substantial impact on results. For inhalation studies, the method and equipment used to generate the test substance were incomplete or confusing but there is no reason to believe there was an impact on animal exposure.	
Low (score = 3)	Deficiencies in reporting of test substance preparation and/or storage conditions are likely to have a substantial impact on results (e.g., available information on physical-chemical properties suggested that stability and/or solubility of test substance in vehicle may be poor). For inhalation studies, there is reason to question the validity of the method used for generating the test substance.	
Unacceptable (score = 4)	Information on preparation and storage was not reported **OR** serious flaws reported with test substance preparation and/or storage conditions will have critical impacts on dose/concentration estimates and make the study unusable (e.g., instability of test substance in exposure medium was reported, or there was heterogeneous distribution of test substance in exposure matrix (for instance, aerosol deposition in exposure chamber, insufficient mixing of dietary matrix)). For inhalation studies, there was no mention of the method and equipment used to generate the test substance, or the method used is atypical and inappropriate.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 8. Consistency of Exposure Administration Were exposures administered consistently across study groups (e.g., same exposure frequency; same time of day; consistent gavage volumes or diet compositions in oral studies; consistent chamber designs, animals/chamber, and comparable particle size characteristics in inhalation studies; consistent application methods and volumes in dermal studies)?		
High (score = 1)	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner (e.g., gavage volume was not excessive).	
Medium (score = 2)	Details of exposure administration were reported, but minor limitations in administration of exposures (e.g., accidental mistakes in dosing) were identified that are unlikely to have a substantial impact on results.	

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	Details of exposure administration were reported, but deficiencies in administration of exposures (e.g., exposed at different times of day) are likely to have a substantial impact on results.	
Unacceptable (score = 4)	Critical exposure details (e.g., methods for generating atmosphere in inhalation studies) were not reported **OR** reported information indicated that exposures were not administered consistently across study groups (e.g., differing particle size), resulting in serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 9. Reporting of Doses/Concentrations Were doses/concentrations reported without ambiguity (e.g., point estimate in addition to a range)? In oral studies, if doses were not reported, was information reported that enabled dose estimation (e.g., test animal dietary intake and BW monitoring data in dietary studies)? In inhalation studies, was test substance vapor/aerosol concentration measured analytically along with nominal and target concentrations?		
High (score = 1)	For oral and dermal studies, administered doses/concentrations, or the information to calculate them, were reported without ambiguity. For inhalation studies, several specific considerations apply: Analytical, nominal and target chamber concentrations were all reported, with high confidence in the accuracy of the actual concentrations; the range of concentrations within a treatment group did not deviate widely (range should be within ±10% for gases and vapors and within ±20% for liquid and solid aerosols). The analytical method (high-performance liquid chromatography, gas chromatography, infrared spectrophotometry, etc.) used to measure chamber test substance and vehicle concentration was reported and appropriate. Actual chamber measurements using gravimetric filters are acceptable when testing dry aerosols and non-volatile liquid aerosols. The particle size distribution data, mass median aerodynamic diameter (MMAD), and geometric standard deviation were reported for all exposed groups (including vehicle controls, when used).	
Medium (score = 2)	For oral and dermal studies, minor uncertainties in reporting of administered doses/concentrations occurred (e.g., dietary or air concentrations were not measured analytically) but are unlikely to have a substantial impact on results. For inhalation studies, several specific considerations apply: With gases only, actual concentrations were not reported, but there is high confidence that the animals were exposed at approximately the reported target concentrations. (There is no comparable medium result for aerosols and vapors if analytical concentrations are not reported.) For inhalation studies (gas, vapor, aerosol), the analytical method used was less than ideal or subject to interference, but, nevertheless, yielded fairly reliable measurements of chamber concentrations. Particle size distribution data were not reported, but MMAD and geometric standard deviation values were reported for all exposed groups (including vehicle controls, when used).	

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	For oral and dermal studies, deficiencies in reporting of administered doses/concentrations occurred (e.g., no information on animal BW or intake were provided) that are likely to have a substantial impact on results. For inhalation studies, several considerations apply: Using aerosols and vapors, a score of low is indicated if actual concentrations are not reported or the analytical method used, such as sampling tubes (e.g., Draeger tubes) provided imprecise measurements. An MMAD is reported but no geometric standard deviation or particle size distribution data were reported.	
Unacceptable (score = 4)	The reported exposure levels could not be validated (e.g., lack of food or water intake data for dietary or water exposures in conjunction with evidence of palatability differences, lack of BW data in conjunction with qualitative evidence for BW differences across groups, inconsistencies in reporting, etc.). This is a serious flaw that makes the study unusable. For inhalation studies, actual concentrations were not reported along with animal responses (or lack of responses) that indicate exposure problems due to faulty test substance generation. Animals were exposed to an aerosol but no MMAD or particle size data were reported.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 10. Exposure Frequency and Duration Were the exposure frequency (hours/day and days/week) and duration of exposure reported and appropriate for this study type and/or outcome(s) of interest?		
High (score = 1)	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest (e.g., inhalation exposure 6 hours/day, gavage 5 days/week, 2-year duration for cancer bioassays).	
Medium (score = 2)	Minor limitations in exposure frequency and duration of exposure were identified (e.g., inhalation exposure of 4 hours/day instead of 6 hours/day in a repeated exposure study) but are unlikely to have a substantial impact on results.	
Low (score = 3)	The duration of exposure and/or exposure frequency differed significantly from typical study designs (e.g., gavage 1 day/week), and these deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The exposure frequency or duration of exposure were not reported **OR** the reported exposure frequency and duration were not suited to the study type and/or outcome(s) of interest (e.g., study length inadequate to evaluate tumorigenicity). These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Metric 11. Number of Exposure Groups and Dose/Concentration Spacing Were the number of exposure groups and dose/concentration spacing justified by study authors (e.g., based on range-finding studies) and adequate to address the purpose of the study (e.g., to evaluate dose-response relationships, identify points of departure, inform MOA/AOP, etc.)?		
High (score = 1)	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study (e.g., the selected doses produce a range of responses).	
Medium (score = 2)	There were minor limitations regarding the number of exposure groups and/or dose/concentration spacing (e.g., unclear if lowest dose was low enough or the highest dose was high enough), but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest (e.g., observation of a dose-response relationship) and the concerns are unlikely to have a substantial impact on results.	
Low (score = 3)	There were deficiencies regarding the number of exposure groups and/or dose/concentration spacing (e.g., narrow spacing between doses with similar responses across groups), and these are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The number of exposure groups and spacing were not reported **OR** dose groups and spacing were not relevant for the assessment (e.g., all doses in a developmental toxicity study produced overt maternal toxicity). These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 12. Exposure Route and Method Were the route and method of exposure reported and suited to the test substance (e.g., was the test substance non-volatile in dietary studies)?		
High (score = 1)	The route and method of exposure were reported and were suited to the test substance. For inhalation studies, a dynamic chamber was used. While dynamic nose-only (or head-only) studies are generally preferred, dynamic whole-body chambers are acceptable for gases and for vapors that do not condense.	
Medium (score = 2)	There were minor limitations regarding the route and method of exposure, but the researchers took appropriate steps to mitigate the problem (e.g., mixed diet fresh each day for volatile compounds). These limitations are unlikely to have a substantial impact on results. For inhalation studies, a dynamic whole-body chamber was used for vapors that might condense or for aerosols.[5]	

[5] This results in a medium score because in addition to inhalation exposure to the test substance, there may also be significant oral exposure due to rodents grooming test substance that adheres to their fur. The combined oral and inhalation exposure results in a lower POD, which makes a test substance appear more toxic than it really is by the inhalation route.

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	There were deficiencies regarding the route and method of exposure that are likely to have a substantial effect on results. Researchers may have attempted to correct the problem, but the success of the mitigating action was unclear. For inhalation studies, there are significant flaws in the design or operation of the inhalation chamber, such as uneven distribution of test substance in a whole-body chamber, having less than 15 air changes/hour in a whole-body chamber, or using a whole-body chamber that is too small for the number and volume of animals exposed.	
Unacceptable (score = 4)	The route or method of exposure was not reported **OR** an inappropriate route or method (e.g., administration of a volatile organic compound via the diet) was used for the test substance without taking steps to correct the problem (e.g., mixing fresh diet). These are serious flaws that makes the study unusable. For inhalation studies, either a static chamber was used, there is no description of the inhalation chamber, or an atypical exposure method was used, such as allowing a container of test substance to evaporate in a room.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 4. Test Animals		
Metric 13. Test Animal Characteristics Were the test animal species, strain, sex, health status, age, and starting BW reported? Was the test animal from a commercial source or in-house colony? Was the test species and strain an appropriate animal model for the evaluation of the specific outcome(s) of interest (e.g., routinely used for similar study types)?		
High (score = 1)	The test animal species, strain, sex, health status, age, and starting BW were reported, and the test animal was obtained from a commercial source or laboratory-maintained colony. The test species and strain were an appropriate animal model for the evaluation of the specific outcome(s) of interest (e.g., routinely used for similar study types).	
Medium (score = 2)	Minor uncertainties in the reporting of test animal characteristics (e.g., health status, age, or starting BW) are unlikely to have a substantial impact on results. The test animals were obtained from a commercial source or in-house colony, and the test species/strain/sex was an appropriate animal model for the evaluation of the specific outcome(s) of interest (e.g., routinely used for similar study types).	
Low (score = 3)	The source of the test animal was not reported **OR** the test animal strain or sex was not reported. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The test animal species was not reported **OR** the test animal (species, strain, sex, life-stage, source) was not appropriate for the evaluation of the specific outcome(s) of interest (e.g., genetically modified animals, strain was uniquely susceptible or resistant to one or more outcome of interest). These are serious flaws that make the study unusable.	
Not rated/applicable		

Confidence Level (Score)	Description	Selected Score
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 14. Adequacy and Consistency of Animal Husbandry Conditions Were all husbandry conditions (e.g., housing, temperature) adequate and the same for control and exposed populations, such that the only difference was exposure to the test substance?		
High (score = 1)	All husbandry conditions were reported (e.g., temperature, humidity, light-dark cycle) and were adequate and the same for control and exposed populations, such that the only difference was exposure.	
Medium (score = 2)	Most husbandry conditions were reported and were adequate and similar for all groups. Some differences in conditions were identified among groups, but these differences were considered minor uncertainties or limitations that are unlikely to have a substantial impact on results.	
Low (score = 3)	Husbandry conditions were not sufficiently reported to evaluate whether husbandry was adequate and whether differences occurred between control and exposed populations. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	There were significant differences in husbandry conditions between control and exposed groups (e.g., temperature, humidity, light-dark cycle) **OR** animal husbandry conditions deviated from customary practices in ways likely to impact study results (e.g., injuries and stress due to cage overcrowding). These are serious flaws that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 15. Number of Animals per Group Was the number of animals per study group appropriate for the study type and outcome analysis?		
High (score = 1)	The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type (e.g., 50/sex/group for rodent cancer bioassay, 10/sex/group for rodent subchronic study).	
Medium (score = 2)	The reported number of animals per study group was lower than the typical number used in studies of the same or similar type (e.g., 30/sex/group for rodent cancer bioassay, 8/sex/group for rodent subchronic study), but it was sufficient for statistical analysis and this minor limitation is unlikely to have a substantial impact on results.	
Low (score = 3)	The reported number of animals per study group was not sufficient for statistical analysis (e.g., varying numbers per group with some groups consisting of only one animal), and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	The number of animals per study group was not reported **OR** the number of animals per study group was insufficient to characterize toxicological effects (e.g., 1-2 animals in each group). These are serious flaws that makes the study unusable.	
Not rated/applicable		

Confidence Level (Score)	Description	Selected Score
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 5. Outcome Assessment		
Metric 16. Outcome Assessment Methodology Did the outcome assessment methodology address or report the intended outcome(s) of interest? Was the outcome assessment methodology (including endpoints and timing of assessment) sensitive for the outcome(s) of interest (e.g., measured endpoints that are able to detect a true health effect or hazard)? Note: Outcome, as addressed in this domain, refers to health effects measured in an animal study (e.g., organ-specific toxicity, reproductive and developmental toxicity).		
High (score = 1)	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.	
Medium (score = 2)	The outcome assessment methodology partially addressed or reported the intended outcomes(s) of interest (e.g., serum chemistry and organ weight evaluated in the absence of histology), but minor uncertainties are unlikely to have a substantial impact on results.	
Low (score = 3)	Significant deficiencies in the reported outcome assessment methodology were identified **OR** due to incomplete reporting, it was unclear whether methods were sensitive for the outcome of interest. This is likely to have a substantial impact on results.	
Unacceptable (score = 4)	The outcome assessment methodology was not reported **OR** the reported outcome assessment methodology was not sensitive for the outcome(s) of interest (e.g., evaluation of endpoints outside the critical window of development, a systemic toxicity study that evaluated only grossly observable endpoints, such as clinical signs and mortality). These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 17. Consistency of Outcome Assessment Was the outcome assessment carried out consistently (i.e., using the same protocol) across study groups (e.g., assessment at the same time after initial exposure in all study groups)?		
High (score = 1)	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.	
Medium (score = 2)	There were minor differences in the timing of outcome assessment across study groups or incomplete reporting of minor details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.	
Low (score = 3)	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported, and these deficiencies are likely to have a substantial impact on results.	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	There were large inconsistencies in the execution of study protocols for outcome assessment across study groups **OR** outcome assessments were not adequately reported for meaningful interpretation of results. These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 18. Sampling Adequacy Was sampling adequate for the outcome(s) of interest, including experimental unit (e.g., litter vs. individual animal weight), number of evaluations per dose group, and endpoint (e.g., number of slides evaluated per organ)?		
High (score = 1)	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest (e.g., litter data provided for developmental studies; endpoints were evaluated in an adequate number of animals in each group).	
Medium (score = 2)	Details regarding sampling for the outcome(s) of interest were reported, but minor limitations were identified in the sampling of the outcome(s) of interest (e.g., histopathology was performed for high-dose group and controls only, and treatment-related changes were observed at the high dose) that are unlikely to have a substantial impact on results.	
Low (score = 3)	Details regarding sampling of outcomes were not reported and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Sampling was not adequate for the outcome(s) of interest (e.g., histopathology was performed on exposed groups but not controls). This is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 19. Blinding of Assessors Were investigators assessing subjective outcomes (i.e., those evaluated using human judgment, including functional observational battery, qualitative neurobehavioral endpoints, histopathological re-evaluations) blinded to treatment group? If blinding was not applied, were quality control/quality assurance procedures for endpoint evaluation cited? Note that blinding is not required for initial histopathology review in accordance with Best Practices recommended by the Society of Toxicologic Pathology. This should be considered when rating this metric.[a] This metric is not rated/applicable for initial histopathology review or if no subjective outcomes were assessed (i.e., only automated measurements were included and/or human judgment was not applied).		

Confidence Level (Score)	Description	Selected Score
High (score = 1)	The study explicitly reported that investigators assessing subjective outcomes (i.e., those evaluated using human judgment, including functional observational battery, qualitative neurobehavioral endpoints, histopathological re-evaluations) were blinded to treatment group or that quality control/quality assurance methods were followed in the absence of blinding.	
Medium (score = 2)	The study reported that blinding was not possible, but steps were taken to minimize bias (e.g., knowledge of study group was restricted to personnel not assessing subjective outcome) and this minor uncertainty is unlikely to have a substantial impact on results. Alternately, blinding was not reported; however, lack of blinding is not expected to have a substantial impact on results.	
Low (score = 3)	The study did not report whether assessors were blinded to treatment group for subjective outcomes, and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Information in the study report did not indicate whether assessors were blinded to treatment group for subjective outcomes or suggested that the assessment of subjective outcomes (e.g., functional observational battery, qualitative neurobehavioral endpoints, histopathological re-evaluations) was performed in a biased fashion (e.g., assessors of subjective outcomes were aware of study groups). This is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 20. Negative Control Response Were the biological responses (e.g., histopathology, litter size, pup viability) of the negative control group(s) adequate?		
High (score = 1)	The biological responses of the negative control group(s) were adequate (e.g., no/low incidence of histopathological lesions).	
Medium (score = 2)	There were minor uncertainties or limitations regarding the biological responses of the negative control group(s) (e.g., differences in outcome between untreated and solvent controls) that are unlikely to have a substantial impact on results.	
Low (score = 3)	The biological responses of the negative control group(s) were reported, but there were deficiencies regarding the control responses that are likely to have a substantial impact on results (e.g., elevated incidence of histopathological lesions).	
Unacceptable (score = 4)	The biological responses of the negative control groups were not reported **OR** there was unacceptable variation in biological responses between control replicates. These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Domain 6. Confounding/Variable Control		
Metric 21. Confounding Variables in Test Design and Procedures Were there confounding differences among the study groups in initial BW or test substance palatability that could influence the outcome assessment (e.g., did palatability issues lead to dehydration and/or malnourishment)? Did reflex bradypnea (i.e., reduced respiration and reduced test substance exposure) induced by respiratory irritants influence outcome assessment? Were normal signs of reflex bradypnea misinterpreted as neurologic, behavioral, or developmental effects (e.g., hypothermia, lethargy, unconsciousness, poor performance in behavioral studies, delayed pup development)?		
High (score = 1)	There were no reported differences among the study groups in initial BW, food or water intake, or respiratory rate that could influence the outcome assessment.	
Medium (score = 2)	The study reported minor differences among the study groups (< 20% difference from control) with respect to initial BW, drinking water and/or food consumption due to palatability issues, or respiratory rate due to reflex bradypnea. These minor uncertainties are unlikely to have a substantial impact on results. Alternately, the lack of reporting of initial BWs, food/water intake, and/or respiratory rate is not likely to have a significant impact on results.	
Low (score = 3)	Initial BW, food/water intake, and respiratory rate were not reported. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	The study reported significant differences among the study groups with respect to initial BW, decreased drinking water/food intake due to palatability issues (≥ 20% difference from control) that could lead to dehydration and/or malnourishment, or reflex bradypnea that could lead to decreased oxygenation of the blood. These are serious flaws that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 22. Health Outcomes Unrelated to Exposure Were there differences among the study groups in animal attrition or health outcomes unrelated to exposure (e.g., infection) that could influence the outcome assessment? Professional judgement should be used to determine whether or not signs of infection would invalidate the study. Criteria for High, Medium and Low are used when the study is still usable.		
High (score = 1)	Details regarding animal attrition and health outcomes unrelated to exposure (e.g., infection) were reported for each study group, and there were no differences among groups that could influence the outcome assessment.	
Medium (score = 2)	Authors reported that one or more study groups experienced disproportionate animal attrition or health outcomes unrelated to exposure (e.g., infection), but data from the remaining exposure groups were valid and the low incidence of attrition is unlikely to have a substantial impact on results **OR** data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted (as indicated by study authors).	

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group, and this deficiency is likely to have a substantial impact on results. **OR** data on attrition and/or health outcomes are reported and could have substantial impact on results.	
Unacceptable (score = 4)	One or more study groups experienced serious animal attrition or health outcomes unrelated to exposure (e.g., infection). This is a serious flaw that makes the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 7. Data Presentation and Analysis		
Metric 23. Statistical Methods Were statistical methods clearly described and appropriate for dataset(s) (e.g., parametric test for normally distributed data)?		
High (score = 1)	Statistical methods were clearly described and appropriate for dataset(s) (e.g., parametric test for normally distributed data). **OR** no statistical analyses, calculation methods, and/or data manipulation were conducted, but sufficient data were provided to conduct an independent statistical analysis.	
Medium (score = 2)	Statistical analysis was described with some omissions that would unlikely have a substantial impact on results.	
Low (score = 3)	Statistical analysis was not described clearly, and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Statistical methods were not appropriate (e.g., parametric test for non-normally distributed data) **OR** statistical analysis was not conducted **AND** data were not provided preventing an independent statistical analysis. These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 24. Reporting of Data Were the data for all outcomes presented? Were data reported by exposure group and sex (if applicable), with numbers of animals affected and numbers of animals evaluated (for quantal data) or group means and variance (for continuous data)? If severity scores were used, was the scoring system clearly articulated?		
High (score = 1)	Data for exposure-related findings were presented for all outcomes by exposure group and sex (if applicable) with quantal and/or continuous presentation and description of severity scores if applicable. Negative findings were reported qualitatively or quantitatively.	

Confidence Level (Score)	Description	Selected Score
Medium (score = 2)	Data for exposure-related findings were reported for most, but not all, outcomes by exposure group and sex (if applicable) with quantal and/or continuous presentation and description of severity scores if applicable. The minor uncertainties in outcome reporting are unlikely to have substantial impact on results.	
Low (score = 3)	Data for exposure-related findings were not shown for each study group, but results were described in the text and/or data were only reported for some outcomes. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	Data presentation was inadequate (e.g., the report does not differentiate among findings in multiple exposure groups) **OR** major inconsistencies were present in reporting of results. These are serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 8. Other (Apply as Needed)		
Metric:		
High (score = 1)		
Medium (score = 2)		
Low (score = 3)		
Unacceptable (score = 4)		
Not rated/applicable		
Reviewer's comments	*Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

[a]Crissman et al., 2004

B.1.8.2. In Vitro Toxicity Studies

Table B-16. Serious Flaws that Would Make *In Vitro* Toxicity Studies Unacceptable

Domain	Metric	Description of Serious Flaw(s) in Data Source[a]
Test Substance	Test Substance Identity	The test substance identity and form (if applicable) could not be determined from the information provided (e.g., nomenclature was unclear and CASRN or structure were not reported) **OR** the components and ratios of mixtures were not characterized.
	Test Substance Source	The test substance was not obtained from a manufacturer **OR** if synthesized or extracted, analytical verification of the test substance was not conducted.
	Test Substance Purity	The nature and quantity of reported impurities were such that study results were likely to be due to one or more of the impurities.
Test Design	Negative Controls	A concurrent negative control group was not included or reported **OR** the reported negative control group was not appropriate (e.g., different cell lines used for controls and test substance exposure).
	Positive Controls	A concurrent positive control or proficiency group was not used (when applicable).
	Assay Procedures	Assay methods and procedures were not reported **OR** assay methods and procedures were not appropriate for the study type (e.g., *in vitro* skin corrosion protocol used for *in vitro* skin irritation assay).
	Standards for Test	Quality control criteria were not reported and/or inadequate data were provided to demonstrate validity, acceptability, and reliability of the test when compared with current standards and guidelines.
Exposure Characterization	Preparation and Storage of Test Substance	Information on preparation and storage was not reported **OR** serious flaws reported with test substance preparation and/or storage conditions will have critical impacts on dose/concentration estimates and make the study unusable (e.g., instability of test substance in exposure media, test substance volatilized rapidly from the open containers that were used as test vessels).
	Consistency of Exposure Administration	Critical exposure details (e.g., amount of test substance used) were not reported **OR** exposures were not administered consistently across and/or within study groups (e.g., 75 mg/cm^2 and 87 mg/cm^2 administered to reconstructed corneas replicate 1 and replicate 2, respectively, in *in vitro* eye irritation test) resulting in serious flaws that make the study unusable.
	Reporting of Doses/Concentrations	The exposure doses/concentrations or amounts of test substance were not reported resulting in serious flaws.
	Exposure Duration	No information on exposure duration(s) was reported **OR** the exposure duration was not appropriate for the study type and/or outcome of interest (e.g., 5 hours for reconstructed epidermis in skin irritation test, 24 hours exposure for bacterial reverse mutation test).

Domain	Metric	Description of Serious Flaw(s) in Data Source[a]
Exposure Characterization (continued)	Number of Exposure Groups and Dose Spacing	The number of exposure groups and dose/concentration spacing were not reported **OR** the number of exposure groups and dose/concentration spacing were not relevant for the assessment (e.g., all concentrations used in an *in vitro* mammalian cell micronucleus test were cytotoxic).
	Metabolic Activation	No information on the characterization and use of a metabolic activation system was reported.
Test Model	Test Model	The test model and descriptive information were not reported **OR** the test model was not appropriate for evaluation of the specific outcome of interest (e.g., bacterial reverse mutation assay to evaluate chromosome aberrations).
	Number per Group	The number of organisms or tissues per study group and/or replicates per study group were not reported **OR** the number of organisms or tissues per study group and/or replicates per study group were insufficient to characterize toxicological effects (e.g., one tissue/test concentration/one exposure time for *in vitro* skin corrosion test, one replicate/strain of bacteria exposed in bacterial reverse mutation assay).
Outcome Assessment	Outcome Assessment Methodology	The outcome assessment methodology was not reported **OR** the assessment methodology was not appropriate for the outcome(s) of interest (e.g., cells were evaluated for chromosomal aberrations immediately after exposure to the test substance instead of after post-exposure incubation period, cytotoxicity not determined prior to CD86/CD expression measurement assay, and labeling antibodies were not tested on proficiency substances in an *in vitro* skin sensitization test in h-CLAT cells).
	Consistency of Outcome Assessment	There were large inconsistencies in the execution of study protocols for outcome assessment across study groups **OR** outcome assessments were not adequately reported for meaningful interpretation of results.
	Sampling Adequacy	Reported sampling was not adequate for the outcome(s) of interest and/or serious uncertainties or limitations were identified in how the study carried out the sampling of the outcome(s) of interest (e.g., replicates from control and test concentrations were evaluated at different times).
	Blinding of Assessors	Information in the study report suggested that the assessment of subjective outcomes was performed in a biased fashion (e.g., assessors of subjective outcomes were aware of study groups).

Domain	Metric	Description of Serious Flaw(s) in Data Source[a]
Confounding/ Variable Control	Confounding Variables in Test Design and Procedures	There were significant differences among the study groups with respect to the strain/batch/lot number of organisms or models used per group or size and/or quality of tissues exposed (e.g., initial number of viable bacterial cells were different for each replicate (10^5 cells in replicate 1, 10^8 cell in replicate 2, and 10^3 cells in replicate 3); tissues from two different lots were used for *in vitro* skin corrosion test, but the control batch quality for one lot was outside of the acceptability range).
	Confounding Variables in Outcomes Unrelated to Exposure	One or more replicates or groups (i.e., negative and positive controls) experienced disproportionate growth or reduction in growth unrelated to exposure (e.g., contamination) such that no outcomes could be assessed.
Data Presentation and Analysis	Data Analysis	Statistical methods, calculation methods, or data manipulation were not appropriate (e.g., Student's t-test used to compare two groups in a multi-group study, parametric test for non-normally distributed data) **OR** statistical analysis was not conducted **AND** data enabling an independent statistical analysis were not provided.
	Data Interpretation	The reported scoring and/or evaluation criteria were inconsistent with established practices resulting in the interpretation of data results that are seriously flawed.
	Cytotoxicity Data	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.
	Reporting of Data	Data presentation was inadequate (e.g., the report did not differentiate among findings in multiple exposure groups, no scores or frequencies were reported), or major inconsistencies were present in reporting of results.

Note:
[a]If the metric does not apply to the study type, the flaw will not be applied to determine unacceptability.

Table B-17. Data Quality Criteria for *In Vitro* Toxicity Studies

Confidence Level (Score)	Description	Selected Score
Domain 1. Test Substance		
Metric 1. Test Substance Identity Was the test substance identified definitively (i.e., established nomenclature, CASRN, physical nature, physiochemical properties, and/or structure reported, including information on the specific form tested (e.g., salt or base, valence state, isomer, if applicable) for materials that may vary in form)? If test substance was a mixture, were mixture components and ratios characterized?		
High (score = 1)	The test substance was identified definitively (i.e., established nomenclature, CASRN, physical nature, physiochemical properties, and/or structure reported, including information on the specific form tested (e.g., salt or base, valence state, isomer, (if applicable)) for materials that may vary in form. For mixtures, the components and ratios were characterized.	
Medium (score = 2)	The test substance and form (if applicable) were identified, and components and ratios of mixtures were characterized, but there were minor uncertainties (e.g., minor characterization details were omitted) that are unlikely to have a substantial impact on results.	

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	The test substance and form (if applicable) were identified, and components and ratios of mixtures were characterized, but there were uncertainties regarding test substance identification or characterization that are likely to have a substantial impact on the results.	
Unacceptable (score = 4)	The test substance identity and form (if applicable) could not be determined from the information provided (e.g., nomenclature was unclear and CASRN or structure were not reported) **OR** the components and ratios of mixtures were not characterized.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 2. Test Substance Source Was the source of the test substance reported, including manufacturer and batch/lot number for materials that may vary in composition? If synthesized or extracted, was test substance identity verified by analytical methods?		
High (score = 1)	The source of the test substance was reported, including manufacturer and batch/lot number for materials that might vary in composition, and its identity was certified by manufacturer and/or verified by analytical methods (melting point, chemical analysis, etc.).	
Medium (score = 2)	The source of the test substance and/or the analytical verification of a synthesized test substance was reported incompletely, but the omitted details are unlikely to have a substantial impact on the results.	
Low (score = 3)	Omitted details on the source of the test substance and/or analytical verification of a synthesized test substance are likely to have a substantial impact on the results.	
Unacceptable (score = 4)	The test substance was not obtained from a manufacturer **OR** if synthesized or extracted, analytical verification of the test substance was not conducted.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 3. Test Substance Purity Was the purity or grade (i.e., analytical, technical) of the test substance reported and adequate to identify its toxicological effects? Were impurities identified? Were impurities present in quantities that could influence the results?		
High (score = 1)	The test substance purity and composition were such that any observed effects were highly likely to be due to the nominal test substance itself (e.g., American Chemical Society grade, analytical grade, reagent grade test substance or a formulation comprising primarily inert ingredients with small amount of active ingredient). Impurities, if identified, were not present in quantities that could influence the results.	

Confidence Level (Score)	Description	Selected Score
Medium (score = 2)	Minor uncertainties or limitations were identified regarding the test substance purity and composition; however, the purity and composition were such that observed effects were more likely than not to be due to the nominal test substance and impurities, if identified, were unlikely to have a substantial impact on the results.	
Low (score = 3)	Purity and/or grade of test substance were not reported **OR** the percentage of the reported purity was such that the observed effects may not have been due to the nominal test substance.	
Unacceptable (score = 4)	The nature and quantity of reported impurities were such that study results were likely to be due to one or more of the impurities.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 2. Test Design		
Metric 4. Negative Controls Was a concurrent negative (untreated, sham-treated, and/or vehicle, as necessary) control group included?		
High (score = 1)	Study authors reported using a concurrent negative control group (untreated, sham-treated, and/or vehicle, as applicable) in which all conditions equal except exposure to test substance.	
Medium (score = 2)	Study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups; however, the identified differences are considered to be minor limitations that are unlikely to have substantial impact on results.	
Low (score = 3)	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported, and the lack of details is likely to have a substantial impact on the results.	
Unacceptable (score = 4)	A concurrent negative control group was not included or reported **OR** the reported negative control group was not appropriate (e.g., different cell lines used for controls and test substance exposure).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 5. Positive Controls Was a concurrent positive or proficiency control group included, *if applicable*, based on study type, and was the response appropriate in this group (e.g., induction of positive effect)? Note: This metric is applicable to studies that require a concurrent positive control.		
High (score = 1)	A concurrent positive control or proficiency control group, if applicable, was used and the intended positive response was induced.	
Medium (score = 2)	A concurrent positive control or proficiency control was used, but there were minor uncertainties (e.g., minor details regarding control exposure or response were omitted) that are unlikely to have a substantial impact on results.	

Confidence Level (Score)	Description	Selected Score
Low (score = 3)	A concurrent positive control or proficiency control was used, but there were uncertainties regarding the control exposure or response that are likely to have a substantial impact on results (e.g., the control response was not described).	
Unacceptable (score = 4)	A concurrent positive control or proficiency group was not used.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 6. Assay Procedures Were assay methods and procedures (e.g., test conditions, cell density culture media and volumes, pre- and post-incubation temperatures, humidity, reaction mix, washing/rinsing methods, incubation with amino acids, slide preparation, instrument used and calibration, wavelengths measured) described in detail and applicable to the study type?		
High (score = 1)	Study authors described the methods and procedures (e.g., test conditions, cell density culture media and volumes, pre- and post-incubation temperatures, humidity, reaction mix, washing/rinsing methods, incubation with amino acids, slide preparation, instrument used and calibration, wavelengths measured) used for the test in detail, and the methods and procedures were applicable for the study type (e.g., protocol for *in vitro* skin irritation test was reported).	
Medium (score = 2)	Methods and procedures were partially described and/or cited in another publication(s), but appeared to be appropriate (e.g., reporting that "calculations were used for enumerating viable and mutant cells" in a mammalian cell gene mutation test using *Hprt* and *xprt* genes instead of inclusion of the equations) to the study type, so the omission is unlikely to have a substantial impact on results.	
Low (score = 3)	The methods and procedures were not well described or deviated from customary practices (e.g., post-incubation time was not stated in a mammalian cell gene mutation test using *Hprt* and *xprt* genes), and this is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Assay methods and procedures were not reported **OR** assay methods and procedures were not appropriate for the study type (e.g., *in vitro* skin corrosion protocol used for *in vitro* skin irritation assay).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Metric 7. Standards for Test For assays with established criteria, were the test validity, acceptability, reliability, and/or quality control criteria reported and consistent with current standards and guidelines? Example acceptability and quality control criteria for an *in vitro* skin corrosion test using the EpiSkin™ (SM) model: Acceptability criteria: negative control optical density values between ≥ 0.6 and ≤ 1.5, variability of the positive control replicates should be ≤ 20% of negative control, difference of viability between 2 tissue replicates should not exceed 30% in the range of 20%–100% viability and for EDs ≥ 0.3; quality control criteria: Only quality control-accepted tissue batches having an IC_{50} range of 1.0–3.0 mg/mL were used.) Note: This metric is generally applicable to studies using reconstructed human cells and may not be applicable to other studies.		
High (score = 1)	The test validity, acceptability, reliability, and/or quality control criteria were reported and consistent with current standards and guidelines,[a] if applicable.	
Medium (score = 2)	Not applicable for this metric.	
Low (score = 3)	Not applicable for this metric.	
Unacceptable (score = 4)	Quality control criteria were not reported and/or inadequate data were provided to demonstrate validity, acceptability, and reliability of the test when compared with current standards and guidelines.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 3. Exposure Characterization		
Metric 8. Preparation and Storage of Test Substance Did the study characterize preparation of the test substance and storage conditions? Were the frequency of preparation and/or storage conditions appropriate to the test substance stability and solubility (if applicable)?		
High (score = 1)	The test substance preparation and/or storage conditions (e.g., test substance stability, homogeneity, mixing temperature, stock concentration, stirring methods, centrifugation/filtration, aerosol/vapor generation method, storage conditions) were reported and appropriate (e.g., stability in exposure media confirmed, volatile test substances prepared and stored in sealed containers) for the test substance.	
Medium (score = 2)	The test substance preparation and storage conditions were reported, but minor limitations in the test substance preparation and/or storage conditions were identified (e.g., test substance formulations were stirred instead of centrifuged for a specific number of rotations per minute) that are unlikely to have a substantial impact on results.	
Low (score = 3)	Deficiencies in reporting of test substance preparation, and/or storage conditions are likely to have a substantial impact on results (e.g., available information on physical-chemical properties suggests that stability and/or solubility of test substance in vehicle or culture media may be poor).	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	Information on preparation and storage was not reported **OR** serious flaws reported with test substance preparation and/or storage conditions will have critical impacts on dose/concentration estimates and make the study unusable (e.g., instability of test substance in exposure media, test substance volatilized rapidly from the open containers that were used as test vessels).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 9. Consistency of Exposure Administration Were exposures administered consistently across study groups (e.g., consistent application methods and volumes, control for evaporation)?		
High (score = 1)	Details of exposure administration were reported, and exposures were administered consistently across study groups in a scientifically sound manner (e.g., consistent application methods and volumes, control for evaporation).	
Medium (score = 2)	Details of exposure administration were reported or inferred from the text, but the minor limitations in administration of exposures (e.g., accidental mistakes in dosing) that were identified are unlikely to have a substantial impact on results.	
Low (score = 3)	Details of exposure administration were reported, but deficiencies in administration of exposures (e.g., non-calibrated instrument used to administer test substance) that were reported or inferred from the text are likely to have a substantial impact on results.	
Unacceptable (score = 4)	Critical exposure details (e.g., amount of test substance used) were not reported **OR** exposures were not administered consistently across and/or within study groups (e.g., 75 mg/cm^2 and 87 mg/cm^2 administered to reconstructed corneas replicate 1 and replicate 2, respectively, in *in vitro* eye irritation test) resulting in serious flaws that make the study unusable.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 10. Reporting of Doses/Concentrations Were exposure doses/concentrations or amounts of test substance reported without ambiguity (e.g., point estimate instead of range, analytical instead of nominal)?		
High (score = 1)	The exposure doses/concentrations or amounts of test substance were reported without ambiguity (e.g., point estimate instead of range, analytical instead of nominal).	
Medium (score = 2)	Not applicable for this metric.	
Low (score = 3)	Not applicable for this metric.	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	The exposure doses/concentrations or amounts of test substance were not reported resulting in serious flaws.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 11. Exposure Duration Was the exposure duration (e.g., minutes, hours, days) reported and appropriate for this study type and/or outcome(s) of interest?		
High (score = 1)	The exposure duration (e.g., minutes, hours, days) was reported and appropriate for the study type and/or outcome(s) of interest (e.g., 60-minute exposure for reconstructed epidermis in skin irritation test, 48–72-hour exposure for bacterial reverse mutation assay).	
Medium (score = 2)	Duration(s) of exposure differed slightly from current standards and guidelines[a] for studies of this type (e.g., 65 minutes for reconstructed epidermis in skin irritation test), but the differences are unlikely to have a substantial impact on results.	
Low (score = 3)	Duration(s) of exposure were not clearly stated (e.g., exposure duration was described only in qualitative terms) or duration(s) differed significantly from studies of the same or similar types. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	No information on exposure duration(s) was reported **OR** the exposure duration was not appropriate for the study type and/or outcome of interest (e.g., 5 hours for reconstructed epidermis in skin irritation test, 24-hour exposure for bacterial reverse mutation test).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 12. Number of Exposure Groups and Concentrations Spacing Were the number of exposure groups and dose/concentration spacing justified by study authors (e.g., based on study type, range-finding study, and/or cytotoxicity studies) and adequate to address the purpose of the study (e.g., to evaluate dose-response relationships, inform MOA/AOP)?		
High (score = 1)	The number of exposure groups and dose/concentration spacing were justified by study authors (e.g., based on study type, range-finding study, and/or cytotoxicity studies) and considered adequate to address the purpose of the study (e.g., to evaluate dose-response relationships, inform MOA/AOP).	
Medium (score = 2)	There were minor limitations regarding the number of exposure groups and/or dose/concentration spacing, but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest (e.g., observation of a dose-response relationship) and the concerns are unlikely to have a substantial impact on results.	
Low (score = 3)	There were deficiencies regarding the number of exposure groups and/or dose/concentration spacing (e.g., one bacterial strain exposed to two concentrations of the test substance in bacterial reverse mutation assay), and these concerns likely had a substantial impact on interpretation of the results.	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	The number of exposure groups and dose/concentration spacing were not reported **OR** the number of exposure groups and dose/concentration spacing were not relevant for the assessment (e.g., all concentrations used in an *in vitro* mammalian cell micronucleus test were cytotoxic).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 13. Metabolic Activation (if Applicable) Were exposures conducted in the presence and absence of a metabolic activation system, if applicable, for the study type? Were the source, method of preparation, concentration or volume in final culture, and quality control information on the metabolic activation system reported?		
High (score = 1)	Study authors reported that exposures were conducted in the presence of metabolic activation, and the type and source, method of preparation, concentration or volume in final culture, and quality control information of the metabolic activation system were described.	
Medium (score = 2)	The presence of a commonly used metabolic activation system (e.g., aroclor-, ethanol-, or phenobarbitial/β-naphthoflavone-induced rat, hamster, or mice liver cells) was reported in the study; however, some details regarding type, composition mix, concentration, or quality control information were not described. These omissions are unlikely to have a substantial impact on the results.	
Low (score = 3)	The presence of a metabolic activation system was reported in the study, but the system described was not validated (e.g., rigorous testing to ensure that it suitable for the purpose for which it is used) or comparable to commonly used systems (e.g., aroclor-, ethanol-, or phenobarbitial/β-naphthoflavone-induced rat, hamster, or mice liver cells).	
Unacceptable (score = 4)	No information on the characterization and use of a metabolic activation system was reported.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 4. Test Model		
Metric 14. Test Model Were the test models (e.g., cell types or lines, tissue models) and descriptive information (e.g., tissue origin, number of passages, karyotype features, doubling times, donor information, biomarkers) reported? Was the test model from a commercial source or an in-house culture? Was the model routinely used for the outcome of interest (e.g., Chinese hamster ovary cells for micronucleus formation)?		
High (score = 1)	The test model (e.g., cell types or lines, tissue models) and descriptive information (e.g., tissue origin, number of passages, karyotype features, doubling times, donor information, biomarkers) were reported, the test model was obtained from a commercial source or laboratory-maintained culture, and the test model was routinely used for the outcome of interest (e.g., Chinese hamster ovary cells for micronucleus formation).	

Confidence Level (Score)	Description	Selected Score
Medium (score = 2)	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. Reporting limitations are unlikely to have a substantial impact on results.	
Low (score = 3)	The test model was reported but no additional details were reported **AND/OR** the test model was not routinely used for the outcome of interest (e.g., feline cell line for micronucleus formation). This is likely to have a substantial impact on results.	
Unacceptable (score = 4)	The test model and descriptive information were not reported **OR** the test model was not appropriate for evaluation of the specific outcome of interest (e.g., bacterial reverse mutation assay to evaluate chromosome aberrations).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 15. Number per Group Was the number of organisms or tissues per study group and/or replicates per study group reported and appropriate for the study type and outcome analysis?		
High (score = 1)	The number of organisms or tissues per study group and/or number of replicates per study group were reported and were appropriate[a] for the study type and outcome analysis and consistent with studies of the same or similar type (e.g., at least two replicates/test substance or three different exposure times for *in vitro* skin corrosion test; three replicates/strain of bacteria in bacterial reverse mutation assay).	
Medium (score = 2)	The number of organisms or tissues per study group and/or replicates per study group were reported but were lower than the typical number used in studies of the same or similar type (e.g., three replicates/strain of bacteria in bacterial reverse mutation assay), but they were sufficient for analysis and unlikely to have a substantial impact on results.	
Low (score = 3)	The number of organisms or tissues per study group and/or replicates per study group were reported but were less than recommended by current standards and guidelines[a] (e.g., one tissue/test concentration/exposure time for *in vitro* skin corrosion test). This is likely to have a substantial impact on results.	
Unacceptable (score = 4)	The number of organisms or tissues per study group and/or replicates per study group were not reported **OR** the number of organisms or tissues per study group and/or replicates per study group were insufficient to characterize toxicological effects (e.g., one tissue/test concentration/one exposure time for *in vitro* skin corrosion test, one replicate/strain of bacteria exposed in bacterial reverse mutation assay).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Domain 5. Outcome Assessment		
Metric 16. Outcome Assessment Methodology Did the outcome assessment methodology address or report the intended outcome(s) of interest? Was the outcome assessment methodology (including endpoints and timing of assessment) sensitive for the outcome(s) of interest (e.g., measured endpoints that are able to detect a true effect)?		
High (score = 1)	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome(s) of interest.	
Medium (score = 2)	The outcome assessment methodology used only partially addressed or reported the intended outcomes(s) of interest (e.g., mutation frequency evaluated in the absence of cytotoxicity in a gene mutation test), but minor uncertainties are unlikely to have a substantial impact on results.	
Low (score = 3)	Significant deficiencies in the reported outcome assessment methodology were identified (e.g., optimum time for expression of chromosomal aberrations after exposure to test compound was not determined) **OR** due to incomplete reporting, it was unclear whether methods were sensitive for the outcome of interest. This is likely to have a substantial impact on results.	
Unacceptable (score = 4)	The outcome assessment methodology was not reported **OR** the assessment methodology was not appropriate for the outcome(s) of interest (e.g., cells were evaluated for chromosomal aberrations immediately after exposure to the test substance instead of after post-exposure incubation period).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 17. Consistency of Outcome Assessment Was the outcome assessment carried out consistently (i.e., using the same protocol) across study groups (e.g., assessment at the same time after initial exposure in all study groups)?		
High (score = 1)	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.	
Medium (score = 2)	There were minor differences in the timing of outcome assessment across study groups, or incomplete reporting of minor details of outcome assessment protocol execution, but these uncertainties or limitations are unlikely to have substantial impact on results.	
Low (score = 3)	Details regarding the execution of the study protocol for outcome assessment (e.g., timing of assessment across groups) were not reported, and these deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	There were large inconsistencies in the execution of study protocols for outcome assessment across study groups **OR** outcome assessments were not adequately reported for meaningful interpretation of results.	
Not rated/applicable		

Confidence Level (Score)	Description	Selected Score
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 18. Sampling Adequacy Was the reported sampling adequate for the outcome(s) of interest, including number of evaluations per exposure group and endpoint (e.g., number of replicates/slides/cells/metaphases evaluated per test concentration)?		
High (score = 1)	The study reported adequate sampling for the outcome(s) of interest, including number of evaluations per exposure group and endpoint (e.g., number of replicates/slides/cells/metaphases (at least 300 well-spread metaphases scored/concentration in a chromosome aberration test)).	
Medium (score = 2)	Details regarding sampling for the outcome(s) of interest were reported, but minor limitations were identified in the reported sampling of the outcome(s) of interest, but those are unlikely to have a substantial impact on results.	
Low (score = 3)	Details regarding sampling of outcomes were not fully reported, and the omissions are likely to have a substantial impact on results.	
Unacceptable (score = 4)	Reported sampling was not adequate for the outcome(s) of interest, and/or serious uncertainties or limitations were identified in how the study carried out the sampling of the outcome(s) of interest (e.g., replicates from control and test concentrations were evaluated at different times).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 19. Blinding of Assessors Were investigators assessing subjective outcomes (i.e., those evaluated using human judgment) blinded to treatment group? This metric is not rated/applicable if no subjective outcomes were assessed (i.e., only automated measurements were included and human judgment was not applied).		
High (score = 1)	The study explicitly reported that investigators assessing subjective outcomes (i.e., those evaluated using human judgment) were blinded to treatment group or that quality control/quality assurance methods were followed in the absence of blinding.	
Medium (score = 2)	The study reported that blinding was not possible, but steps were taken to minimize bias (e.g., knowledge of study group was restricted to personnel not assessing subjective outcome), and this minor uncertainty is unlikely to have a substantial impact on results.	
Low (score = 3)	The study did not report whether assessors were blinded to treatment group for subjective outcomes, and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Information in the study report suggested that the assessment of subjective outcomes was performed in a biased fashion (e.g., assessors of subjective outcomes were aware of study groups).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Domain 6. Confounding/Variable Control		
Metric 20. Confounding Variables in Test Design and Procedures Were there confounding differences among the study groups (in the strain/batch/lot number of organisms); models used (per group, size, and/or quality of tissues exposed); or lot of test substance used that could influence the outcome assessment?		
High (score = 1)	There were no differences reported among study group parameters (e.g., test substance lot or batch, strain/batch/lot number of organisms or models used per group or size, and/or quality of tissues exposed) that could influence the outcome assessment.	
Medium (score = 2)	Minor differences were reported in initial conditions that are unlikely to have a substantial impact on results (e.g., tissues from two different lots were used for *in vitro* skin corrosion test, and quality control data were similar for both lots).	
Low (score = 3)	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	There were significant differences among the study groups with respect to the strain/batch/lot number of organisms or models used per group or size and/or quality of tissues exposed (e.g., initial number of viable bacterial cells were different for each replicate (10^5 cells in replicate 1, 10^8 cell in replicate 2, and 10^3 cells in replicate 3), tissues from two different lots were used for *in vitro* skin corrosion test, but the control batch quality for one lot was outside of the acceptability range).	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 21. Confounding Variables in Outcomes Unrelated to Exposure Were there differences among the study groups unrelated to exposure to test substance (e.g., contamination) that could influence the outcome assessment? Did the test material interfere in the assay (e.g., altering fluorescence or absorbance, signal quenching by heavy metals, altering pH, solubility or stability issues)?		
High (score = 1)	There were no reported differences among the study replicates or groups in test model unrelated to exposure (e.g., contamination), and the test substance did not interfere with the assay (e.g., signal quenching by heavy metals).	
Medium (score = 2)	Authors reported that one or more replicates or groups experienced disproportionate outcomes unrelated to exposure (e.g., contamination), but data from the remaining exposure replicates or groups were valid and unlikely to have a substantial impact on results **OR** data on experienced disproportionate outcomes unrelated to exposure were not reported because only substantial differences among groups were noted (as indicated by study authors). **OR** the test material interfered in the assay, but the interference did not cause substantial differences among the groups.	
Low (score = 3)	Data on outcome differences unrelated to exposure were not reported for each study replicate or group. Assay interference was present or inferred, resulting in large variabilities among the groups. The absence of this information is likely to have a substantial impact on results.	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	One or more replicates or groups (i.e., negative and positive controls experienced disproportionate growth or reduction in growth unrelated to exposure (e.g., contamination), or assay interference occurred such that no outcomes could be assessed.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Domain 7. Data Presentation and Analysis		
Metric 22. Data Analysis Were statistical methods, calculations methods, and/or data manipulation clearly described and appropriate for dataset(s)?		
High (score = 1)	Statistical methods, calculation methods, and/or data manipulation were clearly described and presented for dataset(s) (e.g., frequencies of chromosomal aberrations were statistically analyzed across groups, trend test used to determine dose relationships, or results compared to historical negative control data). **OR** no statistical analyses, calculation methods, and/or data manipulation were conducted, but sufficient data were provided to conduct an independent statistical analysis.	
Medium (score = 2)	Statistical analysis was described with some omissions that would unlikely have a substantial impact on results.	
Low (score = 3)	Statistical analysis was not described clearly, and this deficiency is likely to have a substantial impact on results.	
Unacceptable (score = 4)	Statistical methods were not appropriate (e.g., Student's t-test used to compare two groups in a multi-group study, parametric test for non-normally distributed data) **OR** statistical analysis was not conducted **AND** data were not provided, preventing an independent statistical analysis.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 23. Data Interpretation Were the scoring and/or evaluation criteria reported and consistent with standards and guidelines?		
High (score = 1)	Study authors reported the scoring and/or evaluation criteria (e.g., for determining negative, positive, and equivocal outcomes) for the test and these were consistent with established practices.[a]	
Medium (score = 2)	Scoring and/or evaluation criteria were partially reported (e.g., evaluation criteria were reported following 3- and 60-minute exposures, but not for 240-minute exposure in *in vitro* skin corrosion test), but the omissions are unlikely to have a substantial impact on results.	
Low (score = 3)	Scoring and/or evaluation criteria were not reported, and the omissions are likely to have a substantial impact on interpretation of the results.	

Confidence Level (Score)	Description	Selected Score
Unacceptable (score = 4)	The reported scoring and/or evaluation criteria were inconsistent with established practices, resulting in the interpretation of data results that are seriously flawed.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 24. Cytotoxicity Data Were cytotoxicity endpoints defined, if necessitated by study type, and were methods for measuring cytotoxicity described and commonly used for assessment[a]?		
High (score = 1)	Study authors defined cytotoxicity endpoints (e.g., cell integrity, apoptosis, necrosis, color induction, cell viability, mitotic index), and the methods for measuring cytotoxicity were clearly described and commonly used for assessment.	
Medium (score = 2)	Cytotoxicity endpoints were defined and methods of measurement were partially reported, but the omissions are unlikely to have substantial impact on study results.	
Low (score = 3)	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported, and the omissions are likely to have a substantial impact on the study results.	
Unacceptable (score = 4)	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	
Metric 25. Reporting of Data Were the data for all outcomes presented? Were data reported by exposure group?		
High (score = 1)	Data for exposure-related findings were presented for all outcomes by exposure group. Negative findings were reported qualitatively or quantitatively.	
Medium (score = 2)	Data for exposure-related findings were reported for most, but not all, outcomes by exposure group (e.g., sensitization percentages reported in the absence of incidence data). The minor uncertainties in outcome reporting are unlikely to have substantial impact on results.	
Low (score = 3)	Data for exposure-related findings were not shown for each study group, but results were described in the text and/or data were only reported for some outcomes. These deficiencies are likely to have a substantial impact on results.	
Unacceptable (score = 4)	Data presentation was inadequate (e.g., the report did not differentiate among findings in multiple exposure groups, no scores or frequencies were reported), or major inconsistencies were present in reporting of results.	
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Confidence Level (Score)	Description	Selected Score
Domain 8. Other (Apply as Needed)		
Metric:		
High (score = 1)		
Medium (score = 2)		
Low (score = 3)		
Unacceptable (score = 4)		
Not rated/applicable		
Reviewer's comments	*(Document concerns, uncertainties, limitations, and deficiencies and any additional comments that may highlight study strengths or important elements such as relevance)*	

Note:
[a]For comparison purposes, current standards and guidelines may be reviewed at http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788; https://www.epa.gov/test-guidelines-pesticides-and-toxic-substances; https://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/IngredientsAdditivesGRASPackaging/ucm2006826.htm#TOC.

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Data Evaluation and Scoring Tables

The data evaluation and scoring tables are provided in the subsequent pages for the studies supporting the development of toxicity values for HFPO dimer acid and dimer acid ammonium salt (CASRN 13252-13-6 and CASRN 62037-80-3).

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-17751-1579 RV1: E.I. du Pont de Nemours and Company (2009). Cross-Species Comparison of FRD-902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Test Guideline Not Identified. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: December 8, 2008; Report Revision 1 Completed: February 2, 2009), Newark, Delaware.					
Note:	This study is evaluated in 2 sheets; the table below is for the monkey study. The primate (monkey) portion had additional information on the study protocol attached as Appendix A. The rat portion did not have the protocol information attached.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH		1	2	2
	2. Test substance source	HIGH	Attached a certificate of analysis from DuPont Labs.	1	1	1
	3. Test substance purity	MEDIUM	82.60%	2	1	2
Test Setup	4. Negative controls	Not rated	Not typically used in TK studies.	0	N/A	N/A
	5. Negative control responses	Not rated	Not typically used in TK studies.	0	N/A	N/A
	6. Positive controls	Not rated	Not typical in TK studies.	0	N/A	N/A
	7. Randomized allocation	MEDIUM	The protocol in Appendix A stated "no randomization necessary" for primate study. The animals were within +/- 20% of mean weight of the group, as per OECD TG 417, and all described as healthy so do not expect this has substantial impact on results.	2	1	2
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Prepared before administration of single dose.	1	1	1
	9. Consistency of exposure administration	HIGH		1	1	1
	10. Reporting of doses/ concentrations	HIGH	Administered as i.v. dose.	1	2	2
	11. Exposure frequency and duration	Not rated	A single dose.	0	N/A	N/A

Exposure Characterization (continued)	12. Number of exposure groups and dose spacing	HIGH	Used a single dose group for primates; typical in a TK study.	1	1	1
	13. Exposure Route and Method	HIGH	i.v. is relevant for this study to look at ADME.	1	1	1
Test Organisms	14. Test Animal Characteristics	HIGH	Adequate for primates.	1	2	2
	15. Consistency of Animal Conditions	HIGH	Adequate reporting for primates in the protocol (Appendix A).	1	1	1
	16. Number per Group	HIGH	3 animals per sex were used. As per OCED 417: "the use of both sexes (four males and four females) is strongly recommended", however 3 animals are typical and sufficient for the statistical analysis used, therefore unlikely to have a substantial impact on results.	1	1	1
Outcome Assessment	17. Outcome Assessment Methodology	HIGH	The measurement of parent chemical in blood was described (metabolite measurement not needed based on earlier in vitro study). Analytical method high-performance liquid chromatography adequately described. Level of quantification (LOQ) documented.	1	2	2
	18. Consistency of outcome assessment	HIGH		1	1	1
	19. Sampling adequacy	HIGH	Time course data for blood were presented. Sampling continued sufficiently beyond the time blood concentration was below LOQ.	1	1	1
	20. Blinding of assessors	Not rated	The outcome (blood concentration) is not subjective. Blinding is not typical for a TK study.	0	N/A	N/A

Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	BWs were within +/- 20%, i.v. administration so no palatability issues. No reported differences in respiratory rate. Note concentrations were detected > LOQ at time zero, suggesting some other exposure prior to dosing, or possibly contamination in the method.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	Health outcomes for primates were reported and these effects are not expected to impact the results of the TK study.	1	1	1
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Results of minimal statistical analysis were provided, but the method was not described. The raw data are provided.	2	1	2
	24. Reporting of data	HIGH	All raw data provided.	1	2	2

Sum of scores:		25	28
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.	
Study Reference:	DuPont-17751-1579 RV1: E.I. du Pont de Nemours and Company (2009). Cross-Species Comparison of FRD- 902 Plasma Pharmacokinetics in the Rat and Primate Following Intravenous Dosing. Test Guideline Not Identified. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: December 8, 2008; Report Revision 1 Completed: February 2, 2009), Newark, Delaware.
Note:	This study is evaluated in 2 sheets; the table below is for the rat study. The primate (monkey) portion had additional information on the study protocol attached as Appendix A. The rat portion did not have the protocol information attached.
Number of Hours for Review:	1

Domain	Metric	Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)	Comments	Metric Score	Metric Weighting Factor	Weighted Score
Test Substance	1. Test substance identity	HIGH		1	2	2
	2. Test substance source	HIGH	Attached a certificate of analysis from DuPont Labs.	1	1	1
	3. Test substance purity	MEDIUM	82.6%	2	1	2
Test Setup	4. Negative controls	Not rated	Not typically used in TK studies.	0	N/A	N/A
	5. Negative control responses	Not rated	Not typically used in TK studies.	0	N/A	N/A
	6. Positive controls	Not rated	Not typical in TK studies.	0	N/A	N/A
	7. Randomized allocation	MEDIUM	Not reported. Rat BWs were not given. Expect only healthy animals of similar age were used, so not expecting this has substantial impact on results.	2	1	2
Exposure	8. Preparation and storage of test substance	HIGH	Prepared before administration of single dose.	1	1	1
	9. Consistency of exposure administration	HIGH		1	1	1
	10. Reporting of doses/ concentrations	HIGH	Administered as i.v. dose.	1	2	2
	11. Exposure frequency and duration	Not rated	A single dose.	0	N/A	N/A
	12. Number of exposure groups and dose spacing	HIGH	Used two dose groups, reasonable dose levels.	1	1	1
	13. Exposure route and method	HIGH	i.v. is relevant for this study to look at ADME	1	1	1

Test Organisms	14. Test animal characteristics	MEDIUM	The sex and strain are appropriate and from the in-house colony. Health status, age, BW not reported. Do not expect this will have substantial impact on the outcomes.	2	2	4
	15. Consistency of animal conditions	MEDIUM	Not reported, but expect they were similar to conditions reported for primates and not likely to impact outcomes.	2	1	2
	16. Number per group	HIGH	3 animals per sex were used. As per OCED 417: "the use of both sexes (four males and four females) is strongly recommended", however 3 animals are typical and sufficient for the statistical analysis used, therefore unlikely to have a substantial impact on results.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	The measurement of parent chemical in blood was described (metabolite measurement not needed based on earlier in vitro study). Analytical method high-performance liquid chromatography adequately described. Level of quantification (LOQ) documented.	1	2	2
	18. Consistency of outcome assessment	HIGH		1	1	1
	19. Sampling adequacy	HIGH	Time course data for blood were presented. Sampling continued sufficiently beyond the time blood concentration was below LOQ.	1	1	1
	20. Blinding of assessors	Not rated	The outcome (blood concentration) is not subjective. Blinding is probably not typical for a TK study.	0	N/A	N/A
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	MEDIUM	BWs, health status, respiratory rates not reported; i.v. administration so no palatability issues. Note concentrations were detected > LOQ at time zero, suggesting some other exposure prior to dosing, or possibly contamination in the method.	2	2	4

	22. Health outcomes unrelated to exposure	MEDIUM	Not reported. Based on dose levels and known effects from acute single dose tox studies health effects are not expected to occur that would impact the results of the TK study.	2	1	2
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Results of minimal statistical analysis were provided, but the method was not described. The raw data are provided.	2	1	2
	24. Reporting of data	HIGH	All raw data provided.	1	2	2

Sum of scores:		25	34
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.4		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-18405-1017 RV1: E.I. du Pont de Nemours and Company (2011). H-28548: Absorption, Distribution, Metabolism, and Elimination in the Rat. US EPA OPPTS 870.7485. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: November 3, 2010; Report Revision 1 Completed: April 21, 2011), Newark, Delaware and Wilmington, Delaware.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Addressed on page 42 / 61.	1	2	2
	2. Test substance source	HIGH	Addressed on page 11 / 61.	1	1	1
	3. Test substance purity	MEDIUM	Addressed on page 42 / 61. (84%)	2	1	2
Test Setup	4. Negative controls	Not Rated	Not applicable to this study design.	0	N/A	N/A
	5. Positive controls	Not Rated	Not applicable to this study design.	0	N/A	N/A
	6. Assay procedures	HIGH	Addressed on page 13 / 61.	1	1	1
	7. Standards for test	HIGH	Addressed on page 15 / 61.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Addressed on page 15 / 61.	1	1	1
	9. Consistency of exposure administration	Not Rated	Not applicable to this study design.	0	N/A	N/A
	10. Reporting of doses/ concentrations	HIGH	Addressed on page 12 / 61	1	2	2
	11. Exposure duration	Not Rated	Not applicable to this study design.	0	N/A	N/A
	12. Number of exposure groups and dose spacing	Not Rated	Not applicable to this study design.	0	N/A	N/A
	13. Metabolic activation	Not Rated	Not applicable to this study design.	0	N/A	N/A
Test Model	14. Test model	Not Rated	Not applicable to this study design.	0	N/A	N/A
	15. Number per group	HIGH	Addressed on page 9 / 61.	1	1	1

Outcome Assessment	16. Outcome assessment methodology	HIGH	Addressed on page 14 / 61.	1	2	2
	17. Consistency of outcome assessment	HIGH	Addressed on page 14 / 61.	1	1	1
	18. Sampling adequacy	HIGH	Addressed on page 14 / 61.	1	2	2
	19. Blinding of assessors	Not Rated	Not applicable to this study design.	0	N/A	N/A
Confounding/ Variable Control	20. Confounding variables in test setup and procedures	HIGH	Addressed on page 11 / 61.	1	2	2
	21. Outcomes unrelated to exposure	Not Rated	Not applicable to this study design.	0	N/A	N/A
Data Presentation and Analysis	22. Data analysis	HIGH	Addressed on page 21 / 61.	1	1	1
	23. Data interpretation	HIGH	Addressed on page 22 / 61.	1	2	2
	24. Cytotoxicity data	Not Rated	Not applicable to this study design.	0	N/A	N/A
	25. Reporting of data	HIGH	Individual data provided for all test animals.	1	2	2

Sum of scores:		22	23
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.0		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-18647-1017 RV1: E.I. du Pont de Nemours and Company (2011). H-28548: Absorption, Distribution, Metabolism, and Elimination in the Mouse. US EPA OPPTS 870.7485. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: November 3, 2010; Report Revision 1 Completed: April 21, 2011), Newark, Delaware and Wilmington, Delaware.					
Number of Hours for Review:	2					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH		1	2	2
	2. Test substance source	HIGH		1	1	1
	3. Test substance purity	HIGH	84% adjusted for purity and CoA attached.	1	1	1
Test Setup	4. Negative controls	Not rated	Not typically used in TK studies. Note concentrations were detected > level of quantification (LOQ) at time zero, suggesting some other exposure prior to dosing, or possibly contamination in the method (although very low concentrations)?	0	N/A	N/A
	5. Negative control responses	Not rated	Not typically used in TK studies.	0	N/A	N/A
	6. Positive controls	Not rated	Not typical in TK studies.	0	N/A	N/A
	7. Randomized allocation	HIGH	Adequately described.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH		1	1	1
	9. Consistency of exposure administration	HIGH		1	1	1
	10. Reporting of doses/ concentrations	HIGH		1	2	2
	11. Exposure frequency and duration	HIGH	Used a single dose; appropriate for study type.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Used a single dose group; typical in a TK study.	1	1	1
	13. Exposure route and method	HIGH	Oral gavage is relevant and typical for TK studies.	1	1	1

Test Organisms	14. Test animal characteristics	HIGH		1	2	2
	15. Consistency of animal conditions	HIGH		1	1	1
	16. Number per group	HIGH	5 per group were used; this is adequate as described in OECD TG417.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	The measurement of parent chemical in blood was described (metabolite measurement not needed based on earlier *in vitro* study indicating no metabolism). Analytical method high-performance liquid chromatography adequately described. LOQ documented.	1	2	2
	18. Consistency of outcome assessment	HIGH		1	1	1
	19. Sampling adequacy	HIGH	Time course data for blood were analyzed and sampling continued sufficiently beyond the time blood concentration was below LOQ.	1	1	1
	20. Blinding of assessors	Not rated	The outcome is a blood concentration; it is not subjective. Blinding is probably not typical for a TK study.	0	N/A	N/A
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	BWs were within +/- 20%, oral gavage so no palatability issues. No reported differences in respiratory rate.	1	2	2
	22. Health outcomes unrelated to exposure	MEDIUM	Health outcomes weren't reported. However, we don't expect health effects from a single dose of this amount (based on prior acute studies) so differences in health of the animals are not expected to impact the results of the TK study.	2	1	2
Data Presentation and Analysis	23. Statistical methods	HIGH	Results of statistical analysis and method of calculation were provided. The raw data are also provided for re- analysis or further analysis if needed.	1	1	1
	24. Reporting of data	HIGH	All raw data were provided.	1	2	2

Sum of scores:		26	27
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.0		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-18405-849 RV1. E.I. du Pont de Nemours and Company (2011). H-28548: Toxicokinetic Study in Pregnant Rats. Test Guideline Not Identified. Study conducted by E.I. du Pont de Nemours and Company (Original Report Completed: March 29, 2011; Report Revision 1 Completed: April 11, 2011), Newark, Delaware.					
Number of Hours for Review:	0.75					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Identity of the substance was identified definitively. See page 8 of the report.	1	2	2
	2. Test substance source	HIGH	Test substance was supplied by sponsor. Batch number is H-28548.	1	1	1
	3. Test substance purity	MEDIUM	Purity of the substance was 84%. There is a certificate of analysis that reports other components (water, 12.7%) and perfluorooctanoic acid (150 ppm).	2	1	2
Test Setup	4. Negative controls	MEDIUM	Used deionized water as the negative control. Also they tested the water samples for presence of contaminants (e.g., total bacterial counts, coliforms, lead).	2	2	4
	5. Negative control responses	HIGH		1	1	1
	6. Positive controls	Not rated	The study did not include a positive control. It was not necessary.	0	N/A	N/A
	7. Randomized allocation	HIGH	The study report indicated that the investigators used a computerized randomization procedure to produce homogeneous distribution of BWs across groups within each breeding lot.	1	1	1

Exposure Characterization	8. Preparation and storage of test substance	HIGH	Study characterized test preparation and stability after preparation; said this demonstrated that the test substance was stable at room temperature for up to 12 days.	1	1	1
	9. Consistency of exposure administration	HIGH	Exposure consistently administered. Administered same volume based on BW.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Reported doses (control plus 5 doses).	1	2	2
	11. Exposure frequency and duration	HIGH	Rats exposed daily by oral gavage on GD6 to 20.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Investigators included justification for selection of dose levels.	1	1	1
	13. Exposure route and method	HIGH	Investigators included justification for selection of route of administration.	1	1	1
Test Organisms	14. Test animal characteristics	HIGH	Provided information about test model (rat strain, sex, gestational day at arrival, age at arrival, age at start of study, weight at arrival). Study also reported justification for animal model.	1	2	2
	15. Consistency of animal conditions	HIGH	Animal husbandry conditions were reported (section F of report).	1	1	1
	16. Number per group	HIGH	5 animals per group; above OECD TG 417 recommended minimum of 4 animals per group.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Study report describes the methodology used to conduct the in- life and post-mortem observations.	1	2	2
	18. Consistency of outcome assessment	HIGH	In life and post-mortem observations were carried out consistently.	1	1	1
	19. Sampling adequacy	HIGH	Sampling frequency was reported for mortality/moribundity, clinical observations, BWs, food consumption, blood collection.	1	1	1
	20. Blinding of assessors	MEDIUM	Clinical observations fall within the definition of subjective outcomes. However, the study did not discuss blinding prior to recording clinical observations.	2	1	2

Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	Based on study summary and data tables.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	Based on study summary and data tables.	1	1	1
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Study reported using descriptive statistics for the endpoints, but the description was very brief.	2	1	2
	24. Reporting of data	HIGH	Very well documented study. Data reported in appendices.	1	2	2

Sum of scores:		30	35
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.2		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Gannon, SA; Fasano, WJ; Mawn, MP; Nabb, DL; Buck, RC; Buxton, LW; Jepson, GW; Frame, SR. (2016). Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. Toxicology 340: 1-9. http://dx.doi.org/10.1016/j.tox.2015.12.006					
Note:	This study was reviewed in three parts. The table below pertains to the rat and monkey i.v. dosing section of the study.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Definitive; Chemical Name and CASRN.	1	2	2
	2. Test substance source	HIGH	Reported; from DuPont Chemicals and Fluoro products.	1	1	1
	3. Test substance purity	HIGH	Purity of the substance was 99.4%.	1	1	1
Test Setup	4. Negative controls	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	5. Negative control responses	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	6. Positive controls	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	7. Randomized allocation	MEDIUM	Not indicated.	2	1	2
Exposure Characterization	8. Preparation and storage of test substance	LOW	Not reported in journal article; EPA confirmed it is reported in full Study Summary.	3	1	3
	9. Consistency of exposure administration	HIGH	Exposure consistently administered across dose groups and species based on BW.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Reported doses (1 dose).	1	2	2
	11. Exposure frequency and duration	HIGH	Rats & monkeys exposed ONCE; PK Study.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Reported; 6 rats: 3 of each sex per dose; 6 monkeys: 3 of each sex per dose group.	1	1	1
	13. Exposure route and method	MEDIUM	Reported; IV; via tail vein in rat and via peripheral vein in monkey.	2	1	2

Test Organisms	14. Test animal characteristics	HIGH	Provided species, strain, sex.	1	2	2
	15. Consistency of animal conditions	HIGH	Consistent; cited DuPont Haskell Global Center principles, and AAALAC accreditation.	1	1	1
	16. Number per group	HIGH	3 males + 3 females in rat study per dose (1); 3 males + 3 females in monkey study per dose (1).	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Study describes in detail the methodology (sampling times/frequency) and analytical (high-performance liquid chromatography-mass spectrometry) methods.	1	2	2
	18. Consistency of outcome assessment	HIGH	Consistent across sexes within species and across species in sampling times/methods.	1	1	1
	19. Sampling adequacy	HIGH	Adequate; serum concentrations leveled off at last several time points.	1	1	1
	20. Blinding of assessors	Not rated	Observations are analytically determined; not subjective.	0	N/A	N/A
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	MEDIUM	Limit of detection in IV test is lower (better) than in oral test (see other review); hence, care should be taken in comparing rat oral vs either species IV results.	2	2	4
	22. Health outcomes unrelated to exposure	HIGH	None.	1	1	1
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Described; data tables and graphical representations show inputs and results, respectively. Goodness of fit results not provided in paper.	2	1	2
	24. Reporting of data	MEDIUM	Typical of journal article; results of calculations and graphs provided in article, but underlying data not available for recalculation or modeling. Although paper indicates supplementary data are available on-line, it is simply 3 additional graphs; NOT DATA.	2	2	4

Sum of scores:		26	35
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.4		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Gannon, SA; Fasano, WJ; Mawn, MP; Nabb, DL; Buck, RC; Buxton, LW; Jepson, GW; Frame, SR. (2016). Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. *Toxicology* 340:1-9. http://dx.doi.org/10.1016/j.tox.2015.12.006					
Note:	This study was reviewed in three parts. The table below pertains to the rat and mouse PK section of the study.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Definitive; Chemical Name and CASRN.	1	2	2
	2. Test substance source	HIGH	Reported; from DuPont Chemicals and Fluoro products.	1	1	1
	3. Test substance purity	HIGH	Purity of the substance was 99.4%.	1	1	1
Test Setup	4. Negative controls	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	5. Negative control responses	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	6. Positive controls	Not rated	PK studies - measuring ADME of SUBSTANCE.	0	N/A	N/A
	7. Randomized allocation	MEDIUM	Not indicated.	2	1	2
Exposure Characterization	8. Preparation and storage of test substance	LOW	Not reported in journal article; EPA confirmed it is reported in full Study Summary.	3	1	3
	9. Consistency of exposure administration	HIGH	Exposure consistently administered across dose groups and species based on BW.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Reported doses (2 doses).	1	2	2
	11. Exposure frequency and duration	HIGH	Rats & mice exposed ONCE; PK Study.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Reported; 3 of each sex per dose; MICE: 45 of each sex per dose group.	1	1	1
	13. Exposure route and method	MEDIUM	Reported; oral; method not specified (gavage is usual).	2	1	2

Test Organisms	14. Test animal characteristics	HIGH	Provided species, strain, sex.	1	2	2
	15. Consistency of animal conditions	HIGH	Consistent; cited DuPont Haskell Global Center principles, and AAALAC accreditation.	1	1	1
	16. Number per group	HIGH	3 males + 3 females in rat study per dose (2); 45 males + 45 females in mouse study per dose (2).	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Study describes in detail the methodology (sampling times / frequency) and analytical (high-performance liquid chromatography-mass spectrometry) methods.	1	2	2
	18. Consistency of outcome assessment	HIGH	Consistent across sexes within species and across species in sampling times/methods.	1	1	1
	19. Sampling adequacy	HIGH	Adequate; serum concentrations leveled off at last 2 time points.	1	1	1
	20. Blinding of assessors	Not rated	Observations are analytically determined; not subjective.	0	N/A	N/A
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	MEDIUM	Limit of detection in oral test different than in IV test (see other review); hence, care should be taken in comparing rat oral vs IV; however, absorption and alpha elimination phases appears to be well above LOD; caution comparing beta elimination values...differences could be LOD related.	2	2	4
	22. Health outcomes unrelated to exposure	HIGH	None.	1	1	1
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Described; data tables and graphical representations show inputs and results, respectively. Goodness of fit results not provided in paper.	2	1	2
	24. Reporting of data	MEDIUM	Typical of journal article; results of calculations and graphs provided in article, but underlying data not available for recalculation or modeling. Although paper indicates supplementary data are available on- line, it is simply 3 additional graphs; NOT DATA. EPA able to review full study summaries.	2	2	4

Sum of scores:		26	35
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.3		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Gannon, SA; Fasano, WJ; Mawn, MP; Nabb, DL; Buck, RC; Buxton, LW; Jepson, GW; Frame, SR. (2016). Absorption, distribution, metabolism, excretion, and kinetics of 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt following a single dose in rat, mouse, and cynomolgus monkey. *Toxicology* 340:1-9. http://dx.doi.org/10.1016/j.tox.2015.12.006					
Note:	This study was reviewed in three parts. The table below pertains to the *in vitro* section of the study.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Definitive; Chemical Name and CASRN.	1	2	2
	2. Test substance source	HIGH	Reported; from DuPont Chemicals and Fluoroproducts.	1	1	1
	3. Test substance purity	HIGH	Purity of the substance was 99.4%.	1	1	1
Test Setup	4. Negative controls	HIGH	Heat-inactivated hepatocytes from same prep as those used for metabolism study.	1	2	2
	5. Positive controls	HIGH	Included solvent only control (zero dose).	1	2	2
	6. Assay procedures	HIGH	Non-standard/guideline, but straight forward test.	1	1	1
	7. Standards for test	N/A	Non-standard/guideline test.	0	N/A	N/A
Exposure Characterization	8. Preparation and storage of test substance	LOW	Not reported in journal article; EPA confirmed it is reported in full Study Summary.	3	1	3
	9. Consistency of exposure administration	HIGH	Consistent; all dose with same stock soln; different dilutions.	1	1	1
	10. Reporting of concentrations	HIGH	1 conc for clearance expt; 1 conc for metabolite ID.	1	2	2
	11. Exposure duration	HIGH	Reported; 6 sample points at different durations.	1	2	2
	12. Number of exposure groups and dose spacing	MEDIUM	1 exposure group is fine given this is a screen for in vivo expts; no rationale for concentrations used was provided.	2	1	2
	13. Metabolic activation	HIGH	This was the point of the experiment.	1	1	1
Test Model	14. Test model	HIGH	Primary isolated hepatocytes.	1	2	2
	15. Number per group	HIGH	Multiple time points is the 'group'; used 6.	1	1	1

Outcome Assessment	16. Outcome assessment methodology	HIGH	Study describes in detail the methodology (sampling times/frequency) and analytical (high-performance liquid chromatography-mass spectrometry) methods.	1	2	2
	17. Consistency of outcome assessment	HIGH	There was no metabolism or metabolites in any treatment; Very consistent.	1	1	1
	18. Sampling adequacy	HIGH	6 timepoints.	1	2	2
	19. Blinding of assessors	N/A	Not applicable.	0	N/A	N/A
Confounding/ Variable Control	20. Confounding variables in test setup and procedures	none	None.	0	N/A	N/A
	21. Outcomes unrelated to exposure	HIGH	No aberrant measures at any timepoint reported.	1	1	1
Data Presentation and Analysis	22. Data analysis	N/A	Does not appear to have needed any 'analysis'; the results were straight negative.	0	N/A	N/A
	23. Data interpretation	HIGH		1	2	2
	24. Cytotoxicity data	N/A		0	N/A	N/A
	25. Reporting of data	LOW	NEGATIVE RESULTS: Neither clearance rates nor any chromatograms from metabolite were shown/provided.	3	2	6

Sum of scores:		30	37
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.2		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Sheng, N; Cuir, R; Wang, J; Guo, Y; Wang, J; Dai, J. 2018. Cytotoxicity of novel fluorinated alternatives to long-chain perfluoroalkyl substances to human liver cell line and their binding capacity to human liver fatty acid-binding protein. Arch Toxicol 92:359-369. http://dx.doi.org/10.1007/s00204-017-2055-1					
Number of Hours for Review:	5 minutes					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	Unacceptable	Not from manufacturer and no analysis; no reference to a supplemental file. This designation as "unacceptable" means the remainder of the domains/metrics will not be reviewed/scored.	4	N/A	N/A
	2. Test substance source					
	3. Test substance purity					
Test Setup	4. Negative controls					
	5. Negative control responses					
	6. Positive controls					
	7. Randomized allocation					
Exposure Characterization	8. Preparation and storage of test substance					
	9. Consistency of exposure administration					
	10. Reporting of doses/ concentrations					
	11. Exposure frequency and duration					
	12. Number of exposure groups and dose spacing					
	13. Exposure route and method					

Test Organisms	14. Test animal characteristics					
	15. Consistency of animal conditions					
	16. Number per group					
Outcome Assessment	17. Outcome assessment methodology					
	18. Consistency of outcome assessment					
	19. Sampling adequacy					
	20. Blinding of assessors					
Confounding/ Variable Control	21. Confounding variables in test setup and procedures					
	22. Health outcomes unrelated to exposure					
Data Presentation and Analysis	23. Statistical methods					
	24. Reporting of data					

Sum of scores:		N/A	N/A
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	N/A		
Overall Quality Level:	UNACCEPTABLE		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Wang, J; Wang, X; Sheng, N; Zhou, X, Cui, R; Zhang, H; Dai, J. 2016. RNA-sequencing analysis reveals the hepatotoxic mechanism of perfluoroalkyl alternatives, HFPO2 and HFPO4, following exposure in mice. *Journal of Applied Toxicology* 37:436-444. https://doi.org/10.1002/jat.3376					
Number of Hours for Review:	0.25					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	CAS RN	1	2	2
	2. Test substance source	UNACCEPTABLE	Test materials were synthesized in the author's lab. However, no information on the synthetic process, certificate of analysis, etc., were included to verify the identity of the test materials. The designation of this metric as "unacceptable" means the remaining domains/metrics are not reviewed/scored.	4	N/A	N/A
	3. Test substance purity					
Test Setup	4. Negative controls					
	5. Positive controls					
	6. Assay procedures					
	7. Standards for test					
Exposure Characterization	8. Preparation and storage of test substance					
	9. Consistency of exposure administration					
	10. Reporting of concentrations					
	11. Exposure duration					
	12. Number of exposure groups and dose spacing					
	13. Metabolic activation					
Test Model	14. Test model					
	15. Number per group					

Outcome Assessment	16. Outcome assessment methodology					
	17. Consistency of outcome assessment					
	18. Sampling adequacy					
	19. Blinding of assessors					
Confounding/ Variable Control	20. Confounding variables in test setup and procedures					
	21. Outcomes unrelated to exposure					
Data Presentation and Analysis	22. Data analysis					
	23. Data interpretation					
	24. Cytotoxicity data					
	25. Reporting of data					

Sum of scores:		N/A	N/A
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	4		
Overall Quality Level:	UNACCEPTABLE		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	Rushing, B; Hu, Q; Franklin, J; McMahen, R; Dagnio, Sonia; Higgins, Christopher; Strynar, M; DeWitt, J. 2017. Evaluation of the Immunomodulatory Effects of 2,3,3,3-Tetrafluoro-2-(Heptafluoropropoxy)- Propanoate in C57BL/6 Mice. *Toxicological Sciences* 156:179-189. https://doi.org/10.1093/toxsci/kfw251					
Number of Hours for Review:	1.5					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	MEDIUM	CAS RN provided. Batch/lot number not provided; certificate of analysis by manufacturer not indicated.	2	2	4
	2. Test substance source	HIGH	Manufacturer reported.	1	1	1
	3. Test substance purity	LOW	Purity and/or grade not reported.	3	1	3
Test Setup	4. Negative controls	HIGH	Addressed in Materials and Methods section.	1	2	2
	5. Negative control responses	HIGH	Addressed in Results section.	1	1	1
	6. Positive controls	Not rated	Not relevant to this study type.	0	N/A	N/A
	7. Randomized allocation	HIGH	Addressed in Materials and Methods section.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	MEDIUM	Preparation specified but not storage.	2	1	2
	9. Consistency of exposure administration	HIGH		1	1	1
	10. Reporting of doses/ concentrations	HIGH		1	2	2
	11. Exposure frequency and duration	HIGH		1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Only limited information on dose selection provided in report.	1	1	1
	13. Exposure route and method	HIGH		1	1	1
Test Organisms	14. Test animal characteristics	HIGH		1	2	2
	15. Consistency of animal conditions	HIGH		1	1	1
	16. Number per group	HIGH		1	1	1

Outcome Assessment	17. Outcome assessment methodology	HIGH		1	2	2
	18. Consistency of outcome assessment	HIGH		1	1	1
	19. Sampling adequacy	HIGH		1	1	1
	20. Blinding of assessors	Not rated		0	N/A	N/A
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH		1	2	2
	22. Health outcomes unrelated to exposure	LOW	Not reported.	3	1	3
Data Presentation and Analysis	23. Statistical methods	HIGH		1	1	1
	24. Reporting or data	HIGH		1	2	2

Sum of scores:		29	36
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.2		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-24447 (4 Volumes contained in 4 separate PDF files): E.I. du Pont de Nemours and Company (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Rats with a 28-Day Recovery. OECD TG 407. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: August 22, 2008), Ashland, Ohio.					
Number of Hours for Review:	1.5					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance	HIGH	Definitive-Pg 15; Analyses in Appendix C (in Volume 4 of 4).	1	2	2
	2. Test substance source	HIGH	Test substance, H-28397, was from E.I. duPont Nemours and Company, Newark, NJ (Pg 21).	1	1	1
	3. Test substance purity	MEDIUM	Purity of the substance was 88%. There is a certificate of analysis that reports other components (water, 13.3%) and perfluorooctanoic acid (3.4 ppm).	2	1	2
Test Setup	4. Negative control	HIGH	Used deionized water as the negative control. Tested the water samples for presence of biological contaminants (e.g., total bacterial counts, coliforms, lead).	1	2	2
	5. Negative control responses	HIGH		1	1	1
	6. Positive control	Not rated	The study did not include a positive control. It was not necessary.	0	N/A	N/A
	7. Randomized allocation	HIGH	The study report indicated that the investigators used a computerized randomization procedure to produce homogeneous distribution of BWs across groups within each breeding lot.	1	1	1

Exposure Characterization	8. Preparation and storage of test substance	HIGH	Study characterized test preparation and measured homogeneity and stability after preparation. Test substance was stable at room temperature for 5 hours and refrigerated for up to 10 days.	1	1	1
	9. Consistency of exposure administration	HIGH	Exposure consistently administered; adjusted based on BW. Due to lower than nominal dosing solutions, volume administered was increased for all groups from study day 0-24 (males) or study day 0-23 (females). The increased volume (from 10 µL to 12 µL) did NOT exceed the 2 mL/100 g limit designated in TG 407.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Reported doses (control plus 3 doses).	1	2	2
	11. Exposure frequency and duration	HIGH	Rats exposed daily by oral gavage.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Reported; as per TG407; 10X between doses OK as per TG407.	1	1	1
	13. Exposure route and method	HIGH	Reported; as per TG407.	1	1	1
Test Organisms	14. Test animal characteristics	HIGH	Provided information about test model (rat strain, sex, gestational day at arrival, age at arrival, age at start of study, weight at arrival). Study also reported justification for animal model; as per TG407.	1	2	2
	15. Consistency of animal conditions	HIGH	Consistent; as per TG407 and described in Sections D, E, F and G of Final Report (Volume 1 or 4) and associated appendices.	1	1	1
	16. Number per group	HIGH	20 males + 20 females per vehicle control and high dose groups (exceeds TG407) and 10 males +5 animals per group.	1	1	1

Outcome Assessment	17. Outcome assessment methodology	HIGH	Study report describes the in detail the methodology used to conduct the in-life and post-mortem observations. Outcome measurements meet or exceed all requirements of TG 407.	1	2	2
	18. Consistency of outcome assessment	HIGH	Consistent; as per TG 407.	1	1	1
	19. Sampling adequacy	HIGH	Adequate; as per TG 407.	1	1	1
	20. Blinding of assessors	MEDIUM	Clinical observations fall within the definition of subjective outcomes. The study did not discuss blinding; however, internationally accepted TGs do not require (preferable) this,	2	1	2
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	No confounding variables; study followed TG 407 without exception.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	None	1	1	1
Data Presentation and Analysis	23. Statistical methods	HIGH	Described in Section G of Final Report (Volume 1 of 4). Statistical tests were selected during the design of the study (as per TG 407) in ''Protocol'' found in Appendix I (Volume 4 of 4).	2	1	2
	24. Reporting of data	HIGH	Very well documented study. Summary provide in Final Report (Volume 1 of 4). Individual data provided as per TG 407 - found in Appendices.	1	2	2

Sum of scores:		30	33
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-24459. E.I. du Pont de Nemours and Company (2008). A 28-Day Oral (Gavage) Toxicity Study of H-28397 in Mice with a 28-Day Recovery. OECD Guideline 407. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: August 29, 2008), Ashland, Ohio.					
Number of Hours for Review:	1.25					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Identity of the substance was identified definitively. See page 14 of the report.	1	2	2
	2. Test substance source	HIGH	Test substance was supplied by sponsor. Batch number is H-28397.	1	1	1
	3. Test substance purity	MEDIUM	Purity of the substance was 88%. Test sample had other components (water, 13.3%) and perfluorooctanoic acid (3.4 ppm). Certificate of analysis was provided.	2	1	2
Test Setup	4. Negative controls	MEDIUM	Used deionized water as the negative control. The certificate of analysis indicates that the formulation contains 3.4 ppm of perfluorooctanoic acid as "other components".	2	2	4
	5. Negative control responses	HIGH	Within normal ranges.	1	1	1
	6. Positive controls	Not rated	The study did not include a positive control. It was not necessary.	0	N/A	N/A
	7. Randomized allocation	HIGH	The study report indicated that the investigators used a computerized randomization procedure. (See Section H.)	1	1	1

Exposure Characterization	8. Preparation and storage of test substance	HIGH	Study characterized test preparation. Appendix C has details about the analyses of dosing formulations.	1	1	1
	9. Consistency of exposure administration	HIGH	Exposure consistently administered. Administered same volume based on BW.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Reported doses (control plus 3 doses) as recommended by OECD TG 407.	1	2	2
	11. Exposure frequency and duration	HIGH	Daily by oral gavage for 28 days.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Investigators included justification for selection of dose levels.	1	1	1
	13. Exposure route and method	HIGH	Investigators included justification for selection of route of administration.	1	1	1
Test Organisms	14. Test animal characteristics	HIGH	Information provided about test model (Crl:CD-1 mice). Study also reported justification for animal model.	1	2	2
	15. Consistency of animal conditions	HIGH	Animal husbandry conditions were reported (see Sections D, E, F, G).	1	1	1
	16. Number per group	HIGH	Consistent with OECD Guidelines, there were a minimum of ten animals per sex per group. Some groups had 20 animals per sex per group.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Study report describes the methodology used to conduct the endpoint assessments.	1	2	2
	18. Consistency of outcome assessment	HIGH	Endpoint assessments were carried out consistently.	1	1	1
	19. Sampling adequacy	HIGH	Sampling frequency was reported for the various endpoints measured.	1	1	1
	20. Blinding of assessors	MEDIUM	Included clinical observations which fall within the definition of subjective outcomes. However, blinding prior to recording clinical observations was not addressed.	2	1	2

Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	Minor differences that would not result in substantial impact to results (e.g., food consumption--see page 16 of Part 1).	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	Two unexplained deaths reported. Gross necropsy and histologic findings did not provide explanation for the mortality.	1	1	1
Data Presentation and Analysis	23. Statistical methods	HIGH	They were reported and explained (See Section H).	1	1	1
	24. Reporting of data	HIGH	Very well documented study. All individual animal data reported.	1	2	2

Sum of scores:		30	34
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-17751-1026: E.I. du Pont de Nemours and Company (2009). A 90-Day Oral (Gavage) Toxicity Study of H-28548 in Rats with a 28-Day Recovery. OECD TG 408. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: October 5, 2009), Ashland, Ohio.					
Number of Hours for Review:	1					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Certificate of Analysis on page 1676 / 2076.	1	2	2
	2. Test substance source	HIGH	Provided by manufacturer; Page 24 / 2076.	1	1	1
	3. Test substance purity	MEDIUM	Low level of PFOA as a contaminant (150 ppm; equivalent to 0.015%).	2	1	2
Test Setup	4. Negative controls	HIGH	Page 24 / 2076	1	2	2
	5. Negative control responses	HIGH	All individual animal data provided for all dose groups, including controls.	1	1	1
	6. Positive controls	Not rated	Not applicable for this study design.	0	N/A	N/A
	7. Randomized allocation	HIGH	Page 27 / 2076.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Adequately reported; Page 24 / 2076.	1	1	1
	9. Consistency of exposure administration	HIGH	Adequately described; Page 25 / 2076.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Page 25 / 2076.	1	2	2
	11. Exposure frequency and duration	HIGH	All exposure metrics followed OECD TG 408; Page 25 / 2076.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Based on results from prior, shorter- term studies; page 25 / 2076.	2	1	2
	13. Exposure route and method	HIGH	Page 25 / 2076.	1	1	1

Test Organisms	14. Test animal characteristics	HIGH	Page 56 / 2076.	1	2	2
	15. Consistency of animal conditions	HIGH	Adequate; Page 27 / 2076.	1	1	1
	16. Number per group	HIGH	Page 27 / 2076.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Page 28 / 2076.	1	2	2
	18. Consistency of outcome assessment	HIGH	Page 30 / 2076.	1	1	1
	19. Sampling adequacy	HIGH	Consistent with OECD TG 408; Page 28 / 2076.	1	1	1
	20. Blinding of assessors	HIGH	Page 28 / 2076.	1	1	1
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	None identified; Page 26 / 2076.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	None identified; Page 26 / 2076.	1	1	1
Data Presentation and Analysis	23. Statistical methods	HIGH	Appropriate for study design; Page 34 / 2076.	1	1	1
	24. Reporting of data	HIGH	All individual animal data provided for all dose groups, including controls.	1	2	2

Notes: PFOA = perfluorooctanoic acid.

Sum of scores:		30	32
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-18405-1307: E.I. du Pont de Nemours and Company (2010). H-28548: Subchronic Toxicity 90-Day Gavage Study in Mice. OECD Guideline 408. Study conducted by E.I. du Pont de Nemours and Company (Study Completion Date: February 19, 2010), Newark, Delaware.					
Number of Hours for Review:	1.5					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Certificate of Analysis provided on page 98 / 339.	1	2	2
	2. Test substance source	HIGH	Provided by manufacturer; Page 14 / 339.	1	1	1
	3. Test substance purity	MEDIUM	PFOA contamination noted; Page 9 / 339 (84%).	2	1	2
Test Setup	4. Negative controls	HIGH	Page 10 / 339.	1	2	2
	5. Negative control responses	HIGH	Individual animal pathology data provided for all dose groups, including controls.	1	1	1
	6. Positive controls	Not rated	Not relevant to this study type.	0	N/A	N/A
	7. Randomized allocation	HIGH	Page 16 / 339.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Page 16 / 339.	1	1	1
	9. Consistency of exposure administration	HIGH	Page 16 / 339.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Page 16 / 339.	1	2	2
	11. Exposure frequency and duration	HIGH	Page 16 / 339.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Page 12 / 339.	2	1	2
	13. Exposure route and method	HIGH	Page 16 / 339.	1	1	1
Test Organisms	14. Test animal characteristics	HIGH	Page 14 / 339.	1	2	2
	15. Consistency of animal conditions	HIGH	Page 14 / 339.	1	1	1
	16. Number per group	HIGH	Page 13 / 339.	1	1	1

Outcome Assessment	17. Outcome assessment methodology	HIGH	Adequately described; Page 17 / 339.	1	2	2
	18. Consistency of outcome assessment	HIGH	Page 17 / 339.	1	1	1
	19. Sampling adequacy	HIGH	Page 20 / 339.	1	1	1
	20. Blinding of assessors	HIGH	Appropriate for study type; Page 18 / 339.	1	1	1
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	None; Page 25 / 339.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	None; Page 24 / 339.	1	1	1
Data Presentation and Analysis	23. Statistical methods	HIGH	Appropriate to study type; Page 22 / 339.	1	1	1
	24. Reporting of data	HIGH	All data presented for all dose groups, including controls.	1	2	2

Notes: PFOA = perfluorooctanoic acid.

Sum of scores:		30	32
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult Appendix B, Section B.3 for the metrics to assist in filling out this form.						
Study Reference:	DuPont-18405-841: E.I. du Pont de Nemours and Company (2010). An Oral (Gavage) Prenatal Developmental Toxicity Study of H-28548 in Rats. US EPA OPPTS 850.3700; OECD Guideline 414. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: July 2, 2010), Ashland, Ohio.					
Number of Hours for Review:	1.5					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	Certificate of analysis provided on page 215 / 388.	1	2	2
	2. Test substance source	HIGH	Provided by manufacturer/study sponsor (page 17 / 388).	1	1	1
	3. Test substance purity	MEDIUM	Low level of PFOA as a contaminant (150 ppm; equivalent to 0.015%).	2	1	2
Test Setup	4. Negative controls	HIGH	Page 19 / 388.	1	2	2
	5. Negative control responses	HIGH	All individual animal data provided for all dose groups, including controls.	1	1	1
	6. Positive controls	Not rated	Not applicable to this study design.	0	N/A	N/A
	7. Randomized allocation	HIGH	Page 20 / 388.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Page 17 / 388.	1	1	1
	9. Consistency of exposure administration	HIGH	Adequate (page 18 / 388).	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Page 18 / 388.	1	2	2
	11. Exposure frequency and duration	HIGH	Page 19 / 388.	1	1	1
	12. Number of exposure groups and dose spacing	MEDIUM	Only limited information on dose selection provided in report.	2	1	2
	13. Exposure route and method	HIGH	Adequately reported (page 19 / 388).	1	1	1

Test Organisms	14. Test animal characteristics	HIGH	Page 19 / 388.	1	2	2
	15. Consistency of animal conditions	HIGH	Page 20 / 388.	1	1	1
	16. Number per group	HIGH	Page 19 / 388.	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Page 22 / 388.	1	2	2
	18. Consistency of outcome assessment	HIGH	Page 22 / 388.	1	1	1
	19. Sampling adequacy	HIGH	Page 22 / 388.	1	1	1
	20. Blinding of assessors	HIGH	Adequately reported (page 22 / 388).	1	1	1
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	None reported.	1	2	2
	22. Health outcomes unrelated to exposure	HIGH	None reported.	1	1	1
Data Presentation and Analysis	23. Statistical Methods	HIGH	Appropriate to study methodology (page 25 / 388).	1	1	1
	24. Reporting of data	HIGH	All individual animal data provided for all dose groups, including controls.	1	2	2

Notes: PFOA = perfluorooctanoic acid.

Sum of scores:		30	32
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.1		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Instructions - Please consult the word document for the metrics to assist in filling out this form						
Study Reference:	DuPont-18405-1037: E.I. du Pont de Nemours and Company (2010). An Oral (Gavage) Reproduction/Developmental Toxicity Screening Study of H-28548 in Mice. US EPA OPPTS 870.3550; OECD Guideline 421. Study conducted by WIL Research Laboratories, LLC (Study Completion Date: December 29, 2010), Ashland, Ohio.					
Number of Hours for Review:	2					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	ID'ed by manufacturer.	1	2	2
	2. Test substance source	HIGH	ID'ed by manufacturer.	1	1	1
	3. Test substance purity	MEDIUM	84%; 12.7% water; 150 ppm PFOA (= 0.015%); PFOA content led to decreased confidence but the amount of PFOA is not expected to impact results.	2	1	2
Test Setup	4. Negative controls	HIGH		1	2	2
	5. Negative control responses	HIGH		1	1	1
	6. Positive controls	Not rated		0	N/A	N/A
	7. Randomized allocation	HIGH	The report and protocol described the randomization process; BWs at day 0 were within 20% of mean, as presented in results summary tables.	1	1	1
Exposure Characterization	8. Preparation and storage of test substance	HIGH	Test substance was identified as being stable.	1	1	1
	9. Consistency of exposure administration	MEDIUM	pH increase/dose - highest dose especially; range probably still okay.	2	1	2
	10. Reporting of doses/ concentrations	HIGH		1	2	2
	11. Exposure frequency and duration	HIGH		1	1	1
	12. Number of exposure groups and dose spacing	HIGH		1	1	1
	13. Exposure route and method	HIGH		1	1	1

Test Organisms	14. Test animal characteristics	HIGH		1	2	2
	15. Consistency of animal conditions	HIGH		1	1	1
	16. Number per group	HIGH		1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Went beyond OECD TG 421.	1	2	2
	18. Consistency of outcome assessment	MEDIUM	Consistency re: time of day for measurements among groups not always stated.	2	1	2
	19. Sampling adequacy	HIGH		1	1	1
	20. Blinding of assessors	MEDIUM	The report and protocol did not mention blinding of assessors. Substantial impact from this lack of information is not expected.	2	1	2
Confounding/ Variable Control	21. Confounding variables in test setup and procedures	HIGH	No confounders identified.	1	2	2
	22. Health outcomes unrelated to exposure	MEDIUM	10 mice died early; undetermined causes; more died at the lowest dose (in females this was > 10%) but not expected to have disproportional effect on study.	2	1	2
Data Presentation and Analysis	23. Statistical methods	HIGH		1	1	1
	24. Reporting of data	HIGH		1	2	2

Notes: PFOA = perfluorooctanoic acid.

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Sum of scores:		30	35
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.2		
Overall Quality Level:	HIGH		

Instructions - Please consult the word document for the metrics to assist in filling out this form						
Study Reference:	DuPont-18405-1238 (13 Volumes contained in 10 separate PDF files): E.I. du Pont de Nemours and Company (2010). H-28548: Combined Chronic Toxicity/Oncogenicity Study 2-Year Oral Gavage Study in Rats. US EPA OPPTS 870.4300; OECD Guideline 453. Study conducted by MPI Research, Inc. (Study Completion Date: March 28, 2013), Mattawan, Michigan.					
Number of Hours for Review:	2					
Domain	**Metric**	**Qualitative Determination (i.e., High, Medium, Low, Unacceptable, or Not rated)**	**Comments**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test substance identity	HIGH	ID'ed by manufacturer.	1	2	2
	2. Test substance source	HIGH	ID'ed by manufacturer.	1	1	1
	3. Test substance purity	MEDIUM	84%; 12.7% water; 150 ppm PFOA (= 0.015%); PFOA content led to decreased confidence but the amount of PFOA is not expected to impact results.	2	1	2
Test Setup	4. Negative controls	HIGH	Study report indicates negative controls and treatment groups had same test conditions; no major anomalies reported.	1	2	2
	5. Negative control responses	HIGH	Results appear appropriate for negative controls.	1	1	1
	6. Positive controls	Not rated	Positive controls not necessary according to OECD test guideline 453.	0	N/A	N/A
	7. Randomized allocation	HIGH	Method of randomization fully described/acceptable.	1	1	1

Exposure Characterization	8. Preparation and storage of test substance	HIGH	Well described; substance prepared weekly and was identified as being stable so storage at room temp seemed appropriate.	1	1	1
	9. Consistency of exposure administration	HIGH	Gavage volume was appropriate and controls received vehicle and were treated in same manner as treatment groups.	1	1	1
	10. Reporting of doses/ concentrations	HIGH	Detailed information regarding measured test concentrations was reported in appendix.	1	2	2
	11. Exposure frequency and duration	HIGH	Daily doses given as specified by OECD TG 453; 2-yr study duration appropriate as per OECD TG 453.	1	1	1
	12. Number of exposure groups and dose spacing	HIGH	Dose groups chosen based on previous 90-day study; choices are appropriate; range of effects and results suggest the same.	1	1	1
	13. Exposure route and method	MEDIUM	OECD TG 453 suggests route for environmental chemicals to be via diet or drinking water; however study authors justify their choice by noting gavage is most efficient way to get an accurate dose.	2	1	2
Test Organisms	14. Test animal characteristics	HIGH	BWs at start were within 20% of mean BWs; source of animals adequate; species also adequate as per OECD TG 453.	1	2	2
	15. Consistency of animal conditions	HIGH	Housing conditions for all animals were appropriate, as specified according to OECD TG 453.	1	1	1
	16. Number per group	HIGH	Numbers (80/sex/group) exceed OECD TG 453 recommendation (50/sex/group).	1	1	1
Outcome Assessment	17. Outcome assessment methodology	HIGH	Standard outcomes were reported, as specified by OECD TG 453.	1	2	2
	18. Consistency of outcome assessment	HIGH	Outcomes assessed consistently at same times among test groups and controls.	1	1	1
	19. Sampling adequacy	HIGH	Sampling well described; and is appropriate for study.	1	1	1
	20. Blinding of assessors	MEDIUM	Blinding not mentioned in study; don't expect it to influence results significantly.	2	1	2

Confounding/ Variable Control	21. Confounding variables in test setup and procedures	MEDIUM	Statistically significant differences at week (-1) in food consumption.	2	2	4
	22. Health outcomes unrelated to exposure	HIGH	Disease/infection evaluation well described and negative results reported.	1	1	1
Data Presentation and Analysis	23. Statistical methods	MEDIUM	Statistical methods and use of statistics clearly stated/identified; tumor incidence evaluated both with survival adjusted and unadjusted tests. Statistics were also appropriately re-run for male rats at request of EPA to account for rats that died early and had no tumors. However, no mention of trend tests, which can have greater power (according to OECD guidance document 116).	2	1	2
	24. Reporting of data	HIGH	The study reported both individual animal data (in appendices) and summary tables with statistics (in results of the main report).	1	2	2

Notes: PFOA = perfluorooctanoic acid.

Sum of scores:		30	36
Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:	1.2		
Overall Quality Level:	HIGH		

High	Medium	Low
≥1 and <1.7	≥1.7 and <2.3	≥2.3 and ≤3

Appendix C: Acute and 7-Day Study Summaries

This appendix summarizes studies evaluating acute exposure to 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 technical 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 mg/kg 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 ≤ 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 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 post-exposure. The rats dosed at 2,300 and 3,400 mg/kg no longer exhibited these effects at 2 days and 4 days post-exposure, respectively. Rats dosed with ≥ 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/groups) in a study conducted according to Organization of Economic Cooperation and Development (OECD) TG 425 (Up-and-Down Procedure). 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 post-exposure. 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 (Up-and-Down Procedure). 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 (3 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 rats (3) 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 (Up-and-Down Procedure). 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 were 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, #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 (Up-and-Down Procedure) evaluated toxicity of HFPO 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 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). 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 post-treatment. 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 post-treatment. 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 mg/m^3 of HFPO (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 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 HFPO in air were evaluated for clinical signs of toxicity for 2 days following exposure and rats exposed to 5,200 mg/m^3 of HFPO 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/m3 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. Rat 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). 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 post-exposure (DuPont-24030, 2007).

Eye Irritation. In an OECD TG 405 (OPPTS 870.2400) 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 the 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 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 3,000 mg/kg HFPO 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 D: Genotoxicity Study Summary

Table D-1 provides a summary of the available genotoxicity data for HFPO dimer acid and/or ammonium salt.

Table D-1. Genotoxicity Study Summary

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-19713 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD Guideline 471)	*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 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 Guideline 473)	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	With S9	Positive at 3,391 µg/mL* in 4-hour activated test conditions.
				Without S9	Negative.
			*Doses have been corrected to account for 83% HFPO dimer acid ammonium salt purity.		

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-23219 (2007)	*In vivo* Unscheduled DNA Synthesis Test in Mammalian Cells (OECD Guideline 486)	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 Guideline 476)	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 Guideline 473)	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 Negative control (sterile water) and positive control (mitomycin-C or cyclophosphamide) also included in study	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.
				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.

Study	Assay	Strain/Species	Dosing	Activation	Results
DuPont-22734 RV1 (2008)	*In vitro* Bacterial Reverse Mutation Test (OECD Guideline 471)	*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*	With S9	Negative.
				Without S9	Negative.
			*Doses have been corrected to account for 82.6% HFPO dimer acid ammonium salt purity.		
DuPont-23220 (2007)	*In vivo* Micronucleus and Chromosome Aberration Assay (OECD Guidelines 474 and 475)	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	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*).	
			* Doses have been corrected to account for 82.6% HFPO dimer acid ammonium salt purity.		

Appendix E: Benchmark Dose Modeling

28 Day Oral (Gavage) Toxicity Study in Mice (DuPont-24459 2008)

Increased incidence of single-cell necrosis of hepatocytes and correlative increases in liver enzymes were observed with liver identified as the primary target organ of toxicity. Dichotomous models were used to fit of the incidence of single-cell necrosis in the liver. A benchmark response (BMR) of 10% extra risk was chosen per the EPA's *Benchmark Dose Technical Guidance* (USEPA, 2012). The data used for the modeling are in Table E-1 below.

Table E-1. Single-Cell Necrosis in the Liver Selected for Dose-Response Modeling

Dose (mg/kg bw/day)	Number of Animals (Males)	Incidence of Single-Cell Necrosis
0	10	0
0.1	10	0
3	10	4
30	10	10

Note: mg/kg bw/day = milligrams per kilogram body weight per day.

The BMD modeling results for single-cell necrosis are summarized in Table E-2 and Figures E-1 and E-2. All the models had adequate *p*-values (>0.1). The BMD:BMDL ratios were less than 5 for Gamma, Weibull, LogProbit, Dichotomous-Hill and LogLogistic so these models were not further considered. The remaining models have benchmark dose lower limits (BMDLs) that are sufficiently close so the models with the lowest AICs were considered (these are the Multistage 2 and the Quantal-Linear). Both the Multistage 2 and the Quantal-Linear are selected because the $BMDL_{10}$s of both these models rounded to 1 significant figure are the same 0.3 mg/kg bw/day.

Table E-2. Summary of BMD Modeling Results for Single-Cell Necrosis in Male Mice

Model[a]	Goodness of fit		BMD_{10Pct} (mg/kg/day)	$BMDL_{10Pct}$ (mg/kg/day)	BMD_{10Pct} to $BMDL_{10Pct}$ ratio	Basis for model selection
	p-value	AIC				
Quantal-Linear	0.972	15.918	0.603	0.305	1.97	EPA OPPT selected both the Quantal-Linear and Multistage 2 models. All the models had adequate p-values (>0.1). The BMD:BMDL ratios were > 5 for Gamma, Weibull, LogProbit, Dichotomous-Hill and LogLogistic so these models were not further considered. The remaining models have BMDLs<4-fold difference and the Quantal-Linear and Multistage 2 models had the lowest AICs.
Multistage 2°	1.000	15.472	1.36	0.323	4.23	
Logistic	1.000	17.460	2.72	1.16	2.34	
Probit	1.000	17.460	2.45	1.04	2.37	
Multistage 3°	0.998	17.469	1.45	0.323	4.48	
Gamma	1.000	17.460	1.88	0.323	5.80	
Weibull	1.000	17.460	1.95	0.323	6.04	
LogProbit	1.000	17.460	2.01	0.299	6.72	
Dichotomous-Hill LogLogistic	1.000	17.460	2.42	0.343	7.06	

Notes: OPPT = Office of Pollution Prevention and Toxics.

[a] Selected model in bold; scaled residuals for selected model for doses 0, 0.1, 3, and 30 mg/kg/day were 0, -0.42, -0.05, 0.23, respectively.

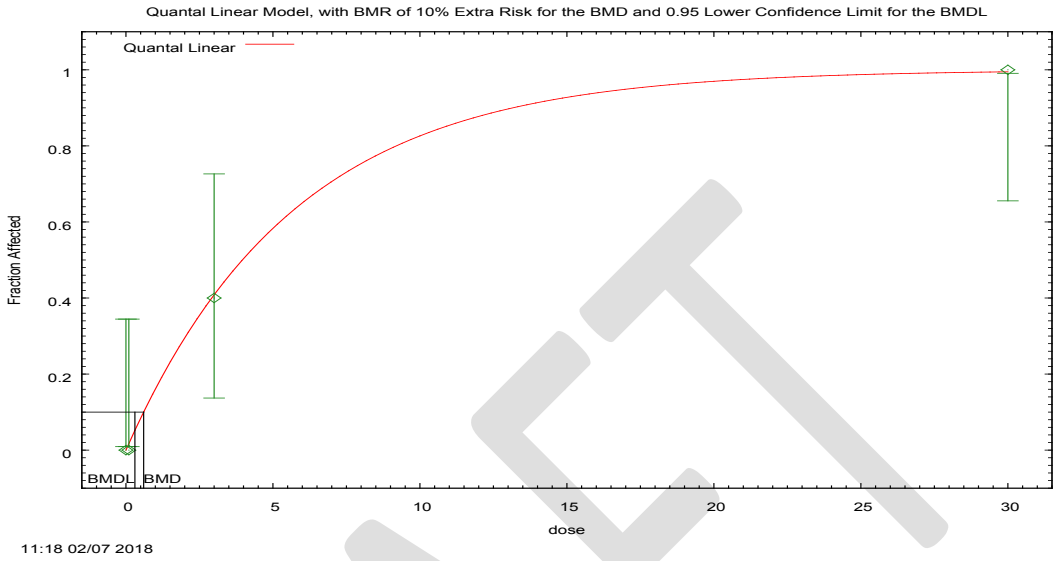

Figure E-1. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Model Quantal-Linear Model for Single-Cell Necrosis in Male Mice; Dose Shown in mg/kg/day

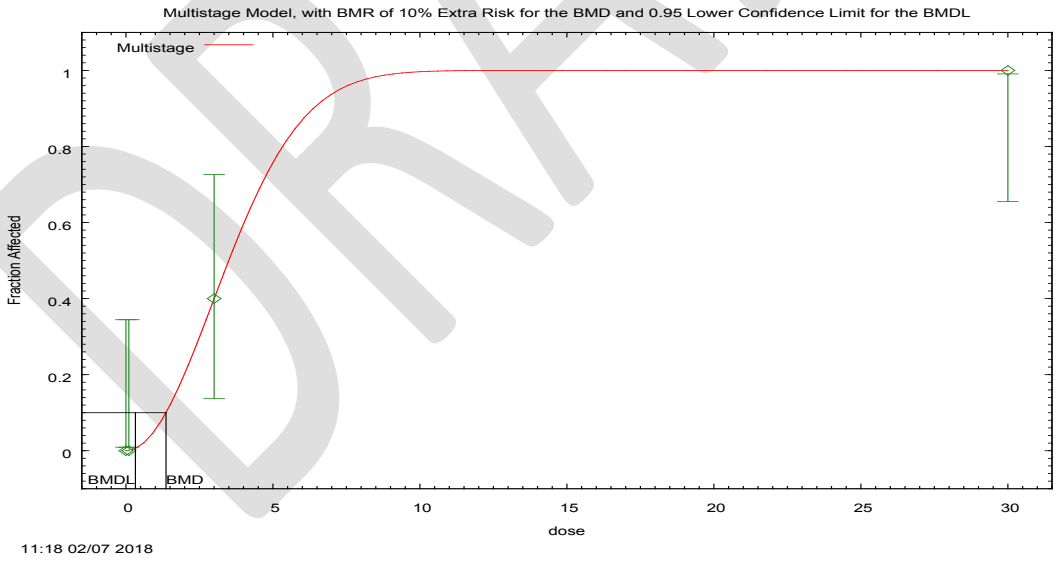

Figure E-2. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Model Multistage 2° Model for Single-Cell Necrosis in Male Mice; Dose Shown in mg/kg/day

Modified Oral Reproductive/Developmental Toxicity Study in Mice (DuPont-18405-1037 2010)

Increased incidence of single-cell necrosis in the liver was observed in the parental males. Dichotomous models were used to fit dose-response data (DuPont-18405-1037, 2010). A BMR

of 10% extra risk was chosen per the EPA's *Benchmark Dose Technical Guidance* (USEPA, 2012). The doses and response data used for the modeling are listed in Table E-3.

Table E-3. Single-Cell Necrosis in the Liver Selected for Dose-Response Modeling

Dose (mg/kg/day)	Number of Animals (Males)	Incidence of Single-Cell Necrosis
0	25	1
0.1	24	1
0.5	24	5
5	24	24

The benchmark dose (BMD) modeling results for single-cell necrosis are summarized in Table E-4 and Figure E-3. The best fitting model was the Multistage 2 model based on adequate *p*-values (>0.1), the BMDLs are sufficiently close, and the Multistage 2 model had the lowest Akaike information criterion (AIC). The Multistage 2, Logistic, and Probit models had the lowest AICs and the AICs were very close to each other. Of these models the Multistage 2 model has the lowest scaled residuals for the dose groups near the BMD and the BMDL and the scaled residuals for the Multistage 2 model are shown in Table E-4. The $BMDL_{10}$ for Multistage 2 model is 0.15 mg/kg/day.

Table E-4. Summary of BMD Modeling Results for Single-Cell Necrosis 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			
Multistage 2°	**0.995**	**45.285**	**0.007**	**-0.078**	**0.368**	**0.151**	EPA OPPT selected the Multistage 2 model. All of the models had adequate *p*-values (> 0.1), the BMDLs are sufficiently close, and the Multistage 2 model had the lowest AIC.
Logistic	0.969	45.337	0.019	-0.181	0.362	0.253	
Probit	0.960	45.358	0.029	-0.212	0.349	0.236	
Weibull	1.000	47.275	0	0	0.407	0.166	
Multistage 3°	1.000	47.275	0	0	0.408	0.145	
Gamma	0.992	47.275	0	0.006	0.399	0.172	
Dichotomous-Hill LogLogistic	0.977	47.275	0	0.021	0.464	0.253	
LogProbit	0.977	47.275	0	0.021	0.443	0.248	
Quantal-Linear	0.261	48.991	-0.756	-0.756	0.162	0.106	

Notes: OPPT = Office of Pollution Prevention and Toxics.
[a] Selected model in bold.

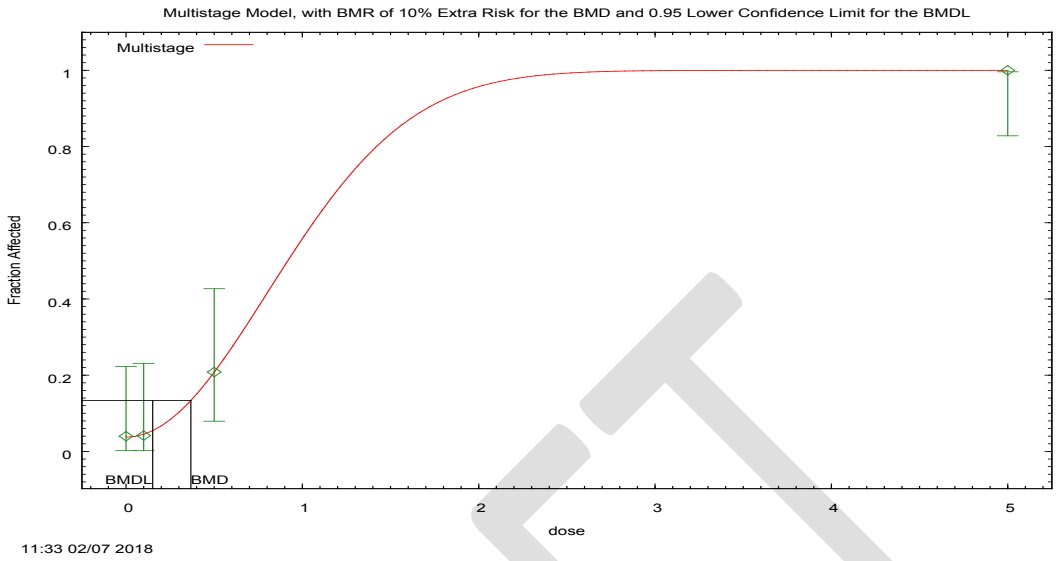

Figure E-3. Plot of Incidence Rate by Dose with Fitted Curve for the Selected Multistage 2° Model for Single-Cell Necrosis in Male Mice; Dose Shown in mg/kg/day

Table E-5 provides a summary of goodness of fit of the BMD modeling results for single-cell necrosis in male mice.

Table E-5. Goodness of Fit Table of BMD Modeling Results for Single-Cell Necrosis in Male Mice

Dose	Est. Prob.	Expected	Observed	Size	Scaled Residual
0	0.0375	0.937	1	25	0.07
0.1	0.0449	1.079	1	24	-0.08
0.5	0.2077	4.985	5	24	0.01
5	1	24	24	24	0