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Toxicological Review of Perfluorohexanoic Acid (PFHxA) and Related Compounds Ammonium and Sodium Perfluorohexanoate (PFHxA-NH_4 and PFHxA-Na)

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ABBREVIATIONS AND ACRONYMS

ADME	absorption, distribution, metabolism, and excretion
AFFF	aqueous film-forming foam
A:G	albumin:globulin ratio
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area under the curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
C_{max}	maximum concentration
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
CBC	complete blood count
CHO	Chinese hamster ovary (cell line cells)
CI	confidence interval
CL	clearance
CL_A	clearance in animals
CL_H	clearance in humans
CPHEA	Center for Public Health and Environmental Assessment
CPN	chronic progressive nephropathy
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
DTXSID	DSSTox substance identifier
eGFR	estimated glomerular filtration rate
EPA	Environmental Protection Agency
ER	extra risk
FTOH	fluorotelomer alcohol
GD	gestation day
GGT	γ-glutamyl transferase
HAWC	Health Assessment Workplace Collaborative
HCT	hematocrit
HED	human equivalent dose
HERO	Health and Environmental Research Online
HGB	hemoglobin
HSA	human serum albumin
IQR	interquartile range
IRIS	Integrated Risk Information System
ISI	Influential Scientific Information
IUR	inhalation unit risk
i.v.	intravenous
LDH	lactate dehydrogenase
LOQ	limit of quantitation
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOEC	lowest observed effect concentration
MCH	mean cell hemoglobin
MCHC	mean cell hemoglobin concentration
MCV	mean cell volume
MOA	mode of action
MW	molecular weight
NCTR	National Center for Toxicological Research
NOAEL	no-observed-adverse-effect level
NPL	National Priorities List
NTP	National Toxicology Program
ORD	Office of Research and Development
OECD	Organisation for Economic Co-operation and Development
OSF	oral slope factor
osRfD	organ/system-specific oral reference dose
PBPK	physiologically based pharmacokinetic
PC	partition coefficient
PECO	populations, exposures, comparators, and outcomes
PFAA	perfluoroalkyl acids
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluorinated carboxylic acid
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PK	pharmacokinetic
PND	postnatal day
POD	point of departure
POD_{HED}	human equivalent dose POD
PPAR	peroxisome proliferated activated receptor
PQAPP	programmatic quality assurance project plan
PT	prothrombin time
QA	quality assurance
QAPP	quality assurance project plan
QMP	quality management plan

RBC	red blood cells
RD	relative deviation
RfC	reference concentration
RfD	oral reference dose
RNA	ribonucleic acid
ROS	reactive oxygen species
RXR	retinoid X receptor
SD	standard deviation
TP	total protein
TRI	Toxics Release Inventory
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid stimulating hormone
UF	uncertainty factor
UF_A	interspecies uncertainty factor
UF_C	composite uncertainty factor
UF_D	evidence base deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL to NOAEL uncertainty factor
UF_S	subchronic to chronic uncertainty factor
V_2	volume of distribution of peripheral compartment (two-compartment PK model)
V_d	volume of distribution

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Perfluorohexanoic acid (PFHxA, CASRN 307-24-4), ammonium perfluorohexanoate (PFHxA-NH_4, CASRN 21615-47-4), and sodium perfluorohexanoate (PFHxA-Na, CASRN 2923-26-4) are members of the group per- and polyfluoroalkyl substances (PFAS). Concerns about PFHxA and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment. PFAS are not naturally occurring in the environment; they are manmade compounds that have been used widely over the past several decades in industrial applications and consumer products because of their resistance to heat, oil, stains, grease, and water. PFAS in the environment are linked to industrial sites, military fire training areas, wastewater treatment plants, and commercial products (Appendix A, Section 2.1.2)

The Integrated Risk Information System (IRIS) Program is developing a series of five PFAS assessments (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [PFHxS], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts) (see December 2018 IRIS Program Outlook) at the request of EPA National Programs. The systematic review protocol (Appendix A) for these five PFAS assessments outlines the related scoping and problem formulation efforts, including a summary of other federal and state assessments of PFHxA. The protocol also lays out the systematic review and dose-response methods used to conduct this review (see also Section 1.2). The systematic review protocol was released for public comment in November 2019 and was updated on the basis of those public comments. Appendix A includes the updated version of the protocol and summarizes the history of the revisions.

Human epidemiological studies have examined possible associations between PFHxA exposure and health outcomes, such as liver enzymes, thyroid hormones, blood lipids, blood pressure, insulin resistance, body mass index, semen parameters, reproductive hormones, and asthma. The ability to draw conclusions regarding these associations is limited by the overall quality of the studies (studies were generally *low* confidence); the few studies per health outcome; and, in some studies, the lack of a quantifiable measure of exposure. No studies were identified that evaluated the association between PFHxA exposure and carcinogenicity in humans.

Animal studies of PFHxA exposure exclusively examined the oral exposure route, and therefore no inhalation assessment was conducted nor was an RfC derived (Section 5.2.2). The available animal studies of oral PFHxA exposure examined a variety of noncancer and cancer endpoints, including those relevant to hepatic, developmental, renal, hematopoietic, endocrine, reproductive, immune, and nervous system effects.

Overall, the available evidence indicates that PFHxA exposure is likely to cause hepatic, developmental, and hematopoietic effects in humans, given relevant exposure circumstances. Specifically, for hepatic effects, the primary support for this hazard conclusion included evidence of increased relative liver weights and increased incidence of hepatocellular hypertrophy in adult rats. These hepatic findings correlated with changes in clinical chemistry (e.g., serum enzymes, blood proteins) and necrosis. For hematopoietic effects, the primary supporting evidence included decreased red blood cell counts, decreased hematocrit values, and increased reticulocyte counts in adult rats. Developmental effects were identified as a hazard based on evidence of decreased offspring body weight and increased perinatal mortality in exposed rats and mice. Selected quantitative data from these identified hazards were used to derive toxicity values (Table ES-1).

In addition, evidence in rats suggests the potential for PFHxA exposure to affect endocrine (i.e., thyroid) responses. Due to limitations in the currently available studies, these data were not considered for use in deriving toxicity values. Although some human and animal evidence was also identified for cardiometabolic, renal, male and female reproductive, immune, and nervous system effects, the currently available ***evidence is inadequate*** to assess whether PFHxA may cause these health effects in humans under relevant exposure circumstances and were not used to derive toxicity values

Table ES-1. Health effects with evidence available to synthesize and draw summary judgments and derived toxicity values

Organ/ System	Integration judgment	Toxicity value	Value (mg/kg-day)	Confid-ence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Hepatic	Evidence indicates (likely)	osRfD	3×10^{-4}	*Medium*	3	10	3	1	3	300	Increased hepatocellular hypertrophy in adult rats (Loveless et al., 2009)
		Subchronic osRfD	9×10^{-4}	*Medium*	3	10	1	1	3	100	Increased hepatocellular hypertrophy in adult rats (Loveless et al., 2009)
Hematopoietic	Evidence indicates (likely)	osRfD	4×10^{-3}	*High*	3	10	1	1	3	100	Decreased red blood cells in adult rats (Klaunig et al., 2015)
		Subchronic osRfD	6×10^{-4}	*High*	3	10	1	1	3	100	Decreased red blood cells in adult rats (Chengelis et al., 2009b)

Organ/ System	Integration judgment	Toxicity value	Value (mg/kg-day)	Confid-ence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Develop-mental	Evidence indicates (likely)	osRfD	4×10^{-4}	*Medium*	3	10	1	1	3	100	Decreased F_1 body weight at PND 0 (Loveless et al., 2009)
		Subchronic osRfD	4×10^{-4}	*Medium*	3	10	1	1	3	100	Decreased F_1 body weight at PND 0 (Loveless et al., 2009)
Overall RfD			4×10^{-4}	*Medium*	3	10	1	1	3	100	Decreased F_1 body weight at PND 0 (Loveless et al., 2009)
Overall Subchronic RfD			4×10^{-4}	*Medium*	3	10	1	1	3	100	Decreased F_1 body weight at PND 0 (Loveless et al., 2009)

RfD = reference dose (in mg/kg-day) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposure; osRfD = organ- or system-specific reference dose (in mg/kg-d); UF_A = animal to human uncertainty factor; UFC = composite uncertainty factor; UF_D = evidence base deficiencies uncertainty factor; UF_H = human variation uncertainty factor; UF_L = LOAEL to NOAEL uncertainty factor; UF_S = subchronic to chronic uncertainty factor.

Chronic Oral Reference Dose (RfD) for Noncancer Effects

From the identified hazards of potential concern (i.e., hepatic, hematopoietic, and developmental toxicity), decreased offspring body weight in neonatal mice (Loveless et al., 2009) was selected as the basis for the RfD of 4×10^{-4} mg/kg-day. A $BMDL_{5RD}$ of 10.62 mg/kg-day was identified for this endpoint and was used as the point of departure (POD). The human equivalent dose POD (POD_{HED}) of 0.039 mg/kg-day was derived by applying the ratio of the clearance between female rats and humans and a normalization from the sodium salt to the free acid using a molecular weight conversion. The overall RfD for PFHxA was calculated by dividing the POD_{HED} by a composite uncertainty factor of 100 to account for pharmacodynamic uncertainty in the extrapolation from rats to humans (UF_A = 3), interindividual differences in human susceptibility (UF_H = 10), and deficiencies in the toxicity evidence base (UF_D = 3). The developmental organ/system-specific (os) RfD is based on the lowest overall POD_{HED} and UF_C; therefore, the selected RfD based on decreased offspring body weight is assumed to be protective of the observed health effects associated with lifetime PFHxA exposure because this is considered a sensitive lifestage and, in the current evidence base, effects on body weight were strongest during the early postnatal window.

Confidence in the Oral Reference Dose (RfD)

The study conducted by Loveless et al. (2009) reported developmental effects following administration of PFHxA sodium salt to pregnant Sprague-Dawley rats dosed by gavage for approximately 70 days prior to cohabitation through gestation and lactation, for a total of 126 days daily gavage with 0, 20, 100, or 500 mg/kg-day sodium PFHxA. This study was rated as *high* confidence based on study evaluation results (click the HAWC link for full study evaluation details) and study design characteristics that make it suitable for deriving toxicity values. The overall confidence in the RfD is *medium* and is primarily driven by *medium* confidence in the overall evidence base for hepatic effects, *high* confidence in the Loveless et al. (2009) study, and *medium* confidence in quantitation of the POD (Table 5-8).

Noncancer Effects Following Inhalation Exposure

No studies that examine toxicity in humans or experimental animals following inhalation exposure and no physiologically based pharmacokinetic (PBPK) models are available to support route-to-route extrapolation; therefore, no RfC was derived.

Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded there is *inadequate information to assess carcinogenic potential* for PFHxA by either oral or inhalation routes of exposure. Therefore, the lack of data on the carcinogenicity of PFHxA precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

Subchronic Oral Reference Dose (RfD) for Noncancer Effects

In addition to providing RfDs for chronic oral exposures in multiple systems, a subchronic RfD was derived for PFHxA. The same study and endpoint (Loveless et al., 2009) and decreased F_1 body weight) was selected as the basis for the subchronic RfD of 4×10^{-4} mg/kg-day (Table ES-1). Details are provided in Section 5.2.1.

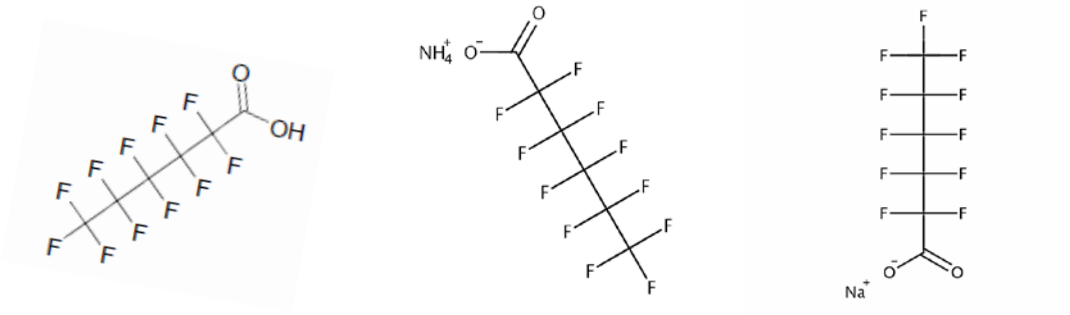
1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS

1.1. BACKGROUND INFORMATION ON PFHxA AND RELATED AMMONIUM AND SODIUM SALTS

Section 1.1 provides a brief overview of aspects of the physiochemical properties, human exposure, and environmental fate characteristics of perfluorohexanoic acid (PFHxA, CASRN 307-24-4), ammonium perfluorohexanoate (PFHxA-NH_4, CASRN 21615-47-4), and sodium perfluorohexanoate (PFHxA-Na, CASRN 2923-26-4).

1.1.1. Physical and Chemical Properties

PFHxA and its related sodium and ammonium PFHxA salts covered in this assessment are members of the group of per- and polyfluoroalkyl substances (PFAS). Concerns about PFHxA and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment (NLM, 2013, 2016, 2017). PFHxA and its related salts are classified as a perfluorinated carboxylic acids (PFCAs) (OECD, 2015). Because PFHxA and its associated salts contain fewer than seven perfluorinated carbon groups, they are considered short-chain PFAS (ATSDR, 2018). The linear chemical structures of these chemicals are presented in Figure 1-1, and select physiochemical properties are provided in Table 1-1.



	PFHxA	PFHxA ammonium salt	PFHxA sodium salt
CASRN	307-24-4	21615-47-4	2923-26-4
DTXSID	3031862	90880232	3052856

Figure 1-1. Linear chemical structures of (from left to right) perfluorohexanoic acid (PFHxA), ammonium perfluorohexanoate (PFHxA-NH_4), and sodium perfluorohexanoate (PFHxA-Na).

Source: EPA CompTox Chemicals Dashboard.

Table 1-1. Physicochemical properties of PFHxA

Property (unit)	PFHxA value	PFHxA-NH_4 value	PFHxA-Na value
Formula	$CF_3(CF_2)_4COOH$	$C_6H_4F_{11}NO_2$	$C_6F_{11}NaO_2$
Molecular weight (g/mol)	314	331	336
Melting point (°C)	12.2[a]	39.2[b]	70.2[b]
Boiling point (°C)	157[a]	156[b]	216[b]
Density (g/cm^3)	1.69[b]	1.72[b]	1.69[b]
Vapor pressure (mm Hg)	0.908[a]	2.00[b]	1.63[b]
Henry's law constant (atm-m^3/mole)	2.35×10^{-10} [(b)]	2.35×10^{-10} [(b)]	2.35×10^{-10} [(b)]
Water solubility (mol/L)	9.34×10^{-5} [(a)]	1.10[b]	8.78×10^{-5} [(a)]
PKa	−0.16[c]	--	--
$LogP_{Octanol\text{-}Water}$	2.85[a]	3.97[b]	0.70[a]
Soil adsorption coefficient (L/kg)	1,070[b]	1070[b]	1070[b]
Bioconcentration factor	49.3[b]	5.47[b]	49.3[b]

[a]U.S. EPA (2018a). CompTox Chemicals Dashboard; access date 2/18/2021. Median or average experimental values.
[b]Average or median predicted values; -- indicates data not available.
[c]Reported by NLM (2016); access date 05/06/2019.

1.1.2. Sources, Production, and Use

PFAS are not naturally occurring in the environment (ATSDR, 2018; U.S. EPA, 2002b, 2007, 2013, 2019c, 2020). They are manmade compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. This class of chemicals has been used in consumer products including stain-resistant fabrics for clothing, carpets, and furniture; nonstick cookware; ski wax; certain leather products; and personal care products (e.g., dental floss, cosmetics, and sunscreen) (ATSDR, 2018; U.S. EPA, 2002b, 2007, 2013, 2019c, 2020). PFAS also have been detected from foam used in firefighting and in industrial surfactants, emulsifiers, wetting agents, additives, and coatings; they are also used in aerospace, automotive, building, and construction industries to reduce friction (ATSDR, 2018; U.S. EPA, 2002b, 2007, 2013, 2019c, 2020). In addition, PFAS have been found at private and federal facilities associated with various material or processes involving aqueous film-forming foam (AFFF), chrome plating, and PFAS production and are associated with other industries using PFAS (e.g., textiles, carpets) (ATSDR, 2018; U.S. EPA, 2002b, 2007, 2013, 2019c, 2020). In AFFF, PFHxA has been detected at concentrations ranging from 0.1 to 0.3 g/L (Baduel et al., 2015; Houtz et al., 2013).

No quantitative PFHxA information on production volume is available (U.S. EPA, 2019a), and EPA's Toxics Release Inventory (TRI) contains no information on releases to the environment from facilities manufacturing, processing, or otherwise using PFHxA (ATSDR, 2018; U.S. EPA, 2018b).

Wang et al. (2014) estimated global emissions of 39 to 1,691 tons of PFHxA from direct and indirect (i.e., degradation of precursors) sources between 1951 and 2030. The lower estimate assumes manufacturers cease production and use of long-chain PFCAs and that their precursors stay consistent with global transition trends. The higher estimate assumes the 2015 emission scenario remains constant until 2030.

1.1.3. Environmental Fate and Transport

PFAS are highly stable and persistent worldwide, and many are found in environmental media (e.g., soils, water, the atmosphere, foods, wildlife, and humans) (U.S. EPA, 2019c) (Appendix A).

Uptake of soil PFAS to plants can occur (ATSDR, 2018), and estimates are available of PFAS accumulation in vegetation when plants are grown in PFAS-contaminated soil. Yoo et al. (2011) estimated grass-soil accumulation factors of 3.4 (grass concentration divided by soil concentration) for PFHxA using samples collected from a site with biosolids-amended soil. Venkatesan and Halden (2014) analyzed archived samples from outdoor mesocosms to investigate the fate over 3 years of PFAS in agricultural soils amended with biosolids. The mean half-life for PFHxA was estimated to be 417 days. Volatilization of PFHxA from moist soil is not expected to be an important fate process (NLM, 2016). PFHxA bioaccumulates in foods grown on PFAS-containing soils. Blaine et al. (2013)

conducted a series of greenhouse and field experiments to investigate the potential for PFAS uptake by lettuce, tomatoes, and corn when grown in industrially impacted and biosolids-amended soils. Blaine et al. (2013) calculated PFHxA bioaccumulation factors of 9.9–11.7 for lettuce and 2.9–6.8 for tomatoes (no bioaccumulation factor was reported for corn).

1.1.4. Potential for Human Exposure and Populations with Potentially Greater Exposure

The general population can be exposed to PFAS via inhalation of air or dust, ingestion of drinking water and food, and dermal contact with PFAS-containing products and during susceptible lifestages (Appendix A). Infants potentially have higher exposure due to greater ingestion of food per body weight. Further, although studies of human breast milk in the U.S. population have not observed PFHxA, it has been detected in human breast milk from France, Korea, and Spain (summarized in Table 5 of Anderson et al. (2019)). Exposure can also occur through hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018).

The oral route of exposure is considered the dominant exposure pathway for the general population (Klaunig et al., 2015), for which contaminated drinking water is likely a significant source of exposure. Due to the high water solubility and mobility of PFAS in groundwater (and potential lack of remediation at some water treatment facilities), populations consuming drinking water from any contaminated watershed could be exposed to PFAS (Shao et al., 2016).

Air and Dust

PFHxA has not been evaluated under the National Air Toxics Assessment program and no additional information on air levels was identified. PFAS, including PFHxA, have been measured in indoor air and dust and might be associated with the indoor use of consumer products such as PFAS-treated carpets or other textiles (ATSDR, 2018). For example, Kato et al. (2009) detected PFHxA in 46.2% of the dust samples collected from 39 homes in the United States, United Kingdom, Germany, and Australia. Karásková et al. (2016) detected PFHxA in all 56 dust samples collected from 41 homes in the Czech Republic, Canada, and the United States at mean concentrations of 12.8, 14.5, and 20.9 ng/g, respectively. Strynar and Lindstrom (2008) analyzed dust samples from 110 homes and 10 daycare centers in North Carolina and Ohio, and detected PFHxA in 92.9% of the samples. Knobeloch et al. (2012) detected PFHxA in 20% of samples of vacuum cleaner dust collected from 39 homes in Wisconsin. PFHxA concentrations ranged from below the reporting limit (1 ng/g) to 180 ng/g. Fraser et al. (2013) analyzed dust samples collected from offices (n = 31), homes (n = 30), and vehicles (n = 13) in Boston, Massachusetts. PFHxA was detected in 68% of the office samples at concentrations ranging from 5.1 to 102 ng/g, 57% of the home samples at concentrations ranging from 4.9 to 1,380 ng/g, and 54% of the vehicle samples at concentrations ranging from 5.0 to 18.2 ng/g.

Water

EPA conducted monitoring for several PFAS in drinking water as part of the third and fifth Unregulated Contaminant Monitoring Rules (UCMR3 and UCMR5) (U.S. EPA, 2016, 2019b). PFHxA was recently added to UCMR5 for public water system monitoring and applies to 2022–2026, with sample collection proposed between 2023 and 2025. Some drinking water PFHxA data are available from other publications. For example, samples from seven municipal wells in Oakdale, Minnesota were analyzed for PFHxA where the concentrations ranged from <0.025 to 0.235 µg/L (U.S. EPA, 2016). PFHxA also was detected in 23% of raw water samples collected from public water systems in New Jersey at concentrations ranging from nondetectable to 0.017 µg/L (Post et al., 2012). In a more recent study of surface waters sampled from 11 waterways in New Jersey, PFHxA was detected in 10 samples, ranging from 0.0015 to 0.026 µg/L (Goodrow et al., 2020).

AFFF Training Sites

PFHxA was detected at an Australian training ground where AFFFs had been used. Baduel et al. (2015) and Bräunig et al. (2017) observed mean concentrations of PFHxA of 0.6 µg/L in water, 8.4 µg/kg dry weight in soil, and 3.0 µg/kg wet weight in grass at an Australian town where the groundwater had been impacted by PFAS from a nearby firefighting training facility. Houtz et al. (2013) analyzed samples of groundwater, soil, and aquifer solids collected at an Air Force firefighting training facility in South Dakota where AFFF had been used. PFAS concentrations in groundwater decreased with increased distance from the burn pit, and PFHxA was detected at a median concentration of 36 µg/L. PFHxA was detected in surficial soil at a median concentration of 11 µg/kg and in aquifer solids at a median concentration of 45 µg/kg.

Military and National Priorities List (NPL) Sites

PFHxA levels in environmental samples have been measured at military and National Priorities List (NPL) sites in the United States. Table 1-2 provides the concentrations at these sites (Anderson et al., 2016; ATSDR, 2018).

Table 1-2. PFHxA levels at 10 military installations and National Priority List sites

Media	PFHxA value	Site	Source
Surface soil Frequency of detection (%) Median (ppb) Maximum (ppb)	 70.33 1.75 51.0	Military[a]	Anderson et al. (2016)
Subsurface soil Frequency of detection (%) Median (ppb) Maximum (ppb)	 65.38 1.04 140	Military[a]	Anderson et al. (2016)

Media	PFHxA value	Site	Source
Sediment Frequency of detection (%) Median (ppb) Maximum (ppb)	 63.64 1.70 710	Military[a]	Anderson et al. (2016)
Surface Water Frequency of detection (%) Median (ppb) Maximum (ppb)	 96.00 0.320 292	Military[a]	Anderson et al. (2016)
Groundwater Frequency of detection (%) Median (ppb) Maximum (ppb)	 94.20 0.820 120	Military[a]	Anderson et al. (2016)
Water (ppb) Median Geometric mean	 0.25 0.10	NPL[b]	ATSDR (2018)
Soil (ppb) Median Geometric mean	 1,175 1,175	NPL[b]	ATSDR (2018)
Air (ppbv) Median Geometric mean	 ND ND	NPL[b]	ATSDR (2018)

[a]Samples collected between March and September 2014 from 10 active U.S. Air Force installations located throughout the United States, including Alaska, with a historic use of AFFFs; data originally reported as µg/kg.
[b]Concentrations found in ATSDR site documents; water and soil values represent data from two NPL sites.

Other Exposures

Schecter et al. (2012) collected 31 food samples from 5 grocery stores in Texas and analyzed them for persistent organic pollutants, including PFHxA. PFHxA was not detected in the samples. Chen et al. (2018) analyzed PFAS in a wide range of foods in Taiwan and detected PFHxA at geometric mean concentrations ranging from 0.03 ng/mL in milk to 1.58 ng/g in liver. Heo et al. (2014) analyzed a variety of foods and beverages in Korea for PFAS. PFHxA was detected in 8.1% of the fish and shellfish samples at a mean concentration of 0.037 ng/g; 8.1% of the dairy samples at a mean concentration of 0.051 ng/g; 9.5% of the beverage samples at a concentration of 0.187 ng/L; 20.5% of the fruit and vegetable samples at a mean concentration of 0.039 ng/g; and 51.3% of the meat samples at a mean concentration of 0.515 ng/g. Heo et al. (2014) also detected PFHxA in tap water in Korea at a mean concentration of 11.7 ng/L; PFHxA was not detected in bottled water. Pérez et al. (2014) analyzed PFAS in 283 food items (38 from Brazil, 35 from Saudi Arabia, 36 from Serbia, and 174 from Spain). PFHxA was detected in 6.0, 21.3, and 13.3% of the samples from Brazil, Saudi Arabia, and Spain, respectively. The mean concentrations of PFHxA were 270, 931, and 418 pg/g, respectively. The study did not find PFHxA in any of the Serbian samples. PFHxA was

detected in microwave popcorn packaging materials at a range of 3.4 to 497 ng/g, but was not detected in the corn or popcorn (Moreta and Tena, 2014).

Stahl et al. (2014) characterized PFAS in freshwater fish from 164 U.S. urban river sites and 157 near-shore Great Lakes sites. PFHxA was not detected in the fish from U.S. urban rivers but was detected in fish from 15% of the Great Lakes sites at a maximum concentration of 0.80 ng/g.

1.2. SUMMARY OF ASSESSMENT METHODS

Section 1.2 summarizes the methods used for developing this assessment. A detailed description of these methods is provided in the PFAS Systematic Review Protocol for the PFDA, PFNA, PFHxA, PFHxS, and PFBA IRIS Assessments in Appendix A and is available online. The protocol includes additional problem formulation details, including the specific aims and key science issues identified for this assessment.

1.2.1. Literature Search and Screening

The detailed search approach, including the query strings and populations, exposures, comparators, and outcomes (PECO) criteria, are provided in Appendix A, Table 3-1. The results of the current literature search and screening efforts are documented in Section 2.1. Briefly, a literature search was first conducted in 2017 and regular yearly updates have been performed (the literature fully considered in the assessment will continue to be updated until shortly before the release of the document for public comment). The literature search queries the following databases (no literature was restricted by language):

- PubMed (National Library of Medicine)
- Web of Science (Thomson Reuters)
- Toxline (moved to PubMed December 2019)
- TSCATS (Toxic Substances Control Act Test Submissions)

In addition, relevant literature not found through evidence base searching was identified by:

- Review of studies cited in U.S. state, U.S. federal, and international assessments, including parallel assessment efforts in progress (e.g., the draft Agency for Toxic Substances and Disease Registry [ATSDR] assessment released publicly in 2018).
- Review of studies submitted to federal regulatory agencies and brought to EPA's attention.
- Identification of studies during screening for other PFAS. For example, searches focused on one of the other four PFAS currently being assessed by the IRIS Program sometimes identified epidemiological studies relevant to PFHxA.

- Other gray literature (i.e., primary studies not indexed in typical evidence bases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to EPA's attention.

All literature, including literature search updates, is tracked in the EPA Health and Environmental Research Online (HERO) database.[1]

The PECO criteria identify the evidence that addresses the specific aims of the assessment and focuses the literature screening, including study inclusion/exclusion. In addition to those studies meeting the PECO criteria, studies containing supplemental material potentially relevant to the specific aims of the assessment were inventoried during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues (Appendix A, Section 2.4) and other major scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as "potentially relevant supplemental material" included the following:

- In vivo mechanistic or mode-of-action studies, including non-PECO routes of exposure (e.g., intraperitoneal injection) and non-PECO populations (e.g., nonmammalian models)
- In vitro and in silico models
- Absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) studies (excluding models)[2]
- Exposure assessment or characterization (no health outcome) studies
- Human case reports or case-series studies
- Studies of other PFAS (e.g., perfluorooctanoic acid [PFOA] and perfluorooctane sulfonate [PFOS])

The literature was screened by two independent reviewers with a process for conflict resolution, first at the title and abstract level and subsequently the full-text level, using structured forms in DistillerSR (Evidence Partners). Literature inventories for studies meeting PECO criteria and studies tagged as "potentially relevant supplemental material" during screening were created to facilitate subsequent review of individual studies or sets of studies by topic-specific experts.

[1]EPA's Health and Environmental Research Online (HERO) database provides access to the scientific literature behind EPA science assessments. The database includes more than 3,000,000 scientific references and data from the peer-reviewed literature EPA uses to develop its risk assessments and related regulatory decisions.

[2]Given the known importance of ADME data, this supplemental tagging was used as the starting point for a separate screening and review of PK data (see Appendix A.9.2 for details).

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiological and animal toxicological studies used in the PFHxA assessment are provided in the systematic review protocol (Appendix A.6). The general approach for evaluating health effect studies meeting PECO criteria is the same for epidemiological and animal toxicological studies although the specifics of applying the approach differ; thus, they are described in detail in Appendices A.6.2 and A.6.3, respectively.

- The key concerns during the review of epidemiological and animal toxicological studies are potential bias (factors that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect; low sensitivity is a bias toward the null when an effect exists). In terms of the process for evaluating individual studies, two or more reviewers independently arrived at judgments about the reliability of the study results (reflected as study confidence determinations; see below) with regard to each outcome or outcome grouping of interest; thus, different judgments were possible for different outcomes within the same study. The results of these reviews were tracked within EPA's version of the Health Assessment Workplace Collaborative (HAWC). To develop these judgments, each reviewer assigned a rating of *good*, *adequate*, *deficient* (or *not reported*, which generally carried the same functional interpretation as *deficient*), or *critically deficient* (listed from best to worst methodological conduct; see Appendix A, Section 6.1 for definitions) to each evaluation domain representing the different characteristics of the study methods that were evaluated on the basis of the criteria outlined in HAWC.

Once all domains were evaluated, the identified strengths and limitations were considered as a whole by the reviewers to reach a final study confidence classification:

- *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.
- *Medium* confidence: Possible deficiencies or concerns were noted, but the limitations are unlikely have a significant impact on the results.
- *Low* confidence: Deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. *Low* confidence results were given less weight compared to *high* or *medium* confidence results during evidence synthesis and integration (see Section 1.2.4).
- *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

Using the HAWC platform (and conflict resolution by an additional reviewer, as needed), the reviewers reached a consensus judgment regarding each evaluation domain and overall (confidence) determination. The specific limitations identified during study evaluation were carried forward to inform the synthesis (Section 1.2.4) within each body of evidence for a given health effect (i.e., study confidence determinations were not used to inform judgments in isolation).

1.2.3. Data Extraction

The detailed data extraction approach is provided in Appendix A.8, and data extraction and content management is carried out using HAWC (see Appendix C). Data extraction elements that may be collected from epidemiological, controlled human exposure, animal toxicological, and in vitro studies are described in HAWC. Not all studies that meet the PECO criteria go through data extraction: For example, studies evaluated as being *uninformative* are not considered further and therefore do not undergo data extraction. The same could be true for *low* confidence studies if enough *medium* and *high* confidence studies (e.g., on an outcome) are available. All findings are considered for extraction, regardless of statistical significance. The level of extraction for specific outcomes within a study might differ (e.g., ranging from a qualitative description to full extraction of dose-response effect size information). For quality control, data extraction is performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction are resolved by discussion or consultation with a third member of the evaluation team.

1.2.4. Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes (see Appendices A.9 and A.10 for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis resulting in a summary discussion of the available evidence that addresses considerations regarding causation adapted from Hill (1965). Mechanistic evidence and other supplemental information is also synthesized to address key science issues or to help inform key decisions regarding the human and animal evidence.

The syntheses focus on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of available studies. Syntheses of the evidence for human and animal health effects are based primarily on studies of *high* and *medium* confidence. *Low* confidence studies might be used if few or no studies with higher confidence are available to help evaluate consistency, or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect. If *low* confidence studies are used, a careful examination of risk of bias and sensitivity with potential impacts on the conclusions of the evidence synthesis is included in the narrative. The synthesis of mechanistic evidence and other supplemental information informs the integration of health effects evidence for hazard identification (i.e., biological plausibility of the available human or animal evidence, inferences regarding human relevance, or the identification of

susceptible populations and lifestages across the human and animal evidence) and for dose-response evaluation.

For each assessed health effect, following the evidence syntheses, integrated judgments are drawn across all lines of evidence. During evidence integration, a structured and documented process is used, as follows:

- Building from the separate syntheses of the human and animal evidence, the strength of the evidence from the available human and animal health effect studies was summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Bradford Hill (Hill, 1965). These summaries incorporate the relevant mechanistic evidence (or mode of action [MOA] understanding) that informs the biological plausibility and coherence within the available human or animal health effect studies.

- The strength of the animal and human evidence was considered together in light of inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed.

- A summary judgment is drawn as to whether the available evidence base for each potential human health effect as a whole: "evidence demonstrates," "evidence indicates (likely)," "evidence suggests," "evidence is inadequate," or "evidence strongly supports no effect" that PFHxA exposure has the potential to cause the health effect in humans.

The decision points within the structured evidence integration process are summarized in an evidence profile table for each assessed health effect.

1.2.5. Dose-Response Analysis

The details for the dose-response analysis completed for this assessment are in Appendix A.11. Briefly, although procedures for dose-response assessments were developed for both noncancer and cancer health hazards, and for both oral and inhalation routes of exposure following exposure to PFHxA, the existing data for PFHxA only supported derivation of an oral reference dose (RfD) for noncancer hazards (see Appendix A.11 for the health hazard conclusions necessary for deriving other values). An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002c). Specifically, for noncancer outcomes this assessment includes dose-response assessments when the evidence integration judgments indicate ***evidence demonstrates*** and ***evidence indicates (likely)***.

Consistent with EPA practice, the PFHxA assessment applied a two-step approach for dose-response assessment that distinguishes analysis of the dose-response data in the range of observation from any inferences about responses at lower, environmentally relevant exposure levels (U.S. EPA, 2005, 2012a). Within the observed dose range, the preferred approach is to use

dose-response modeling to incorporate as much of the data set as possible into the analysis, and considering guidance on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results [see the EPA Benchmark Dose Technical Guidance (U.S. EPA, 2012a)] as elaborated in Appendix A.11. Thus, modeling to derive a POD attempted to include an exposure level near the lower end of the range of observation, without significant extrapolation to lower exposure levels. Extrapolations to exposures lower than the POD involved the application of five uncertainty factors to estimate candidate noncancer toxicity values, as described in Appendix A.11.

Evaluation of these candidate values grouped within a given organ/system were used to derive a single organ/system-specific RfD (osRfD) for each organ/system under consideration. Next, evaluation of these osRfDs, including confidence in the evidence base supporting each potential hazard and other factors (see Appendix A.11), resulted in the selection of a single RfD to cover all health outcomes across all organs/systems. Although this overall RfD represents the focus of the dose-response assessment, the osRfDs can be useful for subsequent cumulative risk assessments. In addition, a less-than-lifetime, "subchronic" RfD was similarly estimated. Uncertainties in these toxicity values are transparently characterized and discussed.

2. SUMMARY OF LITERATURE IDENTIFICATION AND STUDY EVALUATION RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

The evidence base searches yielded 339 unique records, with 18 records identified from posted National Toxicology Program (NTP) study tables and review of reference lists from other authoritative sources (ATSDR, 2018) (see Figure 2-1). Of the 339 studies identified, 194 were excluded at the title and abstract level and 77 were reviewed at the full-text level. Of the 77 screened at the full-text level, 26 were considered to meet the PECO criteria (see Appendix A, Section 4.2.2). The studies meeting PECO at the full-text level included 14 human health effect studies, 6 in vivo animal studies, 3 in vitro genotoxicity studies, and 3 ADME/PK studies. In addition, high-throughput screening data on perfluorohexanoic acid (PFHxA) were available from EPA's CompTox Chemicals Dashboard (U.S. EPA, 2018a). A literature inventory of the included animal toxicological studies is available in an literature inventory heatmap accessible via PFHxA Tableau Link.

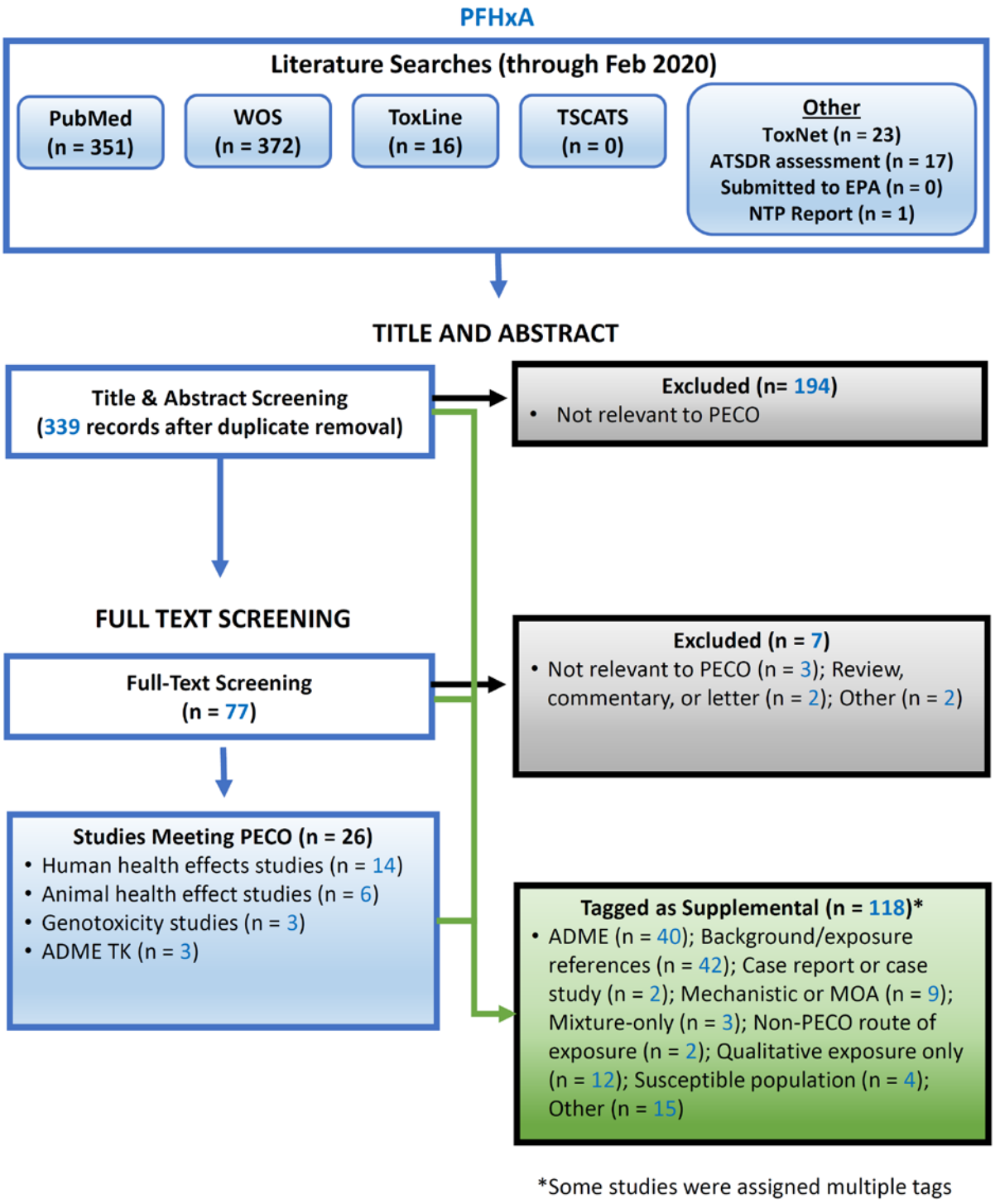


Figure 2-1. Literature search and screening flow diagram for perfluorohexanoic acid (PFHxA) and related compounds ammonium and sodium perfluorohexanoate (PFHxA-NH_4 and PFHxA-Na).

2.2. STUDY EVALUATION RESULTS

Human and animal studies evaluated potential hepatic, developmental, hematopoietic, endocrine, cardiometabolic, renal, reproductive, immune, and nervous system effects, following exposure to PFHxA. The evidence informing these potential health effects is presented and assessed in Sections 3.2.1–3.2.9. Thirteen epidemiological studies were identified that report on the potential association between PFHxA and human health effects. Of these, four were considered *uninformative* due to critical deficiencies in one or more domains, including participant selection, exposure measurement, confounding, or analysis (Jiang et al., 2014; Kim et al., 2016a; Seo et al., 2018; Zhang et al., 2019). The remaining nine studies were rated *medium* (Bao et al., 2017; Dong et al., 2013; Nian et al., 2019; Zeng et al., 2015) or *low* confidence (Fu et al., 2014; Li et al., 2017; Qin et al., 2017; Song et al., 2018; Wang et al., 2019; Zhou et al., 2016).

Of the six unique reports of animal studies meeting PECO criteria, five were considered for dose-response. The remaining study, Kirkpatrick (2005), was considered uninformative due to reporting deficiencies (i.e., all summary data [pages 110–1,334] were missing). The available evidence base of animal toxicity studies on PFHxA and the related ammonium and sodium salts consists of five reports in rats and mice including short-term (NTP, 2018), subchronic (Chengelis et al., 2009b; Loveless et al., 2009), chronic (Klaunig et al., 2015), and reproductive/developmental (Iwai and Hoberman, 2014; Loveless et al., 2009) experiments. These studies were generally well conducted and judged *high* or *medium* confidence. In cases where a study was rated *low* confidence for one or more of the evaluated outcomes, the specific limitations identified during evaluation are discussed in the applicable synthesis section(s).

Detailed rationales for each domain and overall confidence rating are available in HAWC. Results for human studies are available here and animal studies are available here. Graphical representations of the outcome-specific ratings are presented in the organ/system-specific integration sections (in Section 3.2).

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND EVIDENCE INTEGRATION

3.1. PHARMACOKINETICS

Only a few PK studies on PFHxA are available in humans but they provide sufficient data to estimate its half-life. Several studies such as Ericson et al. (2007) reported PFHxA in blood or serum of human populations (e.g., in relation to age and sex) but, because exposure levels are not known for the subjects and the concentrations are not measured over time in specific subjects for whom the exposure level is known to be zero, such observations cannot be used to obtain ADME information. Several other studies that investigate specific aspects of PFHxA ADME in humans are discussed briefly below but were not used in the derivation of toxicity values. One analysis provides an estimate of PFHxA elimination in humans (Russell et al., 2013) using data from an observational study by Nilsson et al. (2013). Luz et al. (2019) describes a reanalysis of these data but based only on the three participants with the most rapid clearance.

Animal experiments in rats, mice, and monkeys have provided valuable information on PK processes of PFHxA. In brief, PFHxA and other perfluoroalkyl acids (PFAA) have similar PK aspects: They are well absorbed following oral exposure and quickly distribute throughout the body (Iwabuchi et al., 2017), particularly to blood, liver, skin, and kidney (Gannon et al., 2011). Dzierlenga et al. (2019) noted that following intravenous (i.v.) administration of 40 mg/kg PFHxA, the PK profiles were generally similar between sexes, but a lower dose-normalized area under the curve (AUC, 3.05 mM·h/mmol/kg), a faster clearance (CL, 327 mL/h-kg), and a lower volume of distribution of peripheral compartment (V_2 = 59.6 mL/kg) was observed in female Sprague-Dawley rats, as compared to their male counterparts (dose-normalized AUC = 7.38 mM·h/(mmol/kg), CL = 136 mL/h-kg, and V_2 = 271 mL/kg, respectively). Likewise, kinetic parameters (e.g., the maximum concentration [C_{max}]) were comparable between sexes following an oral dose of 40 mg/kg, except that females exhibited a lower dose-adjusted AUC/dose and a faster CL. A PK study in mice similarly showed an AUC/dose in male animals 2–3 times higher than in females, indicating slower elimination in males (Gannon et al., 2011). Thus, apparent sex-related quantitative differences in PFHxA PK occur in rats and mice. On the other hand, the AUC in monkeys given a 10 mg/kg i.v. dose of PFHxA was only slightly lower in females than in males (75 vs. 84 mg-h/L), suggesting no significant sex difference in nonhuman primates.

PFHxA is resistant to metabolic transformation, and urinary excretion is the main elimination route, followed by feces (Chengelis et al., 2009a; Gannon et al., 2011; Iwai, 2011).

3.1.1. Absorption

Absorption is rapid in rodents and monkeys (Chengelis et al., 2009a; Gannon et al., 2011; Iwabuchi et al., 2017). PFHxA was extensively absorbed with an average time to reach maximum concentration (T_{max}) of 1 h in Sprague-Dawley rats given 26-day repeated gavage doses of 50, 150 or 300 mg PFHxA/kg (Chengelis et al., 2009a). After gavage at 2 or 100 mg [1-^{14}C]PFHxA/kg using a single dose or 14 daily consecutive doses, Gannon et al. (2011) also observed a short T_{max} of 30 and 15 min, respectively, in male and female Sprague-Dawley rats. Similarly, rapid absorption was also observed in CD-1 mice (Gannon et al., 2011). For female rats and male and female mice, PFHxA absorption does not appear to be saturated between 2 and 100 mg/kg as suggested by dose-normalized $AUC_{0\rightarrow168}$ h, but the data in male rats indicate either a 25% reduction in absorption or a corresponding increase in clearance between these two dose levels (Chengelis et al., 2009a; Gannon et al., 2011).

In a recent PK study by Dzierlenga et al. (2019), Sprague-Dawley rats were given PFHxA, PFOA, and perfluorodecanoic acid (PFDA; C10) by i.v. injection (40 mg/kg) or gavage (40, 80, and 160 mg/kg). Besides collection of blood samples to evaluate the time course of plasma PFHxA at predetermined schedules, liver, kidney, and brain samples were collected to determine the distributions of PFHxA in tissues following 80 mg/kg gavage dose. A two-compartmental model was used to evaluate the PK profiles. Systemic exposure of PFHxA, as assessed by dose-normalized area under the plasma AUC and C_{max}, was generally lower than systemic exposure to PFOA or PFDA. Nevertheless, estimated oral bioavailability for all three PFAAs was >100% (Dzierlenga et al., 2019); this result simply could reflect experimental and analytical uncertainty in estimating the serum concentration AUC from intravenous vs. oral exposure, but also might be due to increased reabsorption from the intestinal lumen by intestinal transporters of material excreted in the bile. The researchers also noticed that T_{max} slightly increased with increasing oral PFHxA dose levels for both sexes. For instance, T_{max} increased from 0.668 ± 0.154 to 0.890 ± 0.134 h (mean ± standard error) and from 0.529 ± 0.184 to 0.695 ± 0.14 h with increased gavage doses of PFHxA for male and female rats, respectively. A similar pattern was observed for PFDA in both male and female rats and for PFOA exposure in male rats, but not in females (for which T_{max} was about constant) (Dzierlenga et al., 2019).

3.1.2. Distribution

PFHxA has an aqueous solubility of 15.7 g/L (Zhou et al., 2010). Computational chemistry predictions conclude that PFHxA and its salts have a $pK_a \leq 0$ (Rayne and Forest, 2010), so it likely exists exclusively in anionic form at physiological pH (Russell et al., 2013). Therefore, it is relatively water soluble, but limited data are available to examine its distribution to various organs and tissues upon exposure in mammalian systems (Gannon et al., 2011; Russell et al., 2013). The largest concentrations were found in liver, skin, heart, lung, and kidney and concentrations peaked within hours (Gannon et al., 2011; Iwabuchi et al., 2017). For example, Gannon et al. (2011) reported

heart, kidneys, liver, and lungs had detectable but not quantifiable concentrations of PFHxA at 24 h in rats dosed with 100 mg/kg (Gannon et al., 2011). Similarly, the highest uptake concentrations occurred in the liver and femur (10 ± 2 and 5 ± 1% of the injected dose, respectively), in male CD-1 mice (Burkemper et al., 2017). As described in detail below, the volume of distribution (V_d) was generally similar (within a factor of 3) among male and female mice, rats, and monkeys (Russell et al., 2013).

Distribution in Humans

The tissue distribution of PFHxA and other PFAAs were analyzed in 99 human autopsy samples (brain, liver, lung, bone, and kidney) (Pérez et al., 2013). Pérez et al. (2013) used the term "accumulation," that implies a steady increase in the amount of a substance in the body tissues over an extended time while exposure continues at a relatively constant level. Because the study data were collected from cadavers, they show only the tissue levels in the individuals at time of death, and thus do not actually demonstrate such accumulation over time. These tissue concentrations could also represent approximate steady-state concentrations achieved in the weeks or months prior to death, with no subsequent accumulation. More generally, these data cannot inform the specific exposure scenarios that might have occurred before the time of death.

Pérez et al. (2013) found PFHxA to be the main PFAA compound in the brain (mean = 180 ng/g tissue weight, median = 141 ng/g). PFHxA was detected in all collected tissue types at levels ranging from below the detection limit to an observed concentration of 569 ng/g in the lung. These observations generally demonstrate the *distribution* of short-chain PFAAs like PFHxA, for which the mean (or median) concentration ranged from 5.6 ng/g (2.7 ng/g) tissue in the kidney to 180 ng/g (141 ng/g) in the brain. The liver and lung had tissue levels somewhat below that in the brain but within the same range, with mean (or median) levels of 115 ng/g (68.3 ng/g) and 50.1 ng/g (207 ng/g), respectively.

Fàbrega et al. (2015) attempted to estimate tissue:blood partition coefficients (PCs) for PFHxA using the data of Pérez et al. (2013). Because Pérez et al. (2013) did not measure or report blood concentrations, Fàbrega et al. (2015) used the mean blood concentration reported 4 years earlier for residents of the same county (Ericson et al., 2007). The resulting set of PCs ranged from 6 (unitless ratio) in the kidney to 202 in the brain, indicating a V_d in the human body around 40 L/kg or higher. In contrast, Chengelis et al. (2009a) estimated V_d of 0.18 and 0.47 L/kg, respectively, in male and in female rats. For monkeys, the individual estimates of V_d Chengelis et al. (2009a) reported varied widely for each sex; for example, the coefficient of variation among the three females was 74%. Therefore, EPA recalculated male and female values for this analysis from the mean values of $AUC_{0-\infty}$ and the beta-phase elimination constant, K_{el}:

$$V_d = \text{dose}/[\text{mean}(AUC_{0-\infty}) \times \text{mean}(K_{el})]. \quad (3\text{-}1)$$

The resulting values of V_d were 0.77 L/kg and 0.35 L/kg for male and female monkeys, respectively. Although the reported values for rats and these re-estimated values for monkeys were within similar ranges, spanning less than a factor of 5, the difference between males and females of each species is larger than expected. The underlying data indicate significant PK differences between males and females of each species.

The average V_d for rats (0.33 L/kg) is only 40% lower than the average for monkeys (0.56 L/kg), a modest species difference that could occur due to differences in the relative lipid content in blood vs. the rest of body. Partitioning or distribution is primarily a function of the physicochemical properties of a tissue vs. blood (lipid content being a significant component) and are typically similar across species, not differing by orders of magnitude as suggested by the difference between the results of Fàbrega et al. (2015) for humans and the animal PC data. This raises a significant question about reasons for the apparent disparity. EPA is unaware of a specific mechanism that could explain this discrepancy, particularly one that differs between monkeys and humans to such a large extent but not between monkeys and rats.

Therefore, the most likely explanation for the differences in the PCs estimated by Fàbrega et al. (2015) are an artifact of combining data from nonmatched human samples Pérez et al. (2013) whereas Ericson et al. (2007) collected data over several years (e.g., due to a change in PFHxA exposure in that population across those times). Thus, these results are considered too uncertain for further analysis of human pharmacokinetics. Instead, the V_d estimated for male and female monkeys by Chengelis et al. (2009a) is assumed to provide the best estimates for men and women, respectively, given the biochemical properties of tissues that determine the relative affinity for PFHxA in tissue vs. blood are more similar between humans and a nonhuman primate than between humans and rats or mice. Because the V_d in monkeys is similar to that in rats (see details below) and an assumption of similar partitioning in humans versus other mammals has been successfully used for many PBPK models, this assumption is considered modest with minimal associated uncertainty.

Zhang et al. (2013a) evaluated the distribution of several PFAS including PFHxA in matched samples of maternal blood, cord blood, placenta, and amniotic fluid among Chinese women. Only 45% of maternal blood samples were above the limit of quantitation (LOQ), with a mean concentration of 0.07 ng/mL, although 87% of cord blood samples were above the LOQ, with a mean of 0.21 ng/mL PFHxA. Only 17% of placenta samples were above the LOQ (mean concentration 0.04 ng/mL) and 45% of amniotic fluid samples (mean concentration 0.19 ng/mL). The authors urge caution in interpreting their results because recovery of PFHxA from test samples was more variable than for most other PFAAs. These data do show, however, that PFHxA distributes into the fetus during pregnancy.

The partitioning of PFHxA and 15 perfluoroalkyl substances (C6–C11) between plasma and blood cells was investigated using blood samples collected from human subjects (n = 60) (Jin et al., 2016). The results showed that although the estimated mass fraction in plasma generally increased

with the carbon chain length, PFHxA appeared to have lowest mass fraction in plasma (0.24) as compared with other PFAA chemicals (0.49 to 0.95). In a study population of 61 adults in Norway, Poothong et al. (2017) also found that although PFHxA was detected in 100% of the whole blood samples, it was not detected in serum or plasma. Given the strong partitioning to whole blood (perhaps due to partitioning into blood cells), the whole blood, rather than serum or plasma, was suggested as a better blood matrix for assessing PFHxA exposure (Poothong et al., 2017).

Role of Plasma Protein Binding

Some evidence suggests plasma protein binding (e.g., serum albumin) could also play a role in PFHxA TK. A study by D'eon et al. (2010) evaluated the molecular interactions of PFHxA and PFOA with human serum albumin (HSA) using nuclear magnetic resonance spectroscopy. They found the interaction of both PFHxA and PFOA with HSA—assessed on the basis of data for selected HSA ligands including oleic acid, phenylbutazone, and ibuprofen—could affect its pharmacokinetics.

Organic anion transporters, a family of transmembrane proteins, had been suggested to play a role in the renal reabsorption of PFAAs (Kudo, 2015; Weaver et al., 2010) (see further discussion for rats below). Weaver et al. (2010) found that renal transport of PFAAs with different chain lengths (C2–C18) could occur via specific transporters (Oat1, Oat2, Oat3, Urat1, and Oatp1a1) that were differentially located in the basolateral membrane and apical membrane in rats (Chinese hamster ovary cell line and kidney RNA from Sprague-Dawley rats). Although PFHxA was capable of inhibiting Oat1-mediated transport of *p*-aminohippurate, the model substrate used for PFAA transport tests, the quantitative role of organic anion transporters in PFHxA PK remains uncertain due to the rapid elimination kinetics of PFHxA (Weaver et al., 2010).

On the other hand, although Bischel et al. (2011) measured the binding of PFHxA to bovine serum albumin in vitro, the measured fraction bound is 99%, which appears quantitatively inconsistent with the empirical observation that the elimination half-life is on the order of 2–3 h in rats, for example. If glomerular filtration could remove only 1% (i.e., the free faction) of PFHxA carried in the corresponding serum flow, the elimination half-life should be much longer. Thus, although plasma protein binding could play some role in PFHxA distribution and elimination, one must be careful in quantitatively interpreting such results. Because it is reversible, protein binding could have a limited impact on distribution and elimination, despite a relatively high faction of plasma protein binding at equilibrium. Therefore, the empirically determined distribution and elimination rates for PFHxA in various species and sexes are used rather than the rate one might predict on the basis of albumin binding.

Distribution in Animal (Rats, Mice, and Monkeys) and In-Vitro Studies

In the study by Chengelis et al. (2009a) described above, both Sprague-Dawley rats and cynomolgus monkeys (3/sex) were also given PFHxA (10 mg/kg) via a single i.v. injection to determine PFHxA PK using noncompartmental analysis. In monkeys they observed a distribution

phase of 8 h and an apparent V_d of 0.77 and 0.35 L/kg in males and females, respectively. In male and female rats, V_d was reported as 0.18 and 0.47 L/kg, respectively, and the distribution phase after gavage dosing was about 1–2 h in both sexes. Serum concentrations of PFHxA were up to 17-fold higher for male than female rats after i.v. dosing, and the AUC after oral dosing was over 4-fold higher in males than females given a 50 mg/kg gavage dose. The half-life in males, however, was only 2.5 times greater than females after i.v. dosing and was similar to that in females after oral dosing. Together these lead to the conclusion of higher V_d for females than for males.

Using a one-compartment model, Iwabuchi et al. (2017) evaluated the distribution of PFHxA and other PFAAs (PFOA, PFOS and perfluorononanoic acid, [PFNA]) in multiple tissues (brain, heart, liver, spleen, kidney, whole blood, and serum) in 6 week old male Wistar rats. The rats were given a single oral dose or 1- and 3-month exposures in drinking water. For the single oral dose, rats were given drinking water containing a mixture of PFAAs by gavage (PFHxA, PFOA, PFOS: 100 µg/kg body weight [BW], PFNA: 50 µg/kg BW). Although the estimated T_{max} for PFHxA was 1 h for all tissues, the T_{max} for other PFAAs was 12 h in the tissues except the brain (72 h) and whole blood (24 h), indicating PFHxA was distributed rapidly throughout the body. Peak concentrations occurred between 15 min and 1 h after dosing, depending on the tissue. Of examined tissues, the highest concentrations of PFHxA were found in the serum and kidney, equivalent to 7.9% and 7.1% of the administered PFHxA, respectively. Note that the peak concentrations measured in liver and brain were roughly 40% (at 15 min) and 1.5% (at 1 h) of the corresponding peak serum levels (4.6% and 0.027% of administered PFHxA dose), respectively. The earlier peak in liver concentration is likely due to initial delivery there from oral absorption, although the results show low delivery to the brain.

For the 1- or 3-month exposures, rats were given a mixture of four PFAA dose levels: 0, 1, 5 and 25 µg/L in drinking water with similar intake rate across dose groups (0.072–0.077 L/kg BW-day) (Iwabuchi et al., 2017). In general, the long-term tissue concentrations of PFHxA predicted on the basis of the data from the single-exposure studies were comparable to that measured after the 1- and 3-month exposures, suggesting that steady-state tissue levels were achieved rather quickly and the tissue distribution of PFHxA remained relatively constant over time (Iwabuchi et al., 2017).

An in vitro study using lung epithelial cells (NCI-H292) and adipocytes (3T3-L1K) made similar observations of no appreciable cellular accumulation and retention of PFHxA (Sanchez Garcia et al., 2018).

3.1.3. Metabolism

Similar to other PFAA compounds, PFHxA is not readily metabolized as evidenced by the findings that no metabolites were recovered from either the liver or urine following oral dosing of mice or rats (Chengelis et al., 2009a; Gannon et al., 2011). Although PFHxA is resistant to metabolism, fluorotelomer-alcohols and sulfonates can undergo biotransformation to form PFHxA

or its glucuronide and sulfate conjugates in rodents and humans (Kabadi et al., 2018; Russell et al., 2015).

3.1.4. Elimination

Existing evidence has consistently suggested PFHxA has a shorter half-life than those of other longer chained PFAAs (e.g., PFOA or PFOS). For instance, approximately 80% of the administered dose of PFHxA appeared in the urine of rats during 24 h post-dosing regardless of sex following i.v. injection (Chengelis et al., 2009a). Daikin Industries (2009a, 2009b) recovered approximately 90% of an oral dose of 50 mg/kg PFHxA, either as a single dose or on the 14th day of dosing by 24 h after the single or last dose in male and female rats and mice. Likewise Dzierlenga et al. (2019) reported that liver and kidney concentrations peaked by 30 min in male rats and by 1 h in female rats after gavage and decreased steadily thereafter (observations at 0.5, 1, 3, 6, 9 and 12 h). The tissues concentrations of PFHxA tended to be very low or not quantifiable 24 h after dosing in both sexes of mice and rats (Gannon et al., 2011; Iwabuchi et al., 2017).

The comparable weight-normalized blood elimination half-life of PFHxA across mammalian species further implies the lack of species-specific roles for renal tissue transporters, either in facilitating elimination or impeding elimination through renal resorption for PFHxA, unlike the situation for some long-chain PFAAs. Gomis et al. (2018) concluded PFHxA had a relatively short elimination half-life and the lowest bioaccumulation among the six PFAAs they evaluated on the basis of applying a one-compartment PK model combined with PK data compiled from previous studies on male rats. In particular, the beta- or elimination-phase half-life ($t_{½,\beta}$) values estimated were: PFHxA = 2.4 h, perfluorobutane sulfonate (PFBS) = 4.7 h, pentafluorobenzoic acid (PFBA) = 9.2 h, ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-propanoate (GenX) = 72 h, PFOA = 136 h, and PFOS = 644 h (Gomis et al., 2018). PK model simulations from a 10-day oral experiment with a dose of 1 mg/kg-day predicted that, as compared to other PFAAs, PFHxA had the lowest serum and liver AUC levels. Likewise, Chengelis et al (2009a) compared PFHxA dosimetry in naïve male and female rats to results after 25 days of dosing (50–300 mg/kg/d) and found no significant difference in the parameters evaluated, with the serum half-life remaining in the range of 2–3 h.

Rat Studies

Iwai (2011) evaluated PFHxA excretion in Sprague-Dawley rats and CD-1 mice treated with single and multiple (4 days) oral dose(s) at 50 mg/kg of [^{14}C] ammonium perfluorohexanoate (APFHx). Urine and feces samples were collected for 0–6 hours (urine only) and 6–24 hours and then followed 24-hour intervals until 72 hours after dosing. Expired air was collected over 0–24 and 24–48 hours following oral exposure. For the single dose administration in rats, 97–100% of administered PFHxA dose was recovered within 24 hours with urine as the major route of elimination (73.0–90.2%), followed by feces (7.0–15.5% of the administered dose). No appreciable PFHxA was found in expired air. Two percent of the dose remained in the gastrointestinal tract and

carcass. Comparable findings were observed with the multiple oral dose administration (14 daily doses) scenarios (Iwai, 2011).

Chengelis et al. (2009a) reported the terminal half-life of PFHxA in serum was about 2.4-fold shorter for female Sprague-Dawley rats than for male rats (0.42 h compared to 1.0 h) with a single dose of 10 mg/kg i.v. injection. Likewise, Gannon et al. (2011) reported elimination half-lives for PFHxA of 1.7 and 1.5 h in male rats and 0.5 and 0.7 h in female rats for doses of 2 and 100 mg/kg, respectively. On the other hand, after repeated oral administration (50–300 mg/kg-day) of PFHxA, Chengelis et al. (2009a) found the serum terminal half-life of PFHxA was generally in the range of 2–3 h regardless of sex. Comparable urinary elimination half-lives following single 10 mg/kg i.v. were also observed (males: 2.1 h; females 2.5 h) (Chengelis et al., 2009a). It is unclear why Chengelis et al. (2009a) obtained different half-lives for males versus females from some of their results, but not in others. Evaluation of the half-life from any PK data set depends on the study design, especially the number and spacing of data points relative to the half-life, the type of PK analysis done, and analytic sensitivity. EPA analyzed PFHxA half-lives that combined data across studies to obtain sex-specific values, described in Section 5.2.1 (Approach for Animal-Human Extrapolation of PFHxA Dosimetry).

As noted above, Daikin Industries (2009a, 2009b) evaluated urinary and fecal excretion in Sprague-Dawley rats after 50 mg/kg oral doses for 1 or 14 days. The elimination pattern is consistent with other studies described here, with approximately 90% of the dose recovered in feces and urine by 24 h. Because excretion was only evaluated at 6 h (urine only), 24 h, and multiple days thereafter, these specific studies are not considered quantitatively informative for evaluation of half-life or clearance.

Russell et al. (2015) conducted PK modeling analysis of 6:2 FTOH inhalation (0.5 or 5 ppm) in rats, including its metabolite PFHxA, as described above. The estimated PFHxA half-lives were 1.3 and 0.5 h in male and female rats, respectively, from single-day exposures, with the estimated yield of PFHxA ranging from 0.5 to 1.9 mol%. The model assumes, however, that the yield of PFHxA from 6:2 FTOH is independent of time. This apparent time-dependence in the half-life could be an artifact of that assumption if induction of metabolism during the dosing period leads to a higher yield with later times. A more comprehensive multiday PK analysis would be needed to demonstrate time-dependent PFHxA clearance unequivocally. Using a noncompartmental PK analysis Kabadi et al. (2018) reanalyzed the 1-day data of Russell et al. (2015) and obtained the same half-life values (1.3 and 0.5 h in males and females).

A recent study by Dzierlenga et al. (2019) and NTP (2017) showed no apparent pattern in $t_{½,\beta}$ among the i.v. (40 mg/kg) and two lower oral doses (40 and 80 mg/kg) for each sex (ranges 5.74–9.3 h for male rats and 2.3–7.3 h for female rats), which likely reflects experimental variability. The $t_{½,\beta}$ for the 160 mg/kg oral dose appeared higher than the other three measurements (13.7 ± 14.2 and 12.2 ± 23.6 h [mean ± standard error of the mean] for males and females, respectively), but a loss of dose-concordance occurred among the PK data starting at 6 h

(i.e., the serum concentrations were similar for all dose levels at 6 h and beyond). Also, the data at the last time point (24 h) varied considerably, resulting in large uncertainty in the estimated terminal half-lives (Dzierlenga et al., 2019).

Similar to the elimination half-life in male Sprague-Dawley rats, the estimated serum elimination half-life of PFHxA in male Wistar rats (6 weeks old) was about 2.6 h for a single dose of 100 µg/kg BW or 2.9 h for exposures in drinking water of 1 or 3 months (Iwabuchi et al., 2017). Using a single-compartment PK model with an elimination constant defined as $k_e = \ln(2)/t_{1/2}$ and obtained from a single-day exposure, the predicted serum concentration after 1 and 3 months of exposure was only 10% higher and 15% lower than the measured concentrations at these time points, respectively. Thus, a systematic change in the half-life or clearance with repeated dosing is not apparent.

In support of the empirical estimates of half-lives described above indicating sex-specific differences in the elimination of PFHxA, the differences can be explained (at least in part) on the basis of available mechanistic information. Specifically, sex hormone-dependent differences occur in expression of transporter proteins in the rat kidney. In rats, kidney Oatp1a1 is expressed at the apical membrane of the proximal tubule (Bergwerk et al., 1996) and mediates sodium-independent transport of thyroid hormones, cholesterol-derived molecules (Hata et al., 2003; Shitara et al., 2002), and PFAS (Han et al., 2012; Yang et al., 2010). In male rats, Oatp1a1 mRNA expression was 2.5-fold greater than in females, undetectable in castrated rats, and inducible in male rats by treatment with estradiol (Kudo et al., 2002).

A separate study (Lu et al., 1996) reported the same sex hormone-dependent effect on Oatp1a1 mRNA expression in castrated males or ovariectomized females treated with testosterone or estradiol. Further, Gotoh et al. (2002) confirmed that Oatp1a1 protein levels were undetectable from female rat kidney and highly expressed in male rat kidney. Because these hormone-dependent transporters are predicted to increase renal resorption of PFHxA in male rats, the implication is that PFAS elimination in female rats should be more rapid compared with male rats. Not all the results above match this expectation, which could reflect a limited activity of the renal transporters toward PFHxA (see next paragraph), or simply aspects of experimental design and sampling that measure the PK parameters better in some studies than others. The empirical results of Chengelis et al. (2009a) and Dzierlenga et al. (2019), however, are consistent with this prediction: higher clearance and shorter half-lives in female rats compared to male rats.

Some evidence also suggests the affinity for Oatp1a1 depends on PFAS chain length. Specifically, Yang et al. (2009) examined the role of PFAS (C4–C12) in inhibiting the uptake of estrone-3-sulfate (ES3) using Oatp1a1-expressing Chinese hamster ovary (CHO) cells. They showed the level of inhibition of E3S uptake increased as the chain length increased; for example, PFHxA inhibited ES3 uptake with an inhibition constant (K_i) of 1,858 µM, as compared with 84 µM for PFOA. This high K_i for PFHxA (i.e., the concentration required to inhibit one-half the Oatp1a1 activity, 584 µg/mL) indicates a low affinity of PFHxA for the transporter and thus leads to

predictions of a low impact of Oatp1a1 expression on PFHxA elimination kinetics, contrary to the empirical PK data discussed above. Chengelis et al. (2009a) clearly showed more rapid elimination in female rats vs. male rats at *serum* concentrations below 40 µg/mL, that is, an order of magnitude or more below the K_i. As most of the water is resorbed from the renal filtrate, however, the concentration of PFHxA in the remaining fluid will increase proportionately. Thus, the PFHxA concentrations in the proximal tubule of these rats (where Oatp1a1 is expressed) could be high enough for significant transporter activity, but below the level of saturation.

Collectively, the evidence provides a biologically plausible explanation for the observed sex-specific PFHxA elimination in rats (i.e., the two- to three-fold longer half-life in male versus female rats), although uncertainties remain (Chengelis et al., 2009b; Gannon et al., 2011; Han et al., 2012). Most notably, whether this apparent sex difference in reuptake exists in humans or in species other than rats is unclear. Organic-anion transporters are known to be under hormonal regulation in rat and mouse kidney, with gender-specific differences in their expression likely regulated by sex-hormone receptors. Some evidence suggests similar sex-related differences in humans (Sabolić et al., 2007). Kudo et al. (2001) demonstrated that the sex-related difference in PFOA elimination in rats was abolished when male rats were castrated, increasing to match that in females, and that its elimination was reduced in both females and castrated males treated with testosterone. This demonstration of hormone-related elimination for PFOA and observations of sex differences in the elimination of other PFAS such as PFNA, PFOA, and PFBS (Chengelis et al., 2009a; Kudo et al., 2001) suggest this is a common underlying mechanism for PFAS elimination.

Mouse Studies

As stated above, Iwai (2011) evaluated PFHxA excretion in CD-1 mice after single and 14-day oral exposures. Results were similar for single and multiple dose administrations. After multiple doses, >95% of the administered PFHxA was recovered within 24 hours with urine as the major route of elimination (77.8–83.4%), followed by feces (9.6–12.9% of the administered dose). Only 0.6–0.9% remained in the gastrointestinal tract and carcass. Gannon et al. (2011) also evaluated PFHxA PK in mice but state they did not report half-lives in mice because the data showed a biphasic clearance pattern that precluded use of the standard noncompartmental modeling.

As noted above, Daikin Industries (2009a, 2009b) evaluated urinary and fecal excretion in CD-1 mice after 50 mg/kg oral doses for 1 or 14 days. The elimination pattern is consistent with Iwai (2011), with approximately 90% of the dose recovered in the urine and feces (total) after 24 h. Because excretion was only evaluated at 6 h (urine only), 24 h, and multiple days after the PFHxA dosing ended, however, the studies cited are not considered quantitatively informative for evaluation of half-life or clearance.

Daikin Industries (2010) evaluated the time-course of PFHxA in female Crl:CD(1CR) mouse plasma after single oral gavage doses of 35, 175, and 350 mg/kg, with concentrations measured at 0.5, 2, 4, 6, 8, and 24 h. The estimated half-life was between 0.9 and 1.2 h for the three dose groups

but lacked a dose-dependent pattern. However, the C_{max}/dose was 2.76, 1.88, and 1.30 kg/L for the 35, 175, and 350 mg/kg doses, respectively, indicating saturation of absorption with higher doses. The $AUC_{0-\infty}$/dose was not dose-dependent, although it varied between 5.1 and 6.5 kg-h/L, indicating that clearance was not dose-dependent.

The plasma time-course data from Gannon et al. (2011) and Daikin Industries (2010) were reevaluated by EPA as described with the derivation of the HED in Section 5.2.1 (Approach for Animal-Human Extrapolation of PFHxA Dosimetry) and Appendix C to obtain overall pharmacokinetic parameters.

Monkey Studies

In the aforementioned study on cynomolgus monkeys by Chengelis et al. (2009a), three males and three females received 10 mg/kg PFHxA by i.v. injection. The mean clearance was nearly the same in both sexes (0.122 L/h-kg in males and 0.136 L/h-kg in females), but the estimated half-life appeared longer in males (5.3 ± 2.5 h) than in females (2.4 ± 1.7 h) with a corresponding apparent difference in V_d (0.989 L/kg in males and 0.474 L/kg in females). The similarity of the clearance values and the nearly identical serum values for males and females after the first 4 h suggest no striking sex differences in the pharmacokinetics of PFHxA in monkeys.

Human Studies

No controlled exposure PK studies of PFHxA elimination in humans are available but Russell et al. (2013) applied PK analysis to biomonitoring data from Nilsson et al. (2013) to estimate the half-life of PFHxA in humans. Specifically, Russell et al. (2013) estimated the apparent half-life of PFHxA in humans by analyzing biomonitoring data collected from professional ski wax technicians and then compared the human estimates of PFHxA elimination to that for mice, rats, and monkeys. For the human monitoring study, blood samples (n = 94) were collected from male professional ski wax technicians (n = 11) and analyzed for PFHxA in plasma and serum. Personal and area air concentration monitoring of the ski wax subjects and facilities demonstrated both the metabolic precursor, 6:2 FTOH, and PFHxA were present in all locations, but the arithmetic mean concentration of 6:2 FTOH ranged from over 100 times higher than PFHxA to almost 100 times lower, across the monitoring locations. A one-compartment model with first-order kinetics was used for PK analyses. The estimated geometric mean half-life of PFHxA was 32 days with a range of 14–49 days in the studied population. PFHxA plasma concentrations declined below the plasma detection limit of 0.05 ng/mL within a period of 2–4 months after exposure ceased, reflecting the relatively rapid elimination rate of PFHxA. In contrast, the half-life of PFHxS in humans was estimated to range from 5 to 9 years (Olsen et al., 2007).

A recent analysis by Luz et al. (2019) found no significant species- or sex-related differences in the elimination kinetics of PFHxA. The PK analysis, however, is attributed to a meeting abstract (Buck and Gannon, 2017) and provides no details of the methods the authors used. The text of Luz et al. (2019) indicates the analysis of Buck and Gannon (2017) used data from only 3 of the 11

subjects of Nilsson et al. (2013), specifically the 3 with the most rapid elimination, reducing the extent to which the conclusion can be assumed to represent the study population as a whole. Luz et al. (2019) state slower *apparent* elimination could occur in some subjects because of ongoing exposure. Although ongoing exposure could cause this effect, it is also possible that elimination in some individuals is slower than others due to interindividual variability. In the absence of independent evidence that ongoing exposure occurred in the eight human subjects of Nilsson et al. (2013) who were excluded in this later analysis, EPA does not consider basing conclusions on human elimination on only the three individuals who had the most rapid elimination appropriate.

EPA examined the data of Nilsson et al. (2013), and the observed seasonal variation appears to show a longer systemic period of exposure (when blood levels are elevated vs. declining) for some individuals than others. Also, the data set includes samples with concentrations below the limit of detection (LOD) that should be treated with an appropriate statistical model to account for the censoring of these data. A detailed description of EPA's analysis is provided in Appendix C.2. Briefly, each ski-wax technician in the study was presumed to have a constant rate of exposure up to a date that is individual and year specific for when exposure stopped and the elimination began. Specifically, we used a one-compartment i.v.-infusion model to fit the data:

$$C(t) = \begin{cases} \frac{A}{tinf \cdot ke} \cdot \left(1 - e^{-ke \cdot t}\right), & if\ t \le tinf \\ \frac{A}{tinf \cdot ke} \cdot \left(1 - e^{-ke \cdot tinf}\right) \cdot e^{-ke \cdot (t - tinf)}, & if\ t > tinf \end{cases} \quad (3\text{-}2)$$

Where $A = dose/V_d$, *tinf* is the time period of exposure (treated as an infusion), and *ke* is the elimination rate. The model is analyzed through hierarchical Bayesian analysis, with *A* and *tinf* estimated independently for each individual technician although the technician-level *ke* is drawn from a population-level distribution. Note blood concentrations were measured only once a month and no other data on exposure is available. Thus, although the model clearly simplifies the exposure estimation, estimating a larger number of parameters reliably would not be possible. As such, the model allows for estimating variation among individuals without subjectively selecting a subset of the technicians for analysis. The resulting distribution of *ke* had a mean (90% confidence interval, CI) of 1.48 (0.89–2.44) month^{-1}. Using an average V_d of 0.7315 L/kg for male and female monkeys from Chengelis et al. (2009a), the resulting mean (CI) for human clearance is CL = $V_d \cdot ke$ = 1.50 (0.90 – 2.48) mL/kg-h.

Xiao et al. (2011) measured the serum concentrations of 10 PFAA chemicals in 227 nonoccupationally exposed individuals aged 0.3–90 years (133 males and 94 females) in China. Significant positive correlations were observed between age and serum levels of PFAA chemicals except for PFBS, PFHpA, and PFHxA. Spearman correlation coefficients between age and serum PFHxA were 0.20, −0.02, and 0.08 for males, females, and the combined data, respectively. Collectively, the findings indicated no age-related accumulation of PFHxA in human bodies, which is consistent with the relatively short half-life.

3.1.5. PBPK Models

No PBPK model is available for PFHxA in rats, mice, or monkeys. Fàbrega et al. (2015) described a PBPK model for multiple PFAS in humans, including PFHxA. However, Fàbrega et al. (2015) state two key parameters that determine the rate of resorption from glomerular filtrate in the kidney were identified using the data from the Ericson et al. (2007) epidemiological survey of PFAS exposure in residents of Catalonia, Spain. Because PFHxA was not detected in any individuals sampled by Ericson et al. (2007), EPA does not consider it possible to reliably identify elimination parameters from that data set. Further, the individual exposure or elimination data needed to associate the blood concentrations of Ericson et al. (2007) with urinary clearance rates are not reported in either paper. Thus, uniquely identifying two parameters with a single combination of average PFHxA exposure and average blood concentration is impossible. Finally, as described above (Distribution in Humans), the tissue: blood partition coefficients Fàbrega et al. (2015) estimated are not considered suitable for the purposes of this assessment due to the 4+-year lag in measurements between collection of the blood samples and the tissue samples and because they are inconsistent with data on PFHxA distribution in other species, including monkeys. Thus, the PBPK model of Fàbrega et al. (2015) is not considered sufficiently suitable for use in this assessment.

3.1.6. Summary

The PFHxA elimination half-lives and clearance values reported in studies described above are important for interpreting and quantifying health outcomes potentially associated with PFHxA exposure, as discussed in later sections of this assessment. The most notable finding was the apparent sex-specific PK differences between male and female mice and rats where female rodents eliminate PFHxA 2–3 times faster than males (Table 3-1). Although monkeys have half-lives and clearance values in the same range as mice and rats, the clearance in female monkeys is only 11% faster than in males. This indicates that the significant sex differences observed in rodents does not appear to apply to primates. Humans have a much longer serum elimination half-life (768 hours) than rodents and monkeys (2–7 hours). The difference could be a consequence of species differences in the expression or activity of the renal transporters that reabsorb PFAS, but this has not been demonstrated. All available PK evidence is summarized below in Table 3-1.

According to EPA's $BW^{0.75}$ guidelines (U.S. EPA, 2011), use of chemical-specific data for dosimetric extrapolation such as the PFHxA-specific data described above is preferable to the default method of $BW^{0.75}$ scaling. That is the case here. For example, using the standard species BWs of 0.25 kg in rats and 80 kg in humans, the half-life in humans is predicted to be 4.2 times greater than rats. Given half-lives in the range of 0.4–14 h among male and female rats (Table 3-1), one would then predict half-lives of 1.6–57 h in humans, 20–200 times lower than the range estimated by Russell et al. (2013) and 10–100 times lower than the range estimated by EPA. Thus, based on the PFHxA-specific PK data, use of $BW^{0.75}$ for dosimetric extrapolation could lead to an

underprediction of human elimination by 1–2 orders of magnitude. Therefore, use of $BW^{0.75}$ as an alternative means of extrapolation is not considered further for PFHxA, and the preferred, data-driven approach will be used for the dosimetric extrapolation.

Table 3-1. Summary of PK evidence for PFHxA

Species/Sex	Study design (dose)	Elimination half-life (beta) (h)	AUC/dose (kg-h/L)	Clearance (mL/h-kg)	Volume of distribution (mL/kg)	Reference
Rats						
Male	Single i.v. dose (10 mg/kg)	1.0	8.7	116	175	Chengelis et al. (2009a)
	Single oral dose (50 mg/kg)	2.2	10.0	NR	NR	
	Single oral dose (150 mg/kg)	2.4	6.1	NR	NR	
	Single oral dose (300 mg/kg)	2.5	8.4	NR	NR	
Female	Single i.v. dose (10 mg/kg)	0.42	1.3	775	466	
	Single oral dose (50 mg/kg)	2.6	2.4	NR	NR	
	Single oral dose (150 mg/kg)	2.2	2.2	NR	NR	
	Single oral dose (300 mg/kg)	2.1	3.5	NR	NR	
Male	Single i.v. dose (40 mg/kg)	8.0 ± 2.2	7.4 ± 0.7	136 ± 13	430 ± 112	Dzierlenga et al. (2019) NTP (2017)
	Single oral dose (40 mg/kg)	9.3 ± 20.8	9.7 ± 1.3	103 ± 13	601 ± 470	
	Single oral dose (80 mg/kg)	5.7 ± 4.6	6.6 ± 0.5	153 ± 11	496 ± 81	
	Single oral dose (160 mg/kg)	14 ± 14	6.8 ± 0.6	147 ± 14	615 ± 367	
Female	Single i.v. dose (40 mg/kg)	7.3 ± 2.0	3.1 ± 0.3	327 ± 33	223 ± 45	
	Single oral dose (40 mg/kg)	2.3 ± 2.1	6.1 ± 1.1	164 ± 29	327± 149	
	Single oral dose (80 mg/kg)	5.5 ± 2.6	3.2 ± 0.4	314 ± 39	560 ± 113	
	Single oral dose (160 mg/kg)	12 ± 24	3.7 ± 0.5	274 ± 37	473 ± 158	
Male	Single oral dose (2 mg/kg)	1.7 ± 0.6	8 ± 1.5	NR	NR	Gannon et al. (2011)
	Single oral dose (100 mg/kg)	1.5 ± 0.2	6.5 ± 1.4	NR	NR	
Female	Single oral dose (2 mg/kg)	0.5 ± 0.1	2.5 ± 0.5	NR	NR	
	Single oral dose (100 mg/kg)	0.7 ± 0.3	2.5 ± 0.7	NR	NR	

Species/Sex	Study design (dose)	Elimination half-life (beta) (h)	AUC/dose (kg-h/L)	Clearance (mL/h-kg)	Volume of distribution (mL/kg)	Reference
Male	Single i.v. dose (0.1 mg/kg)	2.7	9.8	NR	400	Iwabuchi et al. (2017)
Male	Single inhalation* (0.5 ppm)	1.3	ND[†]	107	NR	Kabadi et al. (2018)
	Single inhalation* (5.0 ppm)	1.3	ND[†]	277	NR	
Female	Single inhalation* (0.5 ppm)	0.5	ND[†]	107	NR	
	Single inhalation* (5.0 ppm)	0.5	ND[†]	277	NR	
Mice						
Male	Single oral dose (2 mg/kg)	ND	12	NR	NR	Gannon et al. (2011)
	Single oral dose (100 mg/kg)	ND	12	NR	NR	
Female	Single oral dose (2 mg/kg)	ND	4	NR	NR	
	Single oral dose (100 mg/kg)	ND	6.4	NR	NR	
Monkeys						
Male	Single i.v. dose (10 mg/kg)	5.3 ± 2.5	8.4 ± 1.8	122 ± 24	989 ± 579	Chengelis et al. (2009a)
Female	Single i.v. dose (10 mg/kg)	2.4 ± 1.7	7.5 ± 1.3	136 ± 22	474 ± 349	
Humans						
Males and females	Post-exposure observation	768 (336–1,176) 337 (205–561)	ND	ND 1.50 (0.90–2.48)	ND	Russell et al. (2013) Current analysis

i.v. = intravenous; ND = not determined; NR = not reported.
*6-hour inhalation exposure to 6:2 fluorotelomer alcohol (FTOH)
[†]Dose of PFHxA unknown

3.2. NONCANCER EVIDENCE SYNTHESIS AND INTEGRATION

For each potential health effect discussed below, the synthesis describes the evidence base of available human and animal studies. The PFHxA animal literature inventory summarizes the evidence base on potential health effects (organized by organ or system) from the available *high* and *medium* confidence short-term, developmental, subchronic, and chronic studies in mice and rats (Chengelis et al., 2009b; Iwai and Hoberman, 2014; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Some organs/systems for which data were available (i.e., dermal, musculoskeletal/connective tissue, sensory, ocular) had no evidence of an effect even at the highest administered dose, and others (i.e., respiratory, gastrointestinal system, cardiovascular, and metabolic effects) were limited findings of unclear toxicological relevance (e.g., outcome not necessarily adverse or considered nonspecific). Thus, these data are not synthesized in detail below, but are summarized in the animal literature inventory. Similarly, other effects, including body weights and survival, which had no effect or lowest effect levels at the highest administered dose were not the drivers for hazard identification but were used to aid interpretation of other potential health effects. They are summarized in the animal literature inventory under the appropriate systemic/whole body system. Studies considered suitable for dose-response were given a more detailed summarization of study methods and findings using HAWC. For hepatic changes some individual or constellation of liver endpoints might be considered adaptive in nature. Therefore, to draw inferences regarding the adversity of this type of liver effect, the panel recommendations outlined by Hall et al. (2012) were used to develop conclusions around adversity while also considering that Hall et al. (2012) developed adaptive/adversity criteria in the context liver tumor formation.

3.2.1. Hepatic Effects

Human

Serum Enzymes

Two epidemiological studies report on the relationship between PFHxA exposure and liver enzymes. Of these, one (Jiang et al., 2014) was considered critically deficient in the confounding domain and was considered overall *uninformative*. Based on these deficiencies, the study was excluded from further analysis (Figure 3-1). The remaining study (Nian et al., 2019) was cross-sectional and was classified as *medium* confidence (Figure 3-1). Exposure levels for PFHxA, however, were low (detected in 70% of the study population, adult residents of Shenyang, China, median [interquartile range, IQR] = 0.2 [0.01–0.5]), which would reduce the study's ability to detect an association if present. The study did not observe an association between PFHxA levels and serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total bilirubin, or cholinesterase.

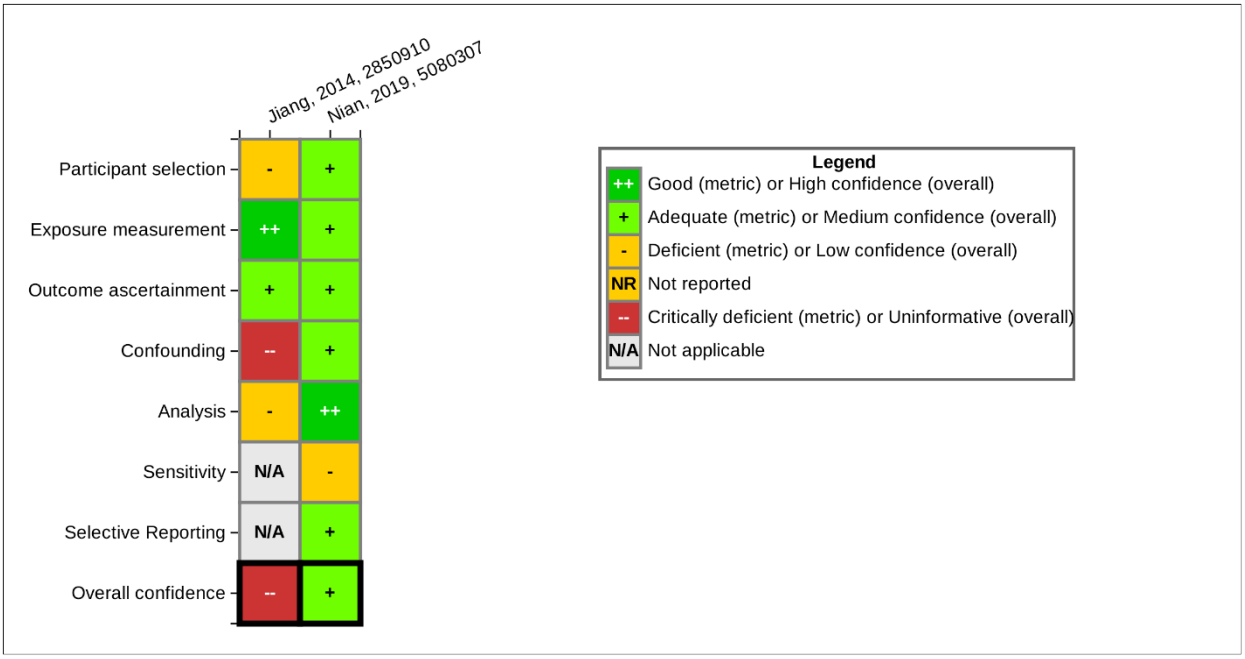


Figure 3-1. Study evaluation for human epidemiological studies reporting hepatic system findings from PFHxA exposures (full details available by clicking the HAWC link). Note that for N/A, critical deficiencies in confounding domains were identified and the study was judged as *uninformative*; thus, the remaining domains were not evaluated.

Animal

Hepatic outcomes were evaluated in multiple short-term, subchronic, or chronic studies in rats and mice (Chengelis et al., 2009b; Iwai and Hoberman, 2014; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Generally, studies were rated as *medium or high* confidence for the hepatic outcomes, but some outcome-specific considerations for study evaluation were influential on the overall confidence ratings for hepatic effects. Histopathology for Chengelis et al. (2009b) was rated *low* confidence because of issues related to observational bias, endpoint sensitivity and specificity, and results presentation. Results of the outcome-specific confidence evaluations are presented in Table 3-2 below, and details are available by clicking the HAWC link.

Table 3-2. Evaluation results for animal studies assessing effects of PFHxA exposure on the hepatic system

Author (year)	Species, strain (sex)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Clinical chemistry	Peroxisomal beta oxidation
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	++	++	NM
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	++	-	++	++
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	++	++	NM

Study evaluation for animal toxicological hepatic endpoints reported from studies with male and female rats receiving by gavage PFHxA[a] or PFHxA sodium salt.[b] Study evaluation details for all outcomes are available by clicking the HAWC link.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Organ Weight

Overall, findings of increased liver weights after oral PFHxA or PFHxA sodium salt exposures in rats were consistent (Figure 3-2; exposure response array link). Relative liver weights (Table 3-3), are generally considered more reliable than absolute liver weights because they take into account large variations in body weight that could skew organ weight interpretation (Hall et al., 2012). Large variations in body weights were not observed after PFHxA exposures in male and female adult rats, and changes in both relative and absolute liver weights were similarly increased and dose responsive. Increases in relative and absolute liver weights were dose-dependently increased in all three short-term and subchronic studies. Statistically significant increases in male rat relative liver weights were observed with oral doses of ≥200–250 mg/kg-day, whereas statistically significant increases in female rats were observed only at ≥500 mg/kg-day. Specifically, in the 28-day study, relative liver weight was increased (14%) in male rats at 250 mg/kg-day, where a similar increase (15%) was observed in female rats at 500 mg/kg-day (NTP, 2018). In the subchronic studies, relative liver weights were increased (22%) at

200 mg/kg-day in males (with no change in females) in one study (Chengelis et al., 2009b), and the other study observed increases of 63% and 37% at 500 mg/kg-day in males and females, respectively (Loveless et al., 2009). Note that PFHxA effects on relative liver weights had resolved by 30 days in the recovery group (Chengelis et al., 2009b). Liver weights were not evaluated in the chronic study (Klaunig et al., 2015).

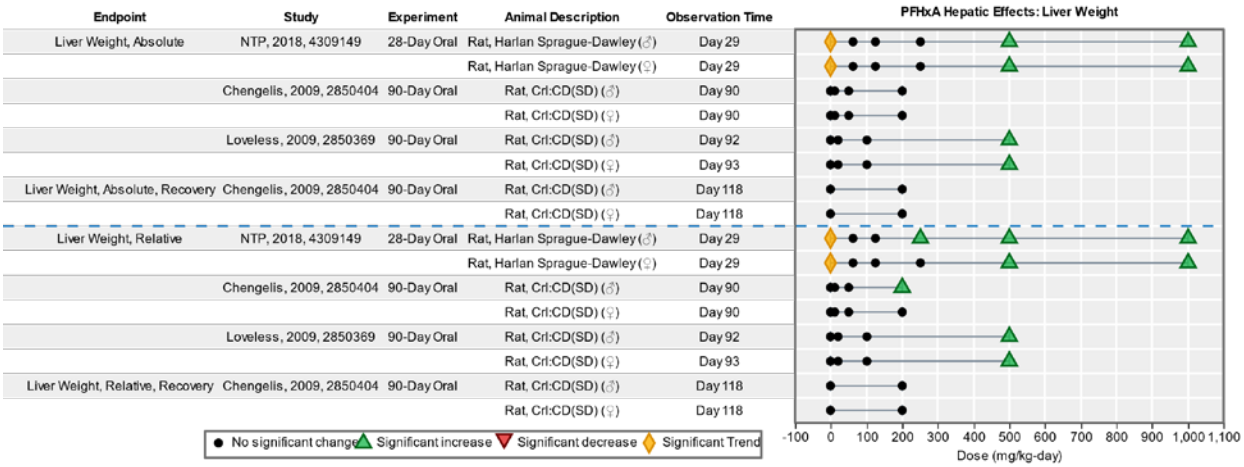


Figure 3-2. Liver weights (absolute and relative) after short-term and subchronic PFHxA exposures (full details available by clicking the HAWC link).

Table 3-3. Percent increase in relative liver weight due to PFHxA exposure in short-term and subchronic oral toxicity studies

Reference	Dose (mg/kg-d)															
	2.5	5	10	15	20	30	35	50	62.5	100	125	175	200	250	500	1,000
28-day female rat (NTP, 2018)									1		2			7	**15**	**47**
28-day male rat (NTP, 2018)									8		7			**14**	**32**	**64**
90-day female rat (Chengelis et al., 2009b)			4					6					5			
90-day male rat (Chengelis et al., 2009b)			1					1					**22**			
90-day female rat (Loveless et al., 2009)					−1					5					**37**	
90-day male rat (Loveless et al., 2009)					0					11					**63**	

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Histopathology

Treatment-related increases in liver weight can result from various changes in hepatic morphology including hyperplasia of any resident liver cell type, cellular hypertrophy, inflammation, fibrosis, increase in hepatocyte size, neoplasia, congestion, or metabolic enzyme induction (Hall et al., 2012; Thoolen et al., 2010; U.S. EPA, 2002a). As shown in Table 3-4 and summarized in the HAWC link, four studies evaluated liver histopathology in rats. One observed effect of PFHxA exposure was hepatocellular hypertrophy that was consistent across the four studies. Hepatic hypertrophy can refer to an increase in liver weight and size; an increase in hepatocyte size caused by abnormal storage of water, glycogen, lipids, or organelle proliferation; and an increase in hepatic enzyme induction (Hall et al., 2012; Thoolen et al., 2010; U.S. EPA, 2002a). Increased hepatocellular hypertrophy was observed in adult male and female rats in the *high* confidence short-term (NTP, 2018) and *high* confidence subchronic (Loveless et al., 2009) studies at doses ≥100–500 mg/kg-day. In the *low* confidence subchronic study, centrilobular hepatocellular hypertrophy was found at 200 mg/kg-day in male rats only (Chengelis et al., 2009b). In the chronic study (Klaunig et al., 2015), no change in hepatocellular hypertrophy was found, although the highest administered dose was 2–10 times lower (100 mg/kg-day in males or 200 mg/kg-day in females) than the highest dose in other studies where effects on hypertrophy were observed. Coherent with findings on liver weight, the observations of hepatocellular hypertrophy were dose-dependent and male rats were more sensitive than females.

Table 3-4. Incidence of hepatocellular hypertrophy findings in adult rats due to PFHxA exposure in short-term and subchronic oral toxicity studies

Reference	Dose (mg/kg-d)									
	10	20	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)				0/10		0/10		0/10	0/10	9/10
28-day, male rat (NTP, 2018)				0/10		0/10		0/10	9/10	10/10
90-day, female rat (Chengelis et al., 2009b)	0/10		0/10				0/10			
90-day, male rat (Chengelis et al., 2009b)	0/10		0/10				7/10			
90-day, female rat (Loveless et al., 2009)		0/10			0/10				5/10	
90-day, male rat (Loveless et al., 2009)		0/10			4/10				10/10	
90-day, female rat, 30-day recovery (Loveless et al., 2009)									4/10	

Reference	Dose (mg/kg-d)									
	10	20	50	62.5	100	125	200	250	500	1,000
90-day, female rat, 90-day recovery (Loveless et al., 2009)									0/10	
90-day, male rat, 30-day recovery (Loveless et al., 2009)									9/10	
90-day, male rat, 90-day recovery (Loveless et al., 2009)									6/10	

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Other pathological findings of PFHxA-mediated hepatic effects included increased hepatocellular necrosis in rats, with a slight increase in male rats ($n = 1/10$ vs. 0/10 in other groups) reported in a subchronic study at 200 mg/kg-day PFHxA, the highest dose tested (Chengelis et al., 2009b). In the chronic study, Klaunig et al. (2015) reported hepatocellular necrosis in females that was characterized as hepatocellular necrosis ($n = 12/70$ vs. 2/60 in controls, $p < 0.05$) or hepatocellular, centrilobular necrosis ($n = 4/70$ vs. 1/60 in controls) in the 200 mg/kg-day dose group (the highest dose tested) in the *high* confidence chronic study. The authors noted most necrosis findings were in animals that died or were euthanized prior to scheduled necropsy and the increased mortality was not treatment related, but was due to mechanical injury, gavage trauma, reflux injury, or spontaneous disease processes (Klaunig et al., 2015). The authors reported no treatment-related increases in hepatocellular necrosis ($n = 6/70$ vs. 4/60 in controls) or necrosis in the centrilobular regions of the liver lobule ($n = 1/46$ vs. 0/42 in controls) in male rats up to the highest dose for that sex, 100 mg/kg-day. Other findings included nonsignificant congestion in males ($n = 23/70$ vs. 15/60 in controls) and females ($n = 8/70$ vs. 11/60 in controls) (Klaunig et al., 2015). Incidence of necrosis were not observed in the short-term study (NTP, 2018), and the subchronic study by Loveless et al. (2009) did not report histological findings other than hepatocellular hypertrophy (no data on necrosis were available).

Other histopathological findings included observations of hepatocellular cytoplasmic alterations ($p < 0.05$) in adult male and female rats at the highest dose (1,000 mg/kg-day in the short-term study (NTP, 2018). All results reported above can be viewed using the HAWC link.

Clinical Chemistry

A clinical chemistry panel measures the proteins, enzymes, chemicals, and waste products in the blood. These measures, when evaluated together and with other biomarkers are informative diagnostic tests of organ function and when interpreted together with histopathology are useful for the assessment of adverse liver effects.

Serum Enzymes

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are often useful indicators of hepatic enzyme induction or hepatocellular damage as increased serum levels are thought to be due to hepatocyte damage resulting in release into the blood, whereas ALP is localized to the bile canalicular membrane and more indicative of hepatobiliary damage (Amacher et al., 1998; Hall et al., 2012). PFHxA effects on the serum enzymes ALT, AST, and ALP included <2-fold increases in serum enzyme across the three short-term and subchronic studies, except for one 2.4-fold increase in male rats at 200 mg/kg-day in the high confidence subchronic study (Chengelis et al., 2009b). No clear pattern of effects on the serum enzymes were reported in the chronic study (Klaunig et al., 2015), but the highest dose was 100 or 200 mg/kg-day PFHxA in male or female rats, respectively. Full study details are available in Figure 3-3 and by clicking the HAWC link. Percent changes are provided in Table 3-5, Table 3-6, and Table 3-7.

Specifically, in the short-term study, ALT, AST, and ALP were increased in a dose-response gradient in adult male rats at doses as low as 500 mg/kg-day (NTP, 2018). In female rats, ALT and AST measures were increased in a dose-response gradient at doses as low as 500 mg/kg-day, whereas ALP was increased only in the highest (1,000 mg/kg-day) dose group (NTP, 2018).

In the subchronic studies, ALT increases were observed only in male rats at PFHxA sodium salt exposures as low as 20 mg/kg-day in one subchronic study (Loveless et al., 2009) and in the highest PFHxA dose group (200 mg/kg-day) in the other subchronic study (Chengelis et al., 2009b). AST was increased in only one subchronic study in males at ≥20 mg/kg-day (Loveless et al., 2009). Chengelis et al. (2009b) reported increased AST in males only in the 200 mg/kg-day dose group that resolved after the 30-day recovery (Table 3-6).

ALP was increased in both subchronic studies with significant increases observed in the highest exposure groups [200 (Loveless et al., 2009) and 500 mg/kg-day (Chengelis et al., 2009b)] that resolved by the 30-day recovery (Chengelis et al., 2009b) (Table 3-7). The chronic study did not include a 13-week endpoint that would have been useful for group mean comparisons with the test measures in the subchronic studies (as clinical pathology test values often change with animal age) (AACC, 1992).

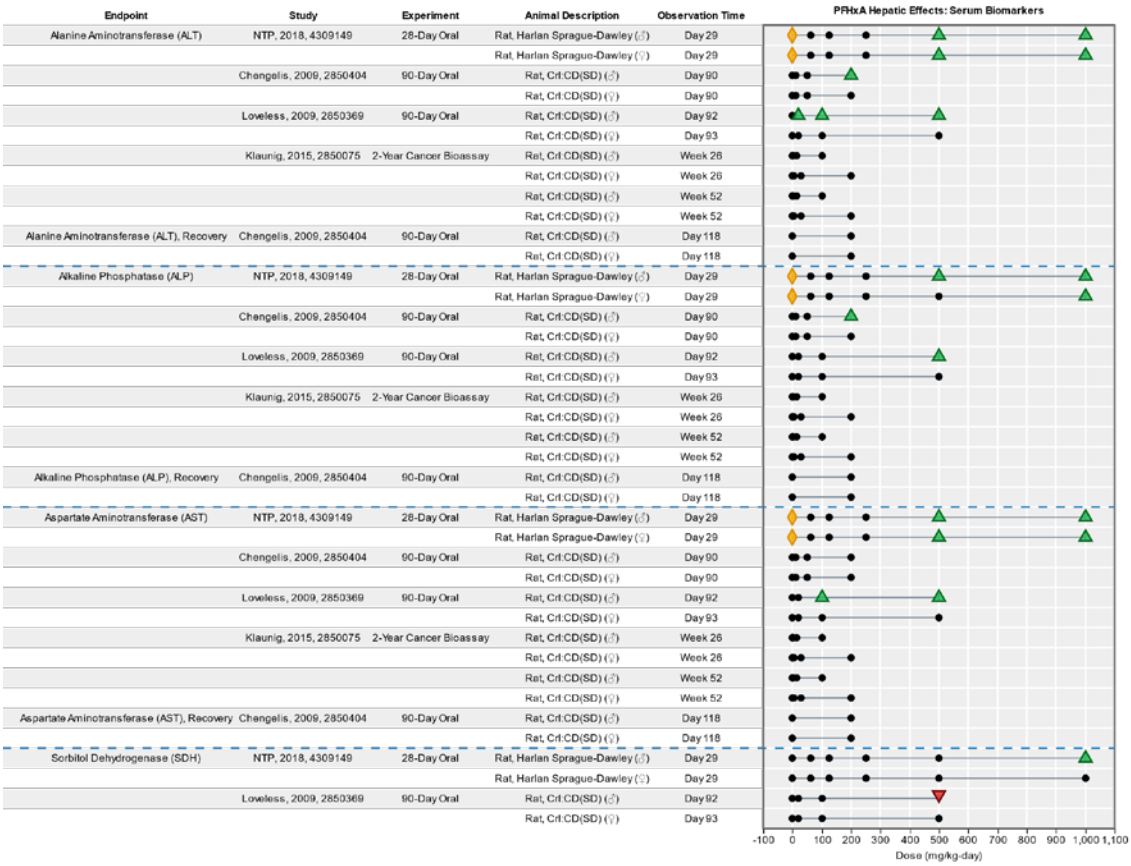


Figure 3-3. Clinical chemistry findings (serum enzymes) after short-term, subchronic, and chronic PFHxA exposures (full details available by clicking the HAWC link).

Table 3-5. Percent change in alanine aminotransferase due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								11		15		10	**35**	**44**
28-day, male rat (NTP, 2018)								4		4		8	**26**	**64**
90-day, female rat (Chengelis et al., 2009b)			60				29				3			
90-day, male rat (Chengelis et al., 2009b)			12				22				**237**			
90-day, female rat (Loveless et al., 2009)					−46				−25				−4	

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
90-day, male rat (Loveless et al., 2009)					**33**				**44**				**56**	
Week 26, female rat (Klaunig et al., 2015)		44				−62					−57			
Week 26, male rat (Klaunig et al., 2015)	10			12					117					
Week 52, female rat (Klaunig et al., 2015)		7				−15					−10			
Week 52, male rat (Klaunig et al., 2015)	194			2					27					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Table 3-6. Percent change in aspartate aminotransferase due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								−1		0		0	**11**	**18**
28-day, male rat (NTP, 2018)								3		1		6	**16**	**36**
90-day, female rat (Chengelis et al., 2009b)			38				18				5			
90-day, male rat (Chengelis et al., 2009b)			−3				16				**9**			
90-day, female rat (Loveless et al., 2009)					−58				−44				−36	
90-day, male rat (Loveless et al., 2009)					74				**25**				**39**	
Week 26, female rat (Klaunig et al., 2015)		−10				−64					−65			
Week 26, male rat (Klaunig et al., 2015)	−4			−2					−63					
Week 52, female rat (Klaunig et al., 2015)		11				−11					−15			

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
Week 52, male rat (Klaunig et al., 2015)	32			−1					13					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Table 3-7. Percent change in alkaline phosphatase due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								8		19		2	7	**38**
28-day, male rat (NTP, 2018)								−4		−2		2	**22**	**51**
90-day, female rat (Chengelis et al., 2009b)			−5				−22				4			
90-day, male rat (Chengelis et al., 2009b)			−2				15				**34**			
90-day, female rat (Loveless et al., 2009)					−16				24				−18	
90-day, male rat (Loveless et al., 2009)					17				20				**60**	
Week 26, female rat (Klaunig et al., 2015)		16				27					7			
Week 26, male rat (Klaunig et al., 2015)	−4			1					−1					
Week 52, female rat (Klaunig et al., 2015)		−18				4					−12			
Week 52, male rat (Klaunig et al., 2015)	−15			−5					−2					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Blood Proteins

The two major classes of proteins in the blood stream, albumin and globulin, are made by the liver (with some globulins also made by the immune system) (Boron and Boulpaep, 2017). Blood proteins are routinely measured in diagnostic panels because changes in blood protein levels,

particularly a decrease, can be indicators of protein loss due to kidney disease or impeded production in the liver, such as in liver disease (Boron and Boulpaep, 2017). Blood protein measures (total protein and globulin) were in general decreased across short-term and subchronic studies, with consistent and coherent dose-dependent findings across study designs. No PFHxA-related treatment effects on blood proteins were found in the chronic study at doses up to 100 or 200 mg/kg-day PFHxA (the highest doses administered) in male or female rats, respectively. The pattern of findings suggests a primary effect on blood globulins (decreased) in response to PFHxA exposure that was driving decreases in total protein and increases in the albumin:globulin ratio (A:G). These findings are discussed below and detailed information can be viewed in Figure 3-4 or by clicking on the HAWC link.

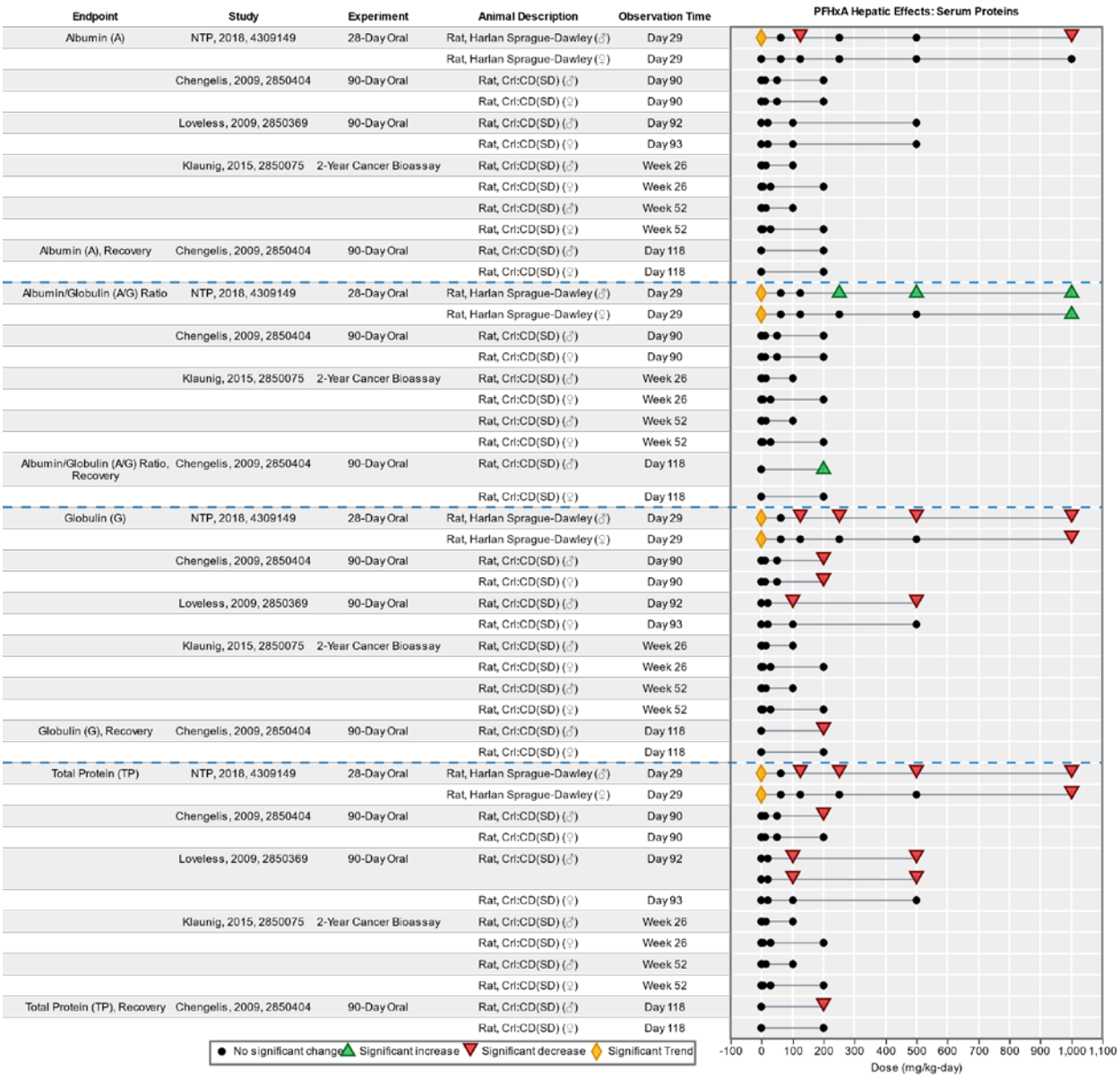


Figure 3-4. Blood protein findings after short-term, subchronic, and chronic PFHxA exposures (full details available by clicking the HAWC link).

Effects on total protein (TP; Table 3-8), the total amount of albumin and globulin found in blood, is associated with chronic liver disease (Whalan, 2014), was decreased up to 20% in male rats receiving a dose ≥125 mg/kg-day in the 28-day study (with a significant trend) (NTP, 2018). A dose-responsive decrease (6–14%, ≥100 mg/kg-day) in TP also was observed in male rats (Chengelis et al., 2009b; Loveless et al., 2009) with decreased levels observed in males (−6%, 200 mg/kg-day) at the 30-day recovery (Chengelis et al., 2009b). Albumin is a major blood protein that binds fatty acids, cations, bilirubin, thyroxine (T4), and other compounds. Decreased albumin levels are associated with decreased synthesis in the liver, increased catabolism, severe diffuse liver

disease, subacute hepatitis, hepatocellular damage, ascites, cirrhosis, and chronic alcoholism (Whalan, 2014). Slight decreases ($p < 0.05$) in albumin were reported only in males exposed for 28 days to 125 mg/kg-day (6% decrease) and 1,000 mg/kg-day (7% decrease) PFHxA (NTP, 2018). The biological significance of this magnitude of change is unclear. No effects on albumin were identified in the subchronic or chronic studies.

Globulin, a collection of blood proteins larger than albumin made by both the liver and immune system, were decreased in all but the 2-year study (Table 3-9). Globulin decreases were observed in both male and female rats treated with PFHxA in the short-term study at ≥250 mg/kg-day and 1,000 mg/kg-day, respectively (NTP, 2018). Consistent with the short-term study, decreases were also observed in both males and females in the highest dose groups [200 (Chengelis et al., 2009b) and 500 mg/kg-day (Loveless et al., 2009)]. Notably, globulin decreases (10%) persisted at the 30-day recovery in males (200 mg/kg-day) and returned to normal in females (Chengelis et al., 2009b).

The decrease in globulin was consistent with increases in A:G, a routine blood test used to screen for liver, kidney, immune, and gastrointestinal function. The A:G was increased in males and females (113–160% at ≥250 mg/kg-day and 142% at 1,000 mg/kg-day) with significant trends in both sexes (NTP, 2018). Chengelis et al. (2009b) observed an increase (10%) at the 30-day recovery in rats receiving an oral dose of 200 mg/kg-day.

Table 3-8. Percent change in total protein (TP) and globulin (G) due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								0		1		−1	−1	**−7**
28-day, male rat (NTP, 2018)								−4		**−7**		**−7**	**−10**	**−20**
90-day, female rat (Chengelis et al., 2009b)			4				3				−4			
90-day, male rat (Chengelis et al., 2009b)			−3				−4				**−6**			
90-day, female rat 30-day recovery (Chengelis et al., 2009b)											−3			
90-day, male rat 30-day recovery (Chengelis et al., 2009b)											**−6**			
90-day, female rat (Loveless et al., 2009)					−1				−1				−3	

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
90-day, male rat (Loveless et al., 2009)					0				**−6**				**−14**	
2-year, female rat (Klaunig et al., 2015)		−1				1					0			
2-year, male rat (Klaunig et al., 2015)	−1			0					−3					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Hepatobiliary Components

Other indicators of potential liver dysfunction or injury included impacts on bile components essential for normal lipid metabolism and red blood cell breakdown. ALP (discussed with serum enzymes and in Table 3-7) is an indicator of bile duct obstruction and was consistently increased in male and female rats in the short-term study (NTP, 2018) and subchronic studies (Chengelis et al., 2009b; Loveless et al., 2009). In the short-term study (NTP, 2018), bile acids were increased at the highest dose (1,000 mg/kg-day) with a significant trend (a possible indication of cholestatic liver injury), and bilirubin was decreased in a dose-response gradient across both the short-term and subchronic (Loveless et al., 2009) studies (Figure 3-5). Lower than normal bilirubin levels are usually not a concern and can be reduced in response to increased conjugation rates after hepatic enzyme induction and excretion into bile (Hall et al., 2012).

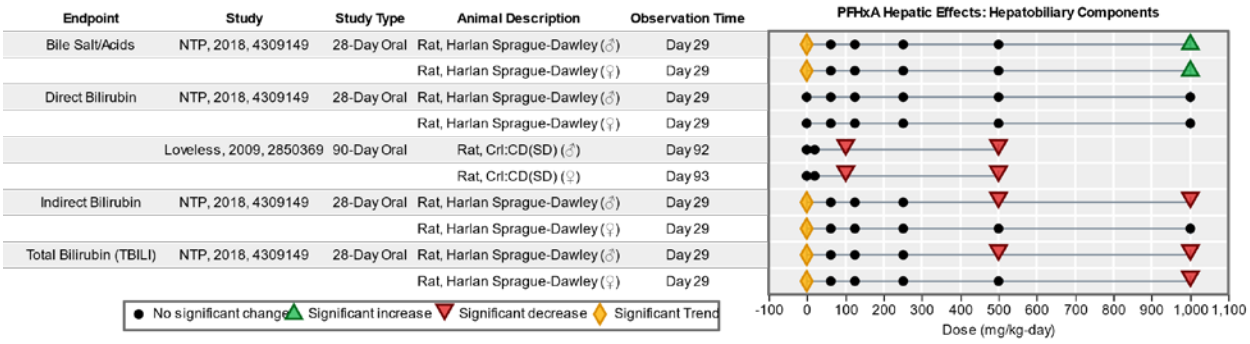


Figure 3-5. Hepatobiliary findings in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Mechanistic Evidence and Supplemental Information

The available evidence base reports increased liver weight, hepatocellular hypertrophy, hepatocellular necrosis, increased (1.5–2.5-fold) serum enzymes, decreased total protein (driven by decreased globulin), and decreased bilirubin effects in rats exposed to PFHxA. Although multiple pathways might be involved in the observed liver effects (e.g., abnormal storage of water, glycogen,

lipids; (Thoolen et al., 2010; U.S. EPA, 2002a); organelle or cellular proliferation; increased metabolizing enzyme induction), the available mechanistic evidence was limited to effects on peroxisomal beta oxidation with some evidence for receptor (e.g., PPARα and CAR) activation.

Peroxisomal beta oxidation

Peroxisomal proliferation can be induced within the peroxisomes to perform beta oxidation of lipids into acetyl CoA and hydrogen peroxide (H_2O_2) (Reddy, 2004). Hydrogen peroxide is a reactive metabolite and can cause oxidative damage to the surrounding tissue. Two subchronic studies measured PFHxA induction of peroxisomal beta oxidation activity in male and female rats (Chengelis et al., 2009b; Loveless et al., 2009) (Figure 3-6) and both were considered *medium* or *high* confidence for this outcome. Chengelis et al. (2009b) reported an increase ($p < 0.05$, 1.37-fold) in males treated with 200 mg/kg-day at 13 weeks. Loveless et al. (2009) found increased peroxisomal beta oxidation activity in both sexes gavaged with 500 mg/kg-day for 10 and 90 days (males, 3.1- and 4.36-fold, respectively; females, 1.45- and 2.67-fold, respectively). Notably, increased activity persisted after the 30-day recovery. Male rats were more sensitive than females, with males in the 100 mg/kg-day group also showing increased peroxisomal beta oxidation .

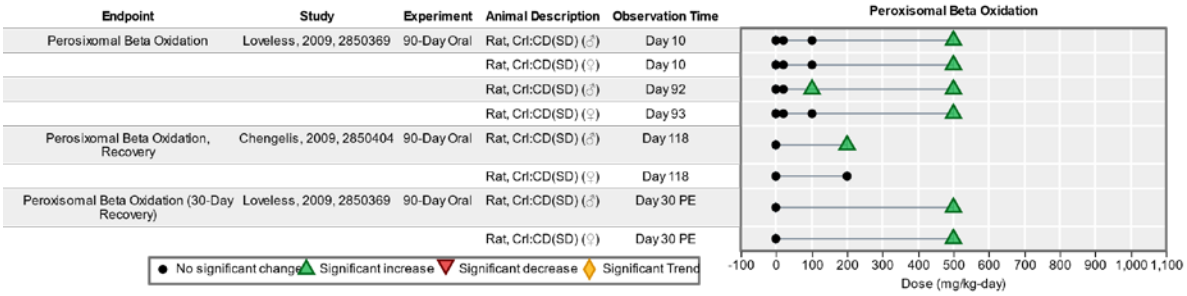


Figure 3-6. Peroxisomal beta oxidation activity in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Receptor Activation

Peroxisomal beta oxidation is inducible by many structurally diverse ligands that activate peroxisome proliferated activated receptors (PPARs). Once ligand-activated PPARα heterodimerizes with retinoid X receptor (RXR) in the nucleus, the PPARα:RXR heterodimer binds to DNA response elements, leading to changes in gene transcription and molecular responses including peroxisomal proliferation.

In vitro high throughput screening assays for PFHxA were accessed from EPA's CompTox Chemicals Dashboard (U.S. EPA, 2018a). Bioactivity data were not available for PFHxA sodium salt or PFHxA ammonium salt. Bioactivity data from ToxCast, Tox21, and EDSP21 data are available for PFHxA in the Chemicals Dashboard (link). PFHxA induced a change from controls in 18 of 717 assay endpoints. All but one AC_{50} value, however, were above the cytotoxicity threshold limit. The one modeled AC_{50} value below the cytotoxicity limit was the thyroid stimulating hormone receptor.

Separately, in other *in vitro* studies, COS1 cells were transfected with reporter gene constructs containing either the mouse or human PPARα ligand binding domain fused to a Gal4 DNA binding domain under control of an SV-40 promoter in a luciferase reporter plasmid. These assays indicated that both mouse and human PPARα are activated by PFHxA treatment (24 h) in a treatment-related manner with PFHxA being a more potent activator of the human (lowest observed effect concentration, LOEC = 10 μM) than the mouse (LOEC = 20 μM) receptor (Wolf et al., 2008).

In a short-term study of *in vivo* PFHxA exposure, NTP (2018) reported significant and dose-related increases in the liver expression of the PPARα-related genes acyl-CoA oxidase(*Acox1*, up to two-fold increase) and cytochrome P450 4a1 (*Cyp4a1*, up to 12.5-fold increase), constitutive androstane receptor (CAR)-related genes cytochrome P450 2b1 (*Cyp2b1*, up to seven-fold increase) and cytochrome P450 2b2 (*Cyp2b2*, up to three-fold increase). NTP (2018) provided further evidence of PPARα activation by PFHxA exposure, with increases in Acyl-CoA oxidase activity (up to 16-fold) in male rats receiving >250 mg/kg-day PFHxA (not measured in females).

Collectively, the in vitro and in vivo results indicate that PFHxA can activate PPARα. The data also suggest this PPARα activation occurs in both rodents and humans to a similar extent (at least in vitro). The data suggest pathways such as PPARα and CAR activation can contribute to some of hepatic changes caused by PFHxA exposure, including hypertrophy. However, PFHxA-specific data informing possible biological pathways leading to the observed hepatic effects are sparse, and many uncertainties remain.

Other PFAS

Although no direct in vivo evidence is available for PFHxA effects in rodents, PFAS exposures in PPARα null and humanized mouse models are available. In Rosen et al. (2017), transcript profiling in male wild-type and null mice identified PFNA, PFOA, PFOS, and PFHxS exposure induced hepatic gene expression profiles similar to agonists for CAR, PPARα, PPARγ, estrogen receptor alpha (ERα), while suppressing signal transducer and activator of transcription 5 B (STAT5B). In the same study, Rosen et al. (2017) also compared transcript profiles between vehicle and PFAS-exposed wild-type and null mice and identified that 11–24% of the genes differentially regulated by PFAS exposure were PPARα independent. In a separate study, Das et al. (2017) reported findings of increased hepatocyte area and decreased DNA content along with increased hepatic triglyceride content and increased hepatocellular lipid content (except for PFNA) indicating hepatocyte hypertrophy and steatosis in adult male SV129 wild-type SV and PPARα null and mice exposed to 10 mg/kg-day PFOA, PFNA, or PFHxS for 7 days. Further, Foreman et al. (2009) also observed increased liver weight, hepatic lipid accumulation, ALT increases >two-fold, and pathologically similar (severity and incidence) hepatocellular hypertrophy in male SV129 wild-type SV and humanized PPARα mice exposed to PFBA. Collectively, these findings suggest pathways in addition to PPARα can mediate the hepatic effects (including increased liver weight and hepatocellular hypertrophy) for those PFAS tested. Based on structural similarity between

PFHxA and PFOA, PFNA, and PFBA it is inferred that PFHxA exposure in these genetic mouse model systems would elicit similar effects.

Consideration for Potentially Adaptive Versus Adverse Responses

Considering the hepatic effects of PFHxA exposure were observed in rodents that have species-specific responses to chemical-induced liver toxicity, the evidence was considered together for potentially adaptive versus adverse responses. For PFHxA, and ammonium or sodium salts, evidence demonstrates increased liver weight, increased hepatocellular hypertrophy, increased ALT/AST/ALP (increases between 1.5- and 3.5-fold), decreased blood protein (driven primarily by decreased globulin), increased peroxisomal beta oxidation activity, the induction of CAR and PPRAα metabolic enzyme gene expression in 28-day and subchronic rodent studies (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018), and activation of mouse and human PPRAα (Wolf et al., 2014; Wolf et al., 2008).

Several biological pathways lead to chemical-induced increases in liver weight and hepatocellular hypertrophy, including hepatocyte swelling due to abnormal storage of water, glycogen, lipids; organelle (i.e., mitochondria, endoplasmic reticulum, peroxisome) proliferation; and increased immune cell infiltration (Thoolen et al., 2010; U.S. EPA, 2002a). Although the available clinical and histopathological data limited evaluation of all these pathways, evidence indicated the hepatocellular changes induced by PFHxA exposure in rodents could become adverse with long-term exposure at doses up to 200 mg/kg-day in female rats (the highest dose tested in males was 100 mg/kg-day) where necrosis was observed with an incidence of 17.1% (12/70). Evidence also showed increased PPARα activation and peroxisomal beta oxidation activity after PFHxA exposure (in the 28-day and subchronic studies described above) that are possibly biological pathways toward hepatocellular hypertrophy and increased liver weight. PPARα activation has been proposed as a potential MOA for the liver effects induced after exposure to some PFAS (Klaunig et al., 2012), but primarily in the context of PPARα-mediated pathways for nongenotoxic carcinogens (Klaunig et al., 2003). Notably, evidence showed that PFHxA exposure did not lead to hepatic carcinogenesis in the *high confidence* chronic study (Klaunig et al., 2015). Further, evidence from other PFAS exposures in genetic mouse models indicated possible pathways leading to increased liver weight and hypertrophy other than PPARα (described above under "other PFAS").

In the absence of a known mechanism leading to increased liver weight, hepatocellular hypertrophy, and necrosis, the evidence for PFHxA-mediated hepatotoxicity was evaluated. There was evidence of increased serum enzymes ALT, AST, and ALP that were dose-responsive in the 28-day study at doses ≥500 mg/kg-day (NTP, 2018). Of these changes in serum enzymes, ALT was increased 3.3-fold and ALP was increased 1.3-fold in male rats receiving a subchronic dose to 200 mg/kg-day PFHxA (Chengelis et al., 2009b). In the other subchronic study (Loveless et al., 2009), ALT was increased (1.56–2.33-fold) at ≥20 mg/kg-day, AST was increased (1.25–1.39-fold) at ≥100 mg/kg-day, and ALP was increased 2.6-fold at 500 mg/kg-day PFHxA sodium salt. Of these

clinical pathological measures, hepatocellular hypertrophy, hepatic congestion, and hepatocellular necrosis were found in rats exposed to PFHxA. Although these changes in serum enzymes were not found in PFHxA-exposed females, the recommendation from Hall et al. (2012) that serum changes in ALT in the range of "2–4 times or higher in individual or group mean data when compared with concurrent controls should raise concern as an indicator of potential hepatic injury unless a clear alternative explanation is found." Hepatic effects were considered adverse based on changes in clinical chemistry accompanied by increased liver weight, increased hepatocellular hypertrophy, decreased total protein, observations of macrocytic anemia (see Section 3.2.4) in the subchronic studies, and increased incidence of necrosis in the chronic study.

Evidence Integration

The human evidence base is limited to a single *medium* confidence study reporting null associations between serum biomarker levels and PFHxA exposure. Based on these data, there is *indeterminate* human evidence of hepatic effects.

The collective hepatic findings in rodents exposed to PFHxA included increased relative liver weight observed with increased hepatocellular hypertrophy at doses as low as 100 mg/kg-day (Loveless et al., 2009) and 200 mg/kg-day (Chengelis et al., 2009b) in male rats. Corroborative evidence for adverse hepatotoxicity included increased serum enzymes, (e.g., ALT increased >2-fold) in the subchronic studies, although a dose-responsive relationship was observed in the short term, but not the subchronic, studies. Serum enzyme changes were not observed in the chronic study (Klaunig et al., 2015). Hepatocellular necrosis was observed in male rats in a *low* confidence subchronic study (Chengelis et al., 2009b) and in the *high* confidence chronic study (female rats) (Klaunig et al., 2015) at 200 mg/kg-day (note that the highest dose tested in males was 100 mg/kg-day, 2-fold less than in females). Other clinical findings altered by PFHxA exposure included decreased bilirubin and decreased total protein mainly driven by decreases in immunoglobulins (see clinical chemistry section above). These findings (i.e., increased liver weight with concurrent hepatocellular hypertrophy, increases in ALT, and decreased protein levels) were considered adverse as they might lead to the necrosis observed in females at 100 mg/kg-day in the chronic study. In general, the pattern of findings suggests a generally increased sensitivity in males as compared to females. Overall, there is *robust* animal evidence of hepatic effects. This judgment is based on four studies in Sprague-Dawley rats that were generally rated *high* confidence on the outcome-specific evaluations.

Although multiple biological pathways could lead to the histopathological findings mentioned above, the PFHxA database for molecular evidence was predominantly limited to PPARα pathways and included *in vitro* assays measuring PFHxA induction of PPARα activity (Wolf et al., 2014; Wolf et al., 2008), peroxisomal beta oxidation activity (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018), changes in gene expression for CAR and PPARα cytochrome P450 gene expression (NTP, 2018), and *in vivo PPARα* knockout and humanized genetic mouse models exposed to PFAS structurally similar to PFHxA (Das et al., 2017; Foreman et al., 2009; Rosen et al.,

2017). Wolf et al. (2008) and Wolf et al. (2014) found evidence for PFHxA activation of human>rodent PPARα receptor activity. Dose-responsive increases in peroxisomal beta oxidation activity were observed in two subchronic studies (Chengelis et al., 2009b; Loveless et al., 2009) at a dose as low as 100 mg/kg-day and this effect persisted after the 30-day recovery (Loveless et al., 2009). Evidence for pathways other than PPARα and CAR were available from genetic PPARα knockout mouse studies evaluating the effects of PFAS exposure (Das et al., 2017; Foreman et al., 2009; Rosen et al., 2017) that found similar levels of increased liver weight and incidence of hepatocellular hypertrophy when comparing between PPARα knockout, humanized, and wild-type mouse models.

Overall, the currently available ***evidence indicates*** that PFHxA likely causes hepatic effects in humans under relevant exposure circumstances. This conclusion is based on studies of animals showing increased liver weight, hepatocellular hypertrophy, increased serum enzymes (>2-fold ALT), and decreased serum globulins generally occurring at ≥ 200 mg/kg-day (with some effects noted at lower doses) within the evidence base of four primarily *high* confidence studies of short-term, subchronic, and chronic PFHxA exposure in (primarily male) Sprague-Dawley rats. The findings in rats were determined to be adverse and relevant to humans, with the likely involvement of both PPARα-dependent and -independent pathways.

Table 3-9. Evidence profile table for hepatic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊕⊕⊙ ***Evidence indicates*** **(likely)** *Primary basis:* Four generally *high* confidence studies in rats ranging from short-term to chronic exposure, generally in males at ≥100 mg/kg-d PFHxA *Human relevance:* Given the induction of human PPARα by PFHxA, as well as support for involvement of both PPARα-dependent and independent pathways, effects in rats are considered potentially relevant to humans *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and lifestages:* No evidence to inform
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Serum Biomarkers *1* low confidence study	• No factors noted	• Low sensitivity	• No association of PFHxA with serum biomarkers	⊙⊙⊙ *Indeterminate*	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Organ Weight *3* high confidence: 28-day 90-day (2 studies)	• *Consistent* increases, all studies and sexes • *Dose-response* in all studies • *Coherence* with histopathology • *Magnitude of effect*, up to 63% • *High* confidence studies	• No factors noted	• Increased liver weight at ≥200 mg/kg-d; stronger in males	⊕⊕⊙ *Moderate* Findings considered adverse based on potential for progression to more severe phenotypes, including necrosis with longer-term exposure, and strong support for liver injury from serum biomarkers	
Histopathology *3* *high* confidence studies in adult rats: 28-day 90-day 2-year	• *Consistent* cellular hypertrophy across studies and sexes • *Coherence* with liver weight • *Dose-response* for hypertrophy	• *Lack of coherence* across sexes (see narrative summary)	• Cellular hypertrophy at ≥100 mg/kg-d; stronger in males • Necrosis in females at 200 mg/kg-d (no change in		

Evidence stream summary and interpretation					Evidence integration summary judgment
1 low confidence study in adult rats: 90-day	• Concerning *severity of effect*—necrosis (with chronic exposure) • *High* confidence studies		males at ≤100 mg/kg-day)		
Serum Biomarkers of Hepatic Injury *4 high* confidence studies in adult rats: 28-day 90-day (2 studies) 2-year	• *Consistent* increases in ALT, AST, and ALP, and decreases in bilirubin, across studies • *Magnitude of effect*, >2-fold ALT • *Dose-response* for total protein • *Coherence* of ALP and bilirubin • *High* confidence studies	• No factors noted	• Increased ALT, AST, ALP, and bile salts/acids at ≥20, ≥100, ≥200, and 500 mg/kg-d, respectively; stronger in males • Decreased total protein and bilirubin at ≥100 mg/kg-day; stronger in males		
Mechanistic evidence and supplemental information					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Molecular Initiating Events—PPARα	*Key findings and interpretation:* • In vitro induction of PPARα activity in transfection studies. Reporter gene activation at lower effective concentrations in human versus mouse constructs. • Induction of PPARα with hepatic effects in a short-term oral exposure study. *Limitations*: Small evidence base investigating PPARα activation by PFHxA exposure.			• Biologically plausible support for PPARα-dependent and independent pathways contributing to hepatic effects of PFHxA	

Evidence stream summary and interpretation			Evidence integration summary judgment
Molecular Initiating Events—Other Pathways	*Key findings and interpretation*: • Indirect evidence supporting activation of PPARα-independent pathways contributing to hepatic effects similar to those observed for PFHxA in PPARα knockout and humanized mice after short-term oral exposure to PFAS other than PFHxA. • In a short-term oral exposure study, PFHxA activated CAR, PPARα, PPARγ, and ERα and suppressed STAT5B. CAR-responsive genes were increased in association with hepatic effects. *Limitations*: Small evidence base with no experiments specifically challenging the role of PPARα in PFHxA-induced hepatic injury.		
Organ Level Effects	*Key findings and interpretation*: • Increased peroxisomal beta oxidation activity that persisted 30 days post-exposure (likely not a transient, adaptive response) in short-term and subchronic rat studies of oral PFHxA exposure. • Indirect evidence of fatty liver, hepatocellular hypertrophy, and hepatomegaly in PPARα KO mice after short-term oral exposure to PFAS other than PFHxA. *Limitations*: Small evidence base and the most compelling in vivo evidence for PPARα-independent pathways with hepatic effects did not specifically test PFHxA.		

3.2.2. Developmental Effects

Human

No studies were identified that evaluated potential developmental effects of PFHxA exposure in humans.

Animal

Three studies examined developmental outcomes, including offspring viability, body weight, and developmental milestones. Rats were exposed to PFHxA sodium salt during gestation (gestation day [GD] 6–20; developmental study) or continuously exposed throughout gestation and lactation (reproductive study) (Loveless et al., 2009). Mice were exposed to PFHxA ammonium salt from GD 6–18 (Iwai and Hoberman, 2014). These studies were rated *high* confidence. The results from outcome-specific, confidence evaluations for all individual reproductive outcomes are presented in Table 3-10, and details are available by clicking the HAWC link. Effects on male and female reproductive system development following developmental exposure are discussed in the male and female reproductive effects sections, respectively (Sections 3.2.6 and 3.2.7).

Table 3-10. Study design characteristics and outcome-specific study confidence for developmental endpoints

Study	Species, strain (sex)	Exposure design	Exposure route and dose	Offspring viability	Offspring body weight	Developmental milestones
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague–Dawley (male and female)	Reproductive study: P0 females dosed 70 d prior to cohabitation, through gestation and lactation (126 days); P0 males dosed for 110 days Developmental study: GD 6–20	Gavage[a] Female: 0, 20, 100, 500 mg/kg-d	++	++	++
Iwai and Hoberman (2014)[c]	Mouse, Crl: CD1(ICR); Charles River Laboratories, Inc.	Developmental study: GD 6–18	Gavage[b] Phase 1: 0, 100, 350, 500 mg/kg-d Phase 2: 0, 7, 35, 175 mg/kg-d	++	++	++

Study evaluation for animal toxicological developmental endpoints reported from studies with rats receiving PFHxA sodium salt[a] or PFHxA ammonium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

[c]Phase 1 was a range finding study used to determine the appropriate dose range for identification of a NOAEL in Phase 2.

++ Outcome rating of high confidence; + outcome rating of medium confidence; - outcome rating of low confidence; -- outcome rating of uninformative; NR, outcome not reported; NM, outcome not measured.

Offspring Mortality

Potential effects of PFHxA exposure on offspring viability were evaluated in a developmental study (Iwai and Hoberman, 2014) and a one-generation reproductive study (Loveless et al., 2009). Mice exposed to PFHxA ammonium salts during gestation (GD 6–18) showed a dose-dependent increase in the incidence of offspring mortalities occurring both pre- and postnatally (Iwai and Hoberman, 2014). Most deaths occurred between postnatal day (PND) 0–7, with a statistically significant increase for the 350 and 500 mg/kg-day dose groups. These early postnatal losses are reflected in treatment-related changes in several measures of offspring viability for the 500 mg/kg-day dose group. Specifically, statistically significant decreases occurred in the viability index for PND 0–4 and PND 0–7, surviving pups per litter were lower on PND 20, and the incidence of total litter loss increased between PND 0–3 (5 of 17 for the 500 mg/kg-day group compared to 1 of 19 dams for concurrent controls). A dose-dependent increase in the number of stillbirths, a measure of prenatal mortality, was also reported across the two phases of the experiment (incidence of 3/241, 5/245 and 19/177 for the 175, 350, and 500 mg/kg-d dose groups, respectively). Results are summarized in Figure 3-7 and Table 3-11.

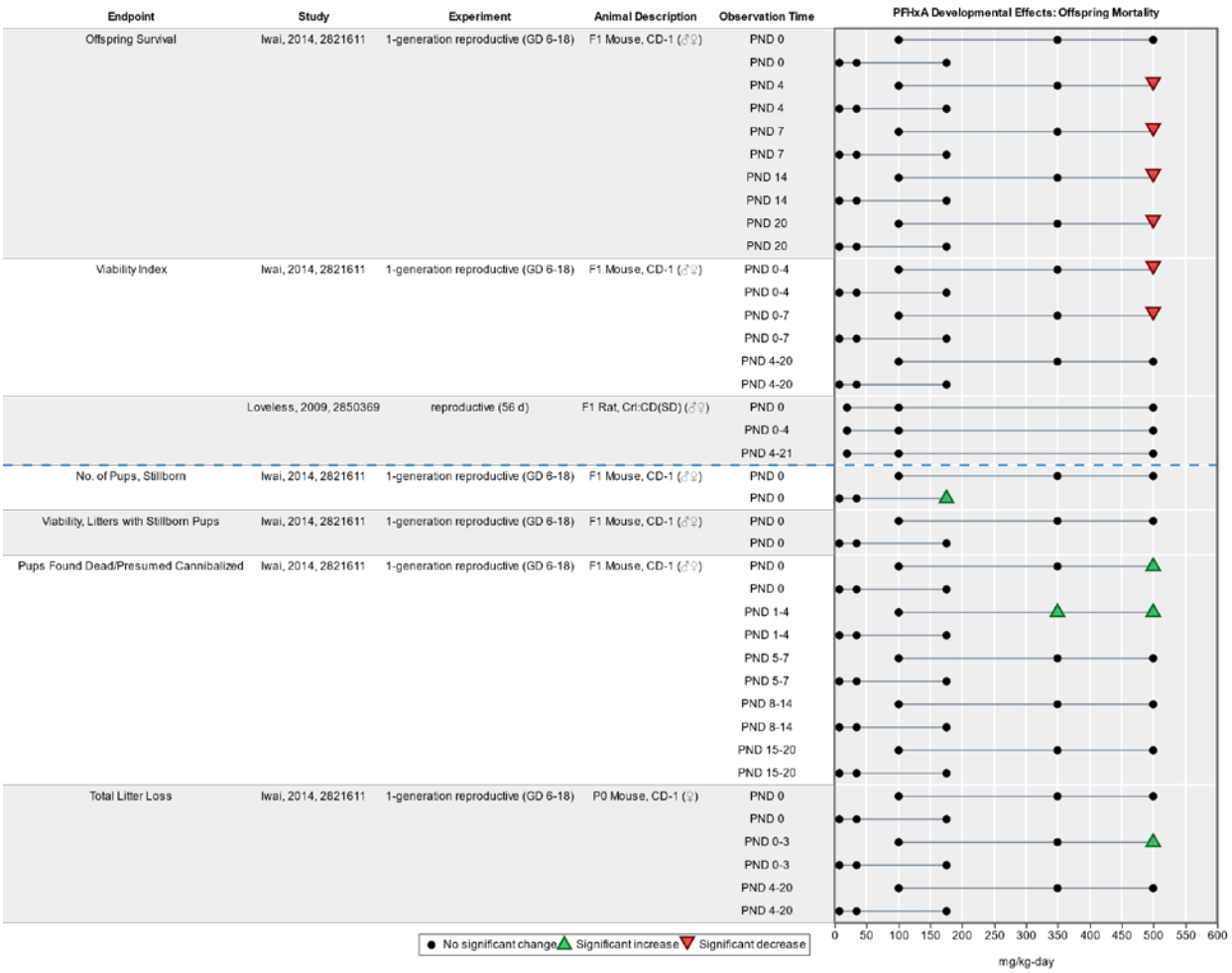


Figure 3-3. Developmental effects on offspring viability in mice exposed to PFHxA ammonium salt (HAWC: PFHxA – Animal Toxicity Developmental Effects link).

The Iwai and Hoberman (2014) study was conducted in two phases. Phase 1 was a range-finding study (100, 350, or 500 mg/kg-d) used to determine the appropriate doses (7, 35, 175 mg/kg-d) to identify a NOAEL in Phase 2.

Table 3-11. Incidence of perinatal mortality following PFHxA ammonium salt exposure in a developmental oral toxicity study

Reference	Dose (mg/kg-d)							
	0 (Phase 1)	**0 (Phase 2)**	**7**	**35**	**100**	**175**	**350**	**500**
Stillbirths, male and female (combined) mice (Iwai and Hoberman, 2014)	**4**	**0**	0	0	0	3	5	**19**

Reference	Dose (mg/kg-d)							
	0 (Phase 1)	**0 (Phase 2)**	**7**	**35**	**100**	**175**	**350**	**500**
Mortalities, PND 0, male and female (combined) mice (Iwai and Hoberman, 2014)	**0**	**0**	0	0	0	4	3	**21**
Mortalities, PNDs 1–4, male and female (combined) mice (Iwai and Hoberman, 2014)	**2**	**1[a]**	3[a]	2	1[a]	0	**13[a]**	**15**
Mortalities, PNDs 5–7, male and female (combined) mice (Iwai and Hoberman, 2014)	**0[a]**	**1**	0[a]	0	1	3	2[a]	0
Mortalities, PNDs 8–14, male and female (combined) mice (Iwai and Hoberman, 2014)	**0**	**0**	0	0	0[a,b]	0[a]	0[a]	0
Mortalities, PNDs 15–20, male and female (combined) mice (Iwai and Hoberman, 2014)	**0**	**0**	0	0	2[b]	1	0	0
Total pups delivered, male and female (combined) mice (Iwai and Hoberman, 2014)	**221**	**249**	211	232	220[b]	241	245	177
Cumulative perinatal mortality/total pups delivered, male and female (combined) mice (Iwai and Hoberman, 2014)	**6/ 221**	**2/ 249**	3/ 211	2/ 232	4/ 220[a]	11/ 241	23/ 245	55/ 177

The Iwai and Hoberman (2014) study was conducted in two phases. Phase 1 was a range-finding study (100, 350, or 500 mg/kg-d) used to determine the appropriate doses (7, 35, 175 mg/kg-d) to identify a NOAEL in Phase 2.
Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors.
[a]Excludes animals that were missing, presumed cannibalized.
[b]Excludes data from litters where the dam died during late lactation; deaths assumed not treatment related.

Offspring Body Weight

Offspring body weights were available from two developmental studies (Iwai and Hoberman, 2014; Loveless et al., 2009) and a one-generation reproductive study (Loveless et al., 2009). In mice, offspring body weights were statistically significantly decreased at PND 0–7 in animals exposed gestationally (GD 6–18) to ≥100 mg/kg-day PFHxA ammonium salt. Reductions in body weight observed at the higher doses across two experimental cohorts with different dose ranges generally persisted throughout lactation. After weaning, body weight deficits persisted in females but not males, however body weight gain during this period was unaffected (Iwai and Hoberman, 2014). Similar results were reported in two separate cohorts of rats exposed to PFHxA sodium salt (Loveless et al., 2009). In the developmental study, fetal body weights (GD 21) of animals exposed gestationally (GD 6–20) to 500 mg/kg-day were decreased by 9% relative to controls, but no effects were observed at the lower doses. In the one-generation reproductive study, a dose-related decrease (4% to 18% less than controls) was found in pup body weights across all dose groups at PND 0. Offspring body weight decrements persisted through PND 21 in the 100 and 500 mg/kg-d dose groups but no treatment-related effects on body weight gains

occurred between PND 21–41 (Loveless et al., 2009). Results are presented in Figure 3-8 and Table 3-12.

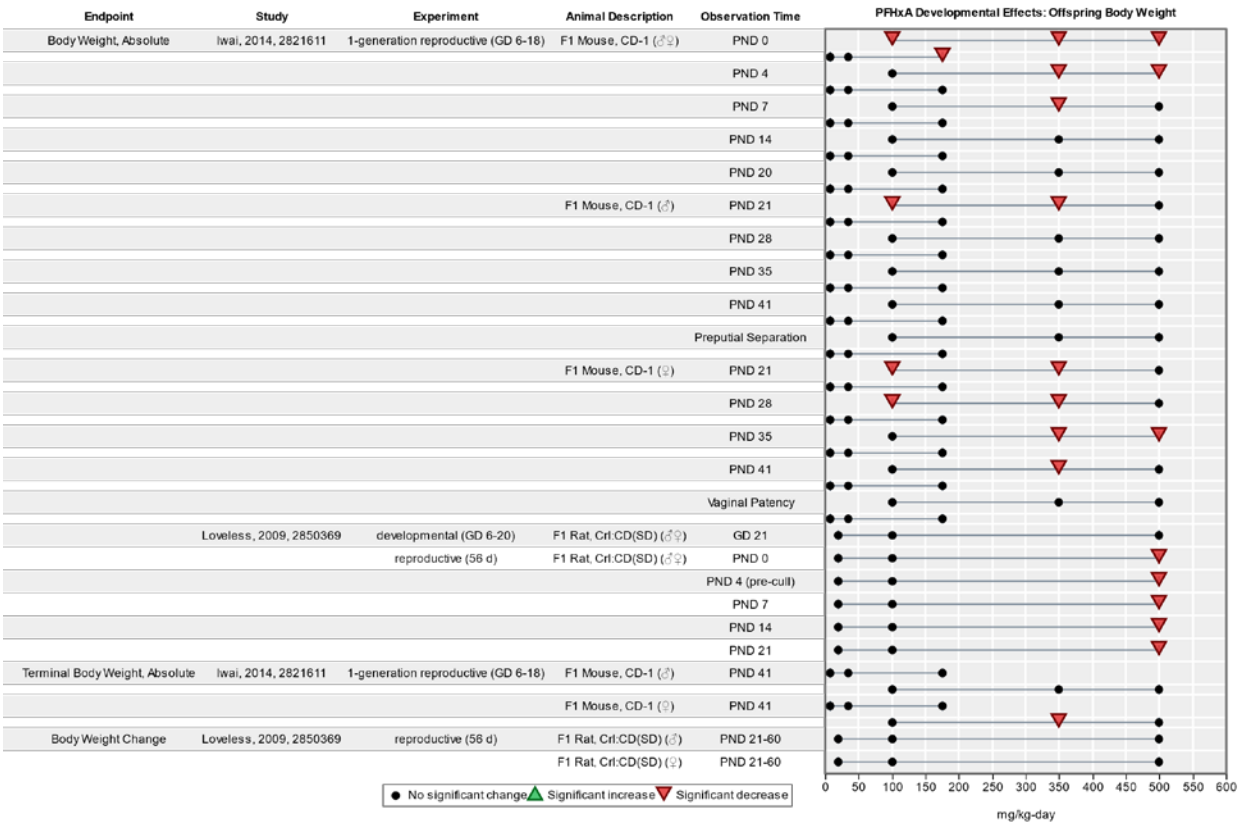


Figure 3-4. Developmental effects on offspring body weight in mice exposed to PFHxA ammonium salt and rats exposed to PFHxA sodium salt (HAWC: PFHxA – Animal Toxicity Developmental Effects link).

Table 3-12. Percent change relative to control in offspring body weight due to PFHxA sodium or ammonium salt exposure in developmental oral toxicity studies

Reference	Dose (mg/kg-d)						
	7	20	35	100	175	350	500
GD 21 (developmental study), male and female (combined) rats (Loveless et al., 2009)		−2		0			−9
PND 0 (one-generation reproductive study), male and female (combined) rats (Loveless et al., 2009)		−4		−11			**−18**
PND 7 (one-generation reproductive study), male and female (combined) rats (Loveless et al., 2009)		0		−6			**−17**
PND 14 (one-generation reproductive study), male and female (combined) rats (Loveless et al., 2009)		3		−6			**−17**

Reference	Dose (mg/kg-d)						
	7	20	35	100	175	350	500
PND 21 (one-generation reproductive study), male and female (combined) rats (Loveless et al., 2009)		3		−5			**−18**
PND 0 , male and female (combined) mice (Iwai and Hoberman, 2014)	0		0	**−6**	**−13**	**−13**	**−13**
PND 4, male and female (combined) mice (Iwai and Hoberman, 2014)	0		7	−7	**−4**	**−27**	**−20**
PND 7, male and female (combined) mice (Iwai and Hoberman, 2014)	0		5	−7	0	**−18**	−11
PND 14, male and female (combined) mice (Iwai and Hoberman, 2014)	−1		3	−8	0	−14	−8
PND 20, male and female (combined) mice (Iwai and Hoberman, 2014)	−2		6	−11	2	−20	−12

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Eye Opening

Potential effects of PFHxA exposure on developmental milestones were evaluated in a developmental study (Iwai and Hoberman, 2014). On PND 14, Iwai and Hoberman (2014) reported a statistically significant delay in eye opening, with less than 50% of pups in the 350 and 500 mg/kg-day PFHxA ammonium salt exposure groups having reached this milestone compared to 85% among vehicle controls (Figure 3-9). Delays in eye opening persisted in the 350 and 500 mg/kg-day dose groups at PND 15 but were not statistically significant. Delays in eye opening can have long-term impacts on vision by interfering with sensory input during the critical window of visual cortex development (Espinosa and Stryker, 2012; Wiesel, 1982). The results are summarized in Figure 3-9 and Table 3-13.

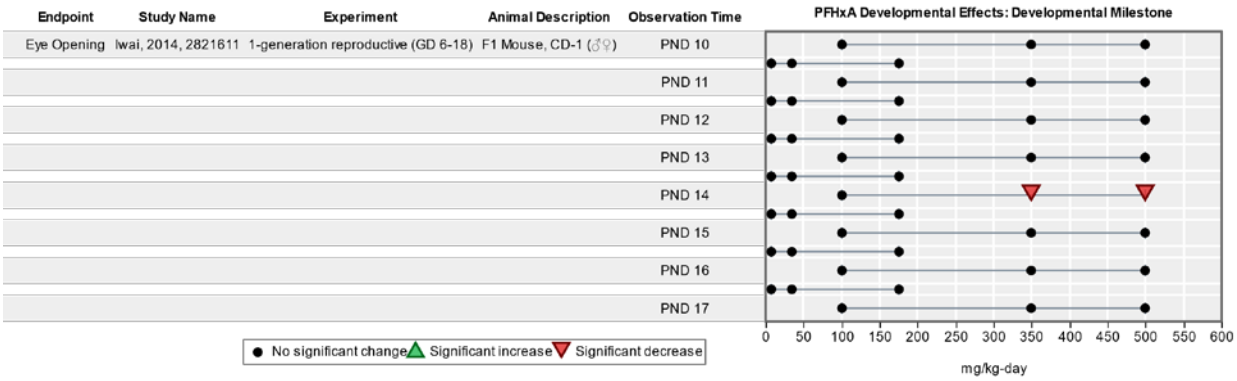


Figure 3-5. Developmental effects on eye opening (percent change relative to control) in mice exposed to PFHxA ammonium salt (HAWC: PFHxA – Animal Toxicity Developmental Eye Effects link).

Table 3-13. Percent change relative to control in eye opening due to PFHxA ammonium salt exposure in a developmental oral toxicity study

Reference	Dose (mg/kg-d)					
	7	35	100	175	350	500
PND 13, male and female (combined) mice (Iwai and Hoberman, 2014)	−6	34	−56	−21	−58	−55
PND 14, male and female (combined) mice (Iwai and Hoberman, 2014)	2	4	−17	−8	**−49**	**−39**
PND 15, male and female (combined) mice (Iwai and Hoberman, 2014)	0	0	−10	−5	−23	−25
PND 16, male and female (combined) mice (Iwai and Hoberman, 2014)	0	0	−1	0	−9	−1

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors.

Malformations and Variations

Potential effects of PFHxA exposure on fetal malformations and variations were evaluated in a single developmental study (Loveless et al., 2009). No treatment-related effects were found on fetal malformations or variations in rats following gestational (GD 6–20) exposure to up to 500 mg/kg-day PFHxA sodium salt.

Evidence Integration

No human studies were identified to inform the potential developmental effects of PFHxA; therefore, there is *indeterminate* human evidence of developmental effects.

In animals, three *high* confidence studies examined developmental effects following maternal perinatal exposure to PFHxA salts (Iwai and Hoberman, 2014; Loveless et al., 2009). Treatment-related effects, including decreased offspring body weight, increased mortality, and delayed eye opening, were observed in mice following exposure to PFHxA ammonium salt as low as 100 mg/kg-day. Notably, no effects on maternal weight gain were observed up to the highest tested

dose of 500 mg/kg-day in this study (Iwai and Hoberman, 2014). Reductions in offspring body weight were also found in the one-generation reproductive and developmental studies in rats. Animals in the reproductive cohort exposed throughout gestation and lactation showed body weight reductions (≥5%) at exposure to ≥100 mg/kg-day that persisted to PND 21, whereas the developmental cohort was reduced (9%) only at the high dose (500 mg/kg-day).

In general, effects on development were greatest in magnitude from PND 0 to PND 7, suggesting that the early postnatal period might be a critical window for developmental changes associated with PFHxA. Although the evidence base is small, the data are strengthened by coherent evidence across outcomes, consistency of effects on body weight across two species/studies, and the severity of some of the outcomes (e.g., increased offspring mortality). In addition, a similar pattern of effects on development (i.e., offspring body weight reductions and delays in developmental milestones) were observed with other PFAS, including PFBS and PFBA, providing additional support for these specific findings.

Reductions in maternal body weights were also noted and might indicate maternal toxicity (U.S. EPA, 1991). For the developmental cohort, total net body weight (i.e., terminal body weight minus the gravid uterine weight) of dams in the high dose group was statistically significantly decreased (5% relative to controls) (Loveless et al., 2009). Effects on total body weight in the gestationally exposed dams were associated with a decrease in maternal weight gain at GD 21 in the 500 mg/kg-day group. No effects on total or net maternal body weights were found in the one-generation reproductive cohort (Loveless et al., 2009) but weight gain of dams exposed to 500 mg/kg-day was statistically significantly reduced. The effect on maternal weight gain was limited to early gestation (GD 0–7). PFHxA sodium salt exposure had no effect on maternal weight gain over the entire gestational window (GD 0–21), and dams in this exposure group showed an increase in body weight during lactation. Also, delays in eye opening in the developmental mouse study were observed only at doses that elicited overt toxicity (i.e., increased offspring mortality) in the pups (Iwai and Hoberman, 2014). Because treatment-related changes in offspring body weight and mortality were observed at doses that did not affect maternal weight gain, maternal toxicity is not expected to be the primary driver of developmental effects. Based on these findings, there is *moderate* animal evidence of developmental effects.

Overall, the currently available ***evidence indicates*** that PFHxA likely causes developmental effects in humans under relevant exposure circumstances. This judgment is based primarily on gestational exposure experiments in mice (and supportive findings in rats), with effects occurring after oral PFHxA exposure at ≥ 100 mg/kg-day. These findings are interpreted as relevant to humans based on similarities in the anatomy and physiology of the developmental system across rodents and humans.

Table 3-14. Evidence profile table for developmental effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊕⊕⊙ ***Evidence indicates (likely)*** *Primary basis:* Three *high* confidence studies in rats and mice including gestational (rats and mice) and continuous one-generational reproductive (rats) exposures at ≥ 100 mg/kg-day PFHxA ammonium or sodium salt. *Human relevance:* Without evidence to the contrary, effects in rats and mice are considered relevant to humans. *Cross stream coherence:* N/A (human evidence indeterminate). *Susceptible populations and lifestages:* The available evidence suggests that
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
• There were no studies available from the PFHxA evidence base.				⊙⊙⊙ ***Indeterminate***	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Offspring Mortality *2 high* confidence studies in rats and mice: • GD 6–18 (mice) • 1-generation reproductive (rats)	• *High* confidence studies • Concerning *severity of effect* – increased mortality	• *Unexplained inconsistency* across species	• Increased pre and postnatal mortality at ≥350 mg/kg-day in mice	⊕⊕⊙ ***Moderate*** Developmental effects observed in multiple *high* confidence studies conducted in two species exposed to different PFHxA salts under different exposure scenarios. Effects were observed at doses that were not associated with frank effects or maternal toxicity.	
Body Weight *3 high* confidence studies in rats and mice: • GD 6–18 (mice) • GD 6–20 (rats) • 1-generation reproductive (rats)	• *High* confidence studies • *Consistency* across studies and species • *Dose-response* observed in mouse study	• Fetal effects observed at doses that are associated with maternal toxicity (i.e., substantial decreases in dam body weight)	• Postnatal body weight decreased at ≥100 mg/kg-day in rats and mice • Fetal body weight decreased at 500 mg/kg-day in rats		
Eye Opening *1 high* confidence study in mice: • GD 6–18	• *High* confidence study	• Effects observed at doses are associated with frank effects in offspring (i.e., offspring mortality)	• Eye opening was delayed in mice prenatally exposed to PFHxA ammonium salt at ≥350 mg/kg-day		

Evidence stream summary and interpretation					Evidence integration summary judgment
Malformations and variations *1 high* confidence study in rats: • GD 6–20	• *High* confidence study.	• No factors noted.	• No fetal malformations or variations observed at ≤500 mg/kg-day		development may be a susceptible lifestage for exposure to PFHxA.
Mechanistic evidence and supplemental information					
Biological events or pathways	**Summary of key findings, limitations, and interpretation**			**Evidence stream judgment**	
• There were no informative studies available from the PFHxA evidence base.					

3.2.3. Renal Effects

Human

Three epidemiological studies investigated the relationship between PFHxA exposure and effects on the renal system. Two were considered *uninformative* due to critical deficiencies in multiple study evaluation domains (Seo et al., 2018; Zhang et al., 2019). The remaining study was a cross-sectional study of primarily government employees in China (Wang et al., 2019) and was classified as *low* confidence primarily due to significant concerns for reverse causality with this population and poor sensitivity because the exposure levels for PFHxA were low. They observed a significant decrease in estimated glomerular filtration rate (eGFR) with higher PFHxA exposure (β: −0.3 change in eGFR as mL/min/1.73 m^2 per 1 ln-unit PFHxA [95% CI: −0.6, −0.01]). No association was observed with chronic kidney disease. Due to the potential for reverse causality and the poor sensitivity, there is substantial uncertainty in the results of this study. A summary of the study evaluations is presented in Figure 3-10, and additional details can be obtained by clicking the HAWC link.

Figure 3-6. Study evaluation for human epidemiological studies reporting findings from PFHxA exposures (full details available by clicking HAWC link).

Animal

Four short-term (28-day), subchronic, or chronic animal studies evaluated potential renal effects of PFHxA or PFHxA sodium salt in rats. Most of the outcome-specific study ratings were rated *high* confidence. For Chengelis et al. (2009b), limitations were identified that influenced some outcome-specific ratings. Specifically, histopathology was rated *low* confidence because of

issues related to observational bias, endpoint sensitivity and specificity, and results presentation. Urinary biomarker outcomes in the same study were rated medium confidence because of results presentation (only qualitative results were reported). The results of the outcome-specific confidence judgments are summarized in Table 3-15, and full study evaluation details can be viewed by clicking the HAWC link.

Table 3-15. Renal endpoints for PFHxA and associated confidence scores from repeated-dose animal toxicity studies

Author (year)	Species, strain (sex)	Exposure design	Exposure route	Blood biomarkers	Urinary biomarkers	Histopathology	Organ weight
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	NM	++	++
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	NR	+	-	++
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	++	++	++	NM

Study evaluation for animal toxicological renal endpoints reported from studies with male and female rats receiving PFHxA[a] or PFHxA sodium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Organ Weight

Increases in relative kidney weight were observed in both sexes in all three studies that reported this endpoint (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). There were statistically significant findings in male rat dose groups at PFHxA doses as low as 10 mg/kg-day in the subchronic study (Chengelis et al., 2009b). With the exception of the results from Chengelis et al. (2009b), effects on relative kidney weights generally showed a weak or no dose-response gradient (Table 3-16). Craig et al. (2015) analyzed oral chemical exposure data extracted from subchronic and chronic rat studies and found a statistically significant correlation between

absolute, but not relative, kidney weight, and kidney histopathology (even at doses where terminal body weights were decreased) for most chemicals (32/35) examined. Absolute kidney weight was increased, but only in one of the three studies reporting on this endpoint (NTP, 2018), and only in male rats at the highest dose group (1,000 mg/kg-day). The decrease in relative, but not absolute, kidney weight could be explained by body weight gain decreases in the affected dose groups: 1,000 mg/kg-day male dose group (13% decrease) (NTP, 2018), 50 and 200 mg/kg-day male dose group (8–11% decrease with similar trends in females (Chengelis et al., 2009b)), and 500 mg/kg-day male dose group (19% decrease, no change in females). Findings and full details of PFHxA effects on kidney weights can be viewed by clicking the HAWC link.

Table 3-16. Percent increase in relative and absolute kidney weight due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Endpoint and reference	Dose (mg/kg-d)									
	10	20	50	62.5	100	125	200	250	500	1,000
Relative kidney weight 28-day, female rat (NTP, 2018)				−2		0		0	3	**12**
Relative kidney weight 28-day, male rat (NTP, 2018)				0		2		2	**12**	**19**
Relative kidney weight 90-day, female rat (Chengelis et al., 2009b)	1		**12**				7			
Relative kidney weight 90-day, male rat (Chengelis et al., 2009b)	**8**		**7**				**9**			
Relative kidney weight 90-day, female rat (Loveless et al., 2009)		−3			5				**16**	
Relative kidney weight 90-day, male rat (Loveless et al., 2009)		0			11				**17**	
Absolute kidney weight 28-day, female rat (NTP, 2018)				−1		1		1	1	**9**
Absolute kidney weight 28-day, male rat (NTP, 2018)				2		0		1	8	3
Absolute kidney weight 90-day, female rat (Chengelis et al., 2009b)	0		7				4			
Absolute kidney weight 90-day, male rat (Chengelis et al., 2009b)	−1		−6				2			
Absolute kidney weight 90-day, female rat (Loveless et al., 2009)		0			1				14	
Absolute kidney weight 90-day, male rat (Loveless et al., 2009)		0			8				4	

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Histopathology

Renal histopathological subchronic findings were qualitatively reported as null (Chengelis et al., 2009b; Loveless et al., 2009). The short-term study findings included increases in minimal chronic progressive nephropathy (CPN) that were significant (incidence 8/10) in the 1,000 mg/kg-day female dose group (Figure 3-11) (NTP, 2018). Male renal histopathological findings from the chronic study were also null, whereas findings for female rats included increased papillary necrosis (17/70 vs. 0/60 in controls) and tubular degeneration (7/70 vs. 1/60 in controls) in the highest dose group 200 mg/kg-day (Klaunig et al., 2015). Full details are available by clicking the HAWC link.

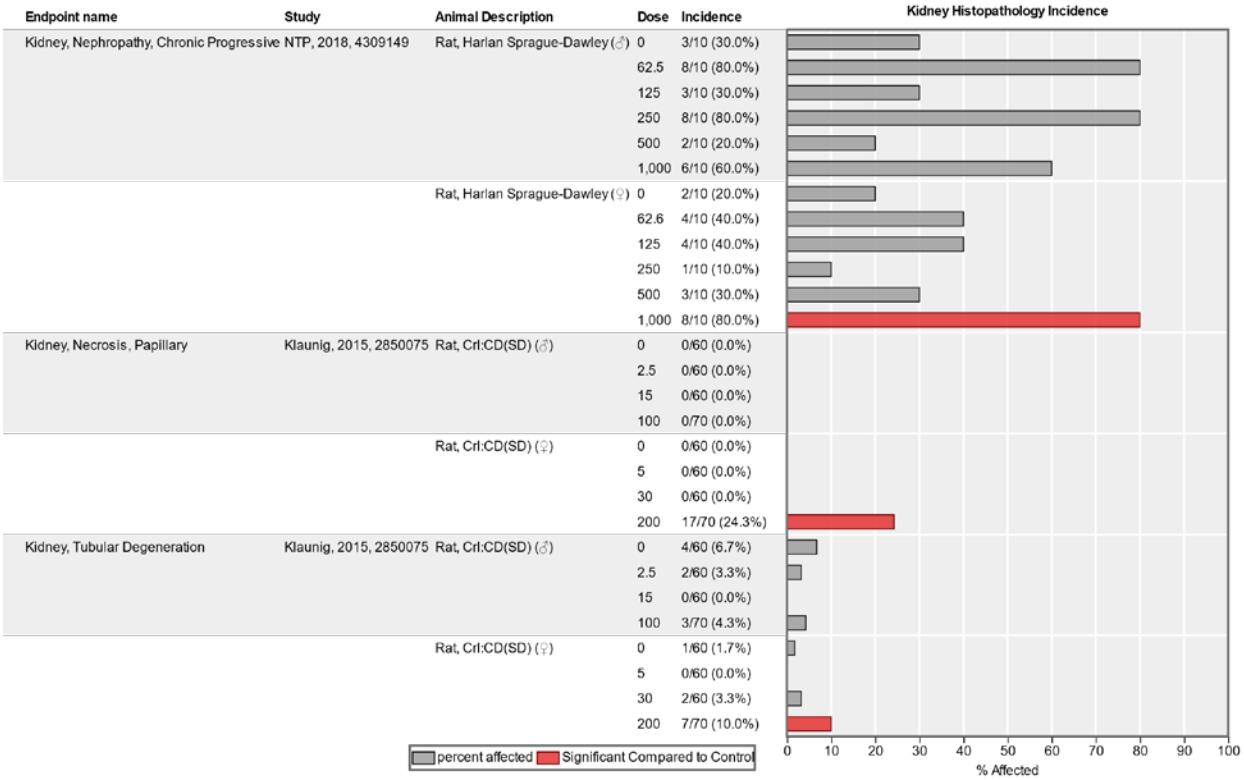


Figure 3-7. Animal toxicological renal histopatholoy after PFHxA exposure (full details available by clicking the HAWC link). Findings from the subchronic studies were reported as null and not included in the above visualization.

Blood and Urinary Biomarkers

Blood biomarkers of renal function were inconsistent across study designs and exposure durations. Both creatinine and blood urea nitrogen (BUN) are removed from the blood by the kidneys and often used as indicators of kidney function. Creatinine is a waste product of creatine metabolism (primarily in muscle) and BUN is a waste product of protein metabolism in the liver. No observations of changes in urea nitrogen or creatinine were reported from Chengelis et al. (2009b) and Klaunig et al. (2015). In the short-term study (NTP, 2018), BUN was unchanged in

both sexes in all dose groups. Changes in creatinine were inconsistent across sexes with null findings in females, whereas a 13% decrease ($p < 0.05$) was found in the male 500 mg/kg-day dose group (NTP, 2018). In a subchronic study, Loveless et al. (2009) reported a sex-specific increase in BUN in the male 200 mg/kg-day dose group, whereas creatinine was decreased in both male and female rats dosed with 200 mg/kg-day PFHxA sodium.

Urinalysis findings included total urine volume and other measures of urine concentrating ability (e.g., specific gravity, urobiloginen) were more consistent than the blood biomarkers, but still difficult to interpret. Urinalysis findings were not measured in the short-term study (NTP, 2018) and were reported as null in a subchronic study (Chengelis et al., 2009b). Findings from the other subchronic study (Loveless et al., 2009) identified changes in urine concentration reflected as decreased (50–88%) urine protein combined with increased (207–300%) total urine volume in males and females in the 500 mg/kg-day dose groups. Coherent with increased urine volume, osmolality was decreased (47%, $p < 0.05$), but only in the male 500 mg/kg-day dose group (Loveless et al., 2009). Urobilinogen and pH findings were null in both male and females in the subchronic study (Loveless et al., 2009). Findings from the chronic study lacked consistency between sexes and did not exhibit a clear dose-response relationship (Klaunig et al., 2015). Specifically, total urine volume was increased in the female 200 mg/kg-day dose group and null in all male dose groups. Urine specific gravity was increased ($p < 0.05$) in the male 15 mg/kg-day dose group and similar to controls in the 100 mg/kg-day dose group, although specific gravity was increased ($p < 0.05$) in the female 200 mg/kg-day dose group. Urine pH was low in males only in the 100 mg/kg-day dose groups at 26 and 52 weeks and attributed by the author to the slightly acidic nature of PFHxA (Klaunig et al., 2015). Total urine volume findings were null in males, whereas an increase was found in female rats from the 200 mg/kg-day dose group at 26 weeks that returned to control levels at 52 weeks study duration (Klaunig et al., 2015). Findings are summarized in Figure 3-12, and full details are available in the HAWC link.

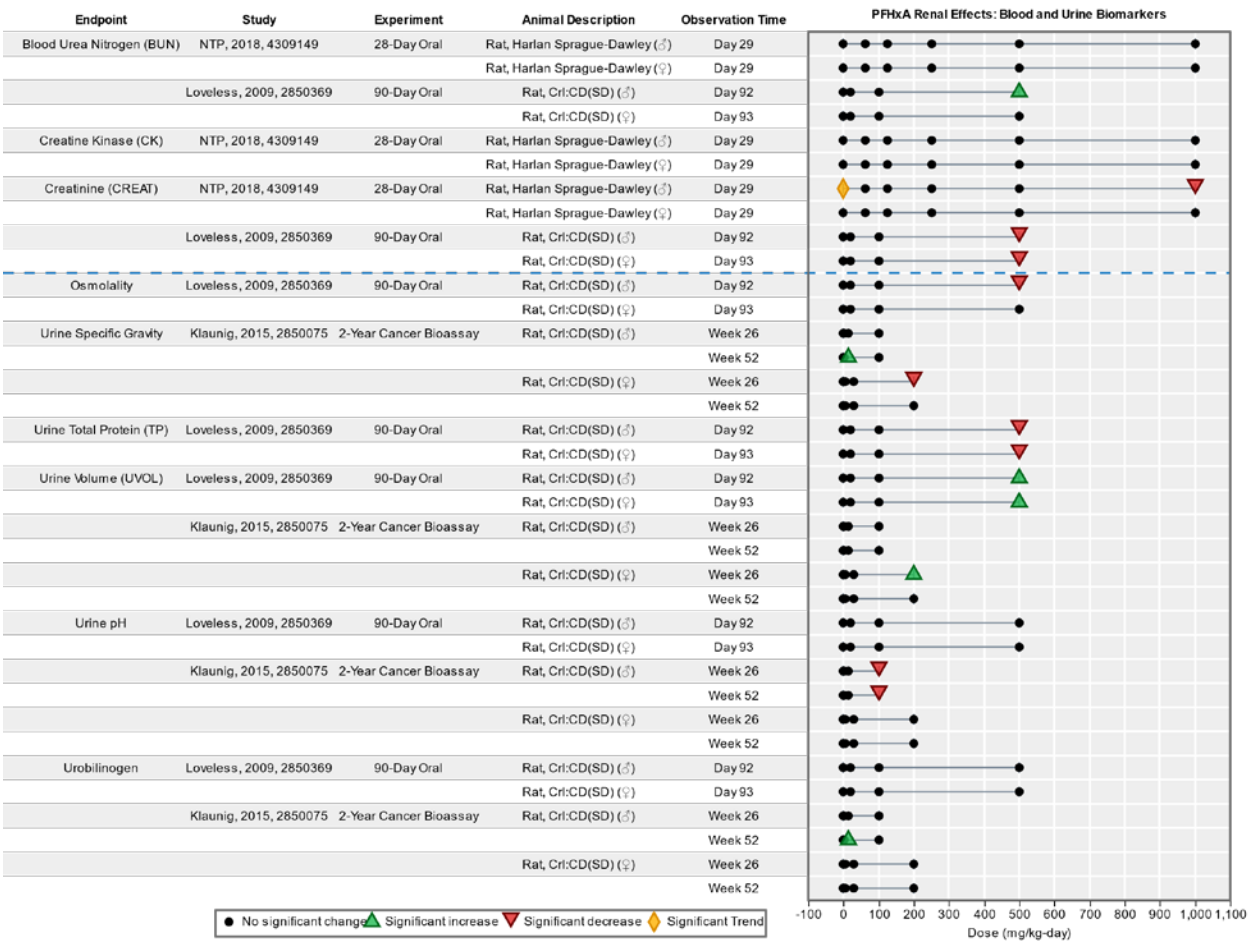


Figure 3-8. PFHxA Effects on blood and urine biomarkers of renal function (full details available by clicking the HAWC link). The dashed blue line divides blood (top) from urinary biomarkers. Note that urea nitrogen (BUN) and creatinine were described as null, but findings were not quantitatively reported.

Evidence Integration

The human evidence was limited to a single *low* confidence study reporting an inverse association between PFHxA exposure and eGFR, although notable uncertainty in this result exists due to potential for reverse causality. Based on these data, there is *indeterminate* human evidence for renal effects.

The evidence base for renal effects in experimental animals was drawn from one short-term study, two subchronic studies, and one chronic study. Findings were, in general, null except for histopathology and some urinary biomarkers. Kidney histopathology was the most significant finding in the short term and chronic studies. In the short-term study, increased incidence of CPN was observed in female rats at the highest dose (1,000 mg/kg-day PFHxA). Histopathological findings were null in both subchronic studies at doses up to 500 mg/kg-day. In the chronic study, the incidence of papillary necrosis and tubular degeneration were increased in females compared to controls at the highest dose (200 mg/kg-day, twice the highest male dose). Some changes

occurred in urinary biomarkers (decreased urine pH, increased urine volume) and potentially correlated changes were observed in female histopathology in the chronic study. However, inconsistencies between sexes and across studies at similar observation times were notable. Based on these results, there is *slight* animal evidence of renal effects.

Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA may causes renal effects in humans under relevant exposure circumstances (Table 3-17).

Table 3-17. Evidence profile table for renal effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊙⊙⊙ ***Evidence inadequate*** *Primary basis:* Indeterminate evidence in humans and animal evidence is largely null or lacking biological coherence. *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible lifestages:* No evidence to inform
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Low Confidence 1 low confidence study	• No factors noted	• Low sensitivity	• Weak association of PFHxA with decrease in estimated eGFR	⊙⊙⊙ *Indeterminate*	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgement**	
Organ Weight 3 high confidence studies in adult rats: • 28-day • 90-day (2 studies)	• *Consistent* increases, all studies	• *Lack of coherence* across sexes (see Section xx)	• Increased relative kidney weight at ≥10 mg/kg-d. • Increase absolute kidney weight at 1000 mg/kg-d; 28-day study, males only	⊕⊙⊙ *Slight* Findings of adversity were considered uncertain based on lack of coherence between effects (organ weight, histopathology, blood and urine biomarkers) inconsistency between sexes, and lack of coherence across exposure designs	
Histopathology *3 high* confidence studies in adult rats: • 28-day • 90-day • 2-year *1 low* confidence study in adult rats: 90-day	• *Large magnitude of effect*, up to 24.3% for papillary necrosis; up to 80% for chronic progressive nephropathy	• *Unexplained inconsistency* between exposure durations. • *Lack of dose-response*	• Increased incidence papillary necrosis, tubular degeneration, chronic progressive nephropathy at ≥200 mg/kg-d; female rats only, 28-day and chronic studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
Blood Biomarkers *4 high* confidence studies in adult rats: • 28-day • 90-day (2 studies) • 2-year	• No factors noted	• *Unexplained inconsistency* across exposure durations, sexes • *Lack of coherence* with other histopathological findings; chronic study	• Increased BUN at 500 mg/kg-d; males only, 90-day study. • Decreased creatinine at ≥500 mg/kg-d), both sexes, 1 subchronic study • Decreased creatine at 1,000 mg/kg-day; males only, 28-day study • No treatment related creatinine kinase findings; both sexes, 28-day study		
Urinary Biomarkers *3 high* confidence studies in adult rats: • 28-day • 90-day • 2-year *1* medium confidence study in adult rats: • 90-day	• *Coherence* of urine protein, urine volume, urine specific gravity, and decreased osmolality	• *Unexplained inconsistency* between exposure durations and sexes • *Lack of dose-response gradient.* • *Lack of coherence* with histopathological findings.	• Decreased osmolality 500 mg/kg-day; males only, 1 subchronic study • Decreased urine protein and increased urine volume in at 500 kg/kg-d; both sexes, 1 subchronic study • Increased total urine volume at ≥200 mg/kg-day; both sexes -- 1 subchronic study, females only, 1 2-year study • Decreased urine pH at 100 mg/kg-day; males only, 1 2-year study • No treatment related findings for urobilinogen; both sexes, 1 subchronic study and 1 2-year study		

Evidence stream summary and interpretation			Evidence integration summary judgment
Mechanistic evidence and supplemental information			
Biological events or pathways	**Primary evidence evaluated** **Key findings, interpretation, and limitations**	**Evidence stream judgement**	
Molecular Initiating Events—Oatp1a1	*Key findings and interpretation:* Sex hormone-dependent regulation of Oatp1a1 mRNA and protein level (see Section 3.1.4).	Sex-specific Oatp1a1 expression leading to sex-specific PFHxA elimination	

1

3.2.4. Hematopoietic Effects

Hematology is a subgroup of clinical pathological parameters concerned with morphology, physiology, and pathology of blood and blood-forming tissues. Hematological parameters are measured using blood tests such as complete blood counts (CBC) and a clinical chemistry panel. The CBC measures three primary types of blood cells (red blood cells, white blood cells, and erythrocytes), whereas the clinical chemistry panel measures the proteins, enzymes, chemicals, and waste products in the blood. Hematological measures, when evaluated together and with information on other biomarkers, are informative diagnostic tests for blood-forming tissues (i.e., bone marrow, spleen, liver) and organ function. In animals, blood tests are influenced by the feeding protocol, blood collection site, animal age, and other factors.

Human Studies

One human study (Jiang et al., 2014) evaluated blood counts in samples drawn from a population of 141 pregnant women living in Tianjin, China. The study was considered *uninformative*, however, due to lack of consideration of confounding in the analysis and inadequate reporting of population selection criteria. Full study evaluation for Jiang et al. (2014) is available by clicking the HAWC link.

Animal Studies

Several animal toxicological studies were available that assessed hematopoietic parameters including a *high* confidence short-term study (NTP, 2018), *high* confidence (Chengelis et al., 2009b) and *high* confidence (Loveless et al., 2009) subchronic studies, and a *high* confidence chronic study (Klaunig et al., 2015). Cell counts for the blood components associated with immune system responses are summarized under in Immune Effects, Section 3.2.8. Study findings are discussed below and summarized in Table 3-18 (full details are available by clicking the HAWC link), and summary details are available in PFHxA Tableau visualization.

Table 3-18. Hematopoietic endpoints for PFHxA and associated confidence scores from repeated-dose animal toxicity studies

Author (year)	Species, strain (sex)	Exposure design	Exposure route and dose range	Hematology and hemostasis
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	++
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	++

Study evaluation for animal toxicological hematopoietic endpoints reported from studies with male and female rats receiving PFHxA[a] or PFHxA sodium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Hematology

Several findings were consistent (i.e., decreased red blood cells [RBCs], hematocrit, and hemoglobin) across studies and study designs that, when interpreted together, suggest PFHxA related adverse hematological effects such as anemia (Figure 3-13). Indications were also present that red blood cells were swollen and made up a larger proportion of the blood volume (increased mean cell volume [MCV, a measure of the average red blood cell size]). These changes were correlated with potential secondary erythrogenic responses to PFHxA exposure including increased reticulocyte (immature RBCs) counts that were consistently increased >10% across study designs and exposure durations, even in the females (that received a dose twice the male dose) in the chronic studies (Klaunig et al., 2015). Specifically, a dose-responsive decrease occurred in red blood cells (Table 3-19), hematocrit (Table 3-20), and hemoglobin (Table 3-21) in the short-term study with decreases at doses ranging from 62.5 mg/kg-day in male rats to 250 mg/kg-day in female rats (NTP, 2018). These findings also were observed in both subchronic studies in the highest dose groups (200 mg/kg-day in males only (Chengelis et al., 2009b) and 500 mg/kg-day in both sexes (Loveless et al., 2009)). Of note, decreases in both hemoglobin and hematocrit were 1.5–2-fold greater in the subchronic study (Loveless et al., 2009) than in the 28-day study (NTP, 2018) for both males and females at the same dose (500 mg/kg-day).

Findings from the 2-year chronic study (Klaunig et al., 2015) were generally null or observed at dose levels ≥100 mg/kg-day (100 mg/kg-day in males and 200 mg/kg-day in females) at 25 and 51 weeks. Measures of hematology beyond 52 weeks in the chronic study might be complicated due to natural diseases occurring in rodents and test variability leading to decreased sensitivity and increasing variability with the results (AACC, 1992). Klaunig et al. (2015) did, however, qualitatively evaluate blood and reported no PFHxA treatment effects on blood smear morphology. Loveless et al. (2009) also evaluated blood smears up to test day 92 with PFHxA sodium salt exposure and noted nucleated blood cells in smears indicative of bone marrow damage or stress, but only for 1 female and 1 male.

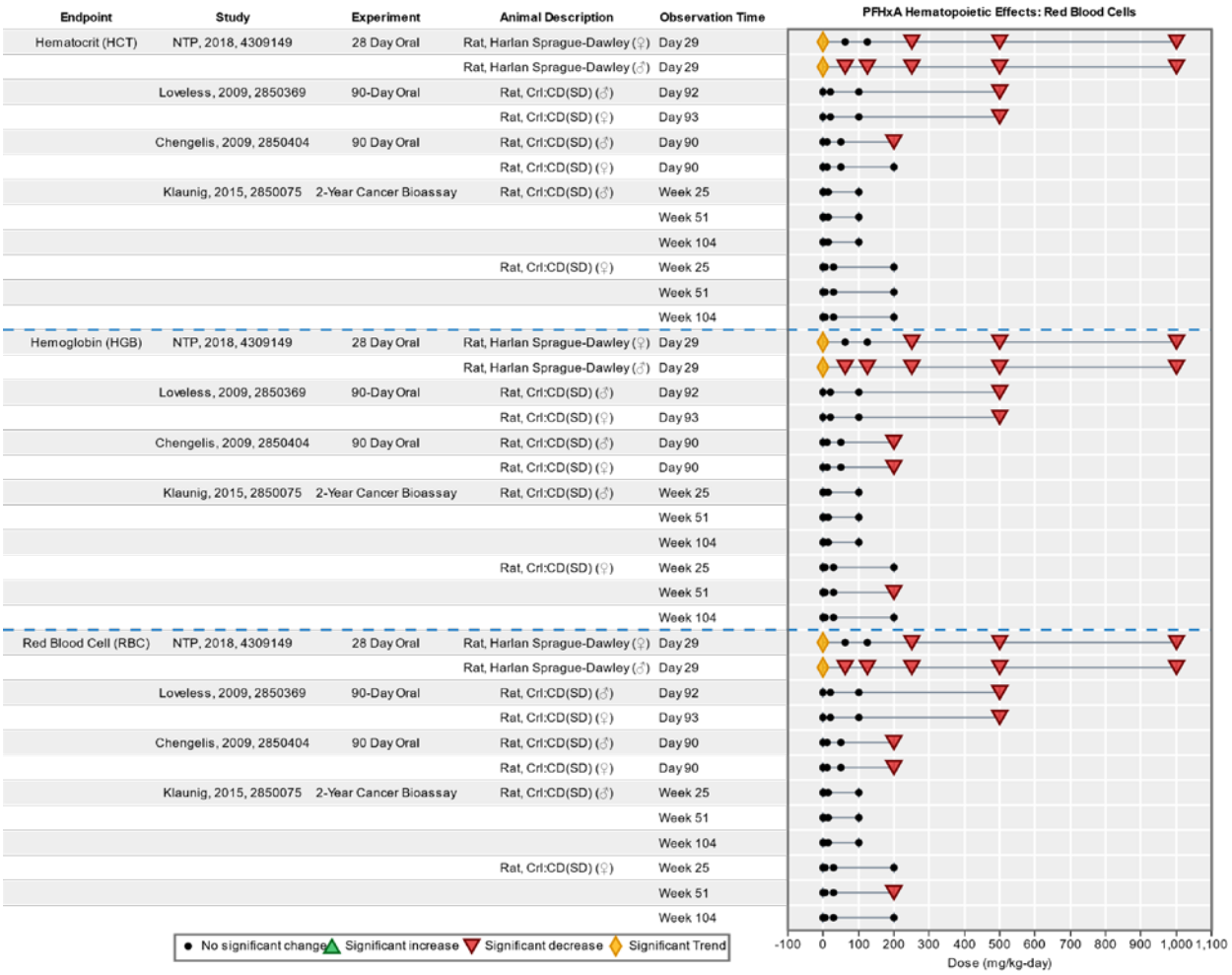


Figure 3-9. Hematological findings (hematocrit [HCT], hemoglobin [HGB], and red blood cells [RBC]) in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Table 3-19. Percent change in red blood cells due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								−1		2		−7	**−10**	**−26**
28-day, male rat (NTP, 2018)								−5		−5		**−9**	**−23**	**−48**
90-day, female rat (Chengelis et al., 2009b)			−1				−3				**−8**			
90-day, male rat (Chengelis et al., 2009b)			−1				0				**−8**			
90-day, female rat (Loveless et al., 2009)					2				0				**−18**	
90-day, male rat (Loveless et al., 2009)					1				−5				**−31**	
2-year, female rat (Klaunig et al., 2015), Week 25		4				−2					−1			
2-year, male rat (Klaunig et al., 2015), Week 25	−3			−3					−4					
2-year, female rat (Klaunig et al., 2015), Week 51		1				0					**−8**			
2-year, male rat (Klaunig et al., 2015), Week 51	−4			−6					−4					
2-year, female rat (Klaunig et al., 2015), Week 104		−1				−2					1			
2-year, male rat (Klaunig et al., 2015), Week 104	−7			−1					−8					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

The red blood cell mass parameter (MCHC, the average weight of hemoglobin in a specified volume of red blood cells) was decreased in both sexes in the short-term (NTP, 2018) and subchronic studies (Loveless et al., 2009) (Figure 3-14). Null findings for MCHC were observed in the other subchronic study (Chengelis et al., 2009b) and the chronic study (Klaunig et al., 2015). The maximum dose in Chengelis et al. (2009b) and Klaunig et al. (2015), however, was 200 mg/kg--day in females and for the chronic 100 mg/kg-day for males, versus >500 mg/kg-day in the other studies. MCV, a measure of average blood volume of RBCs was increased in both a short-term and a subchronic study (Loveless et al., 2009; NTP, 2018).

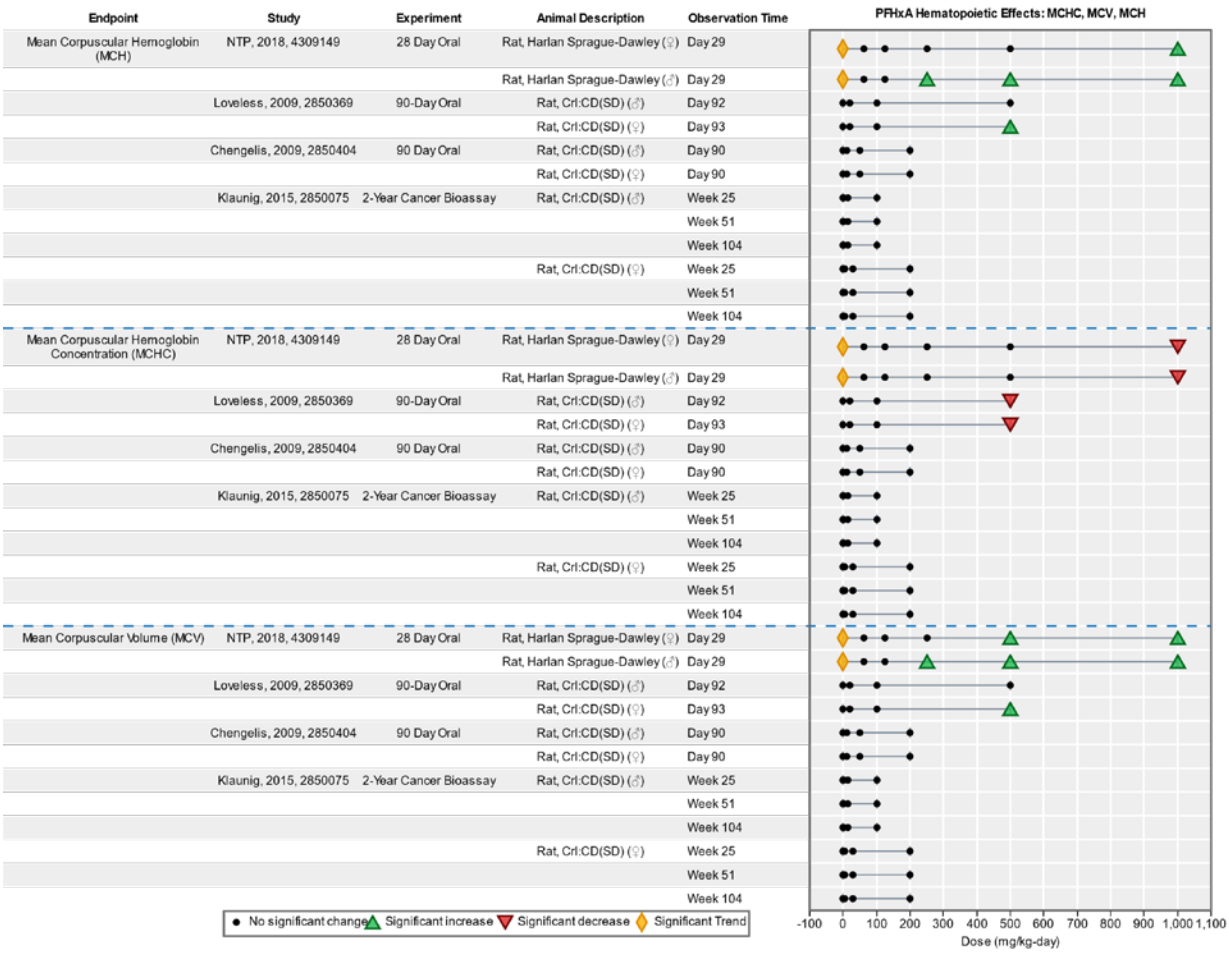


Figure 3-10. Hematological findings (mean cell hemoglobin [MCH], mean cell hemoglobin concentration [MCHC], and mean cell volume [MCV]) in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Table 3-20. Percent change in hematocrit due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								−1		0		**−7**	**−8**	**−17**
28-day, male rat (NTP, 2018)								−4		**−6**		**−6**	**−17**	**−30**
90-day, female rat (Chengelis et al., 2009b)			0				−5				−6			
90-day, male rat (Chengelis et al., 2009b)			−3				−3				−8			
90-day, female rat (Loveless et al., 2009)					1				0				**−13**	
90-day, male rat (Loveless et al., 2009)					0				−6				**−31**	
2-year, female rat (Klaunig et al., 2015), Week 25		3				0					0			
2-year, male rat (Klaunig et al., 2015), Week 25	−1			−3					−3					
2-year, female rat (Klaunig et al., 2015), Week 51		1				0					−4			
2-year, male rat (Klaunig et al., 2015), Week 51	−5			−4					−3					
2-year, female rat (Klaunig et al., 2015), Week 104		0				−1					1			
2-year, male rat (Klaunig et al., 2015), Week 104	−9			−5					−8					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Table 3-21. Percent change in hemoglobin due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								0		−1		**−6**	**−8**	**−19**
28-day, male rat (NTP, 2018)								−3		**−5**		**−6**	**−19**	**−40**
90-day, female rat (Chengelis et al., 2009b)			1				−3				−6			
90-day, male rat (Chengelis et al., 2009b)			−1				−1				−8			
90-day, female rat (Loveless et al., 2009)					1				0				**−15**	
90-day, male rat (Loveless et al., 2009)					1				−71				**−36**	
2-year, female rat (Klaunig et al., 2015), Week 25		3				1					−1			
2-year, male rat (Klaunig et al., 2015), Week 25	−1			−2					−3					
2-year, female rat (Klaunig et al., 2015), Week 51		1				0					**−5**			
2-year, male rat (Klaunig et al., 2015), Week 51	−6			−5					−3					

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
2-year, female rat (Klaunig et al., 2015), Week 104		0				0					−1			
2-year, male rat (Klaunig et al., 2015), Week 104	−9			-4					−9					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Increased reticulocyte (immature RBCs formed during the erythroid regenerative process) counts were consistently found across all four animal toxicological studies (Table 3-22 and Figure 3-15) and correlated with decreases in RBCs. PFHxA treatment-related increases in reticulocyte counts were potentially a compensatory biological response to the PFHxA anemia effect. Reticulocytes were increased (>10%) across all study designs and exposure durations at 200 mg/kg-day (Chengelis et al., 2009b; Klaunig et al., 2015), 250 mg/kg-day (NTP, 2018), or 500 mg-kg/day (Loveless et al., 2009). Reticulocyte levels also were measured by Klaunig et al. (2015), but only decreased in female rats that received double the dose of males. The observation of increased reticulocytes was correlated with histological findings of increased splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia incidence in both the males and females dosed with 500 mg/kg-day (Loveless et al., 2009; NTP, 2018) (summary details are available in PFHxA Tableau visualization). Collectively, the histological findings considered together with red blood cell parameters suggest PFHxA interacts with the erythropoietic pathways including outcomes such as anemia that can lead to compensatory erythrogenic responses in the bone marrow and spleen.

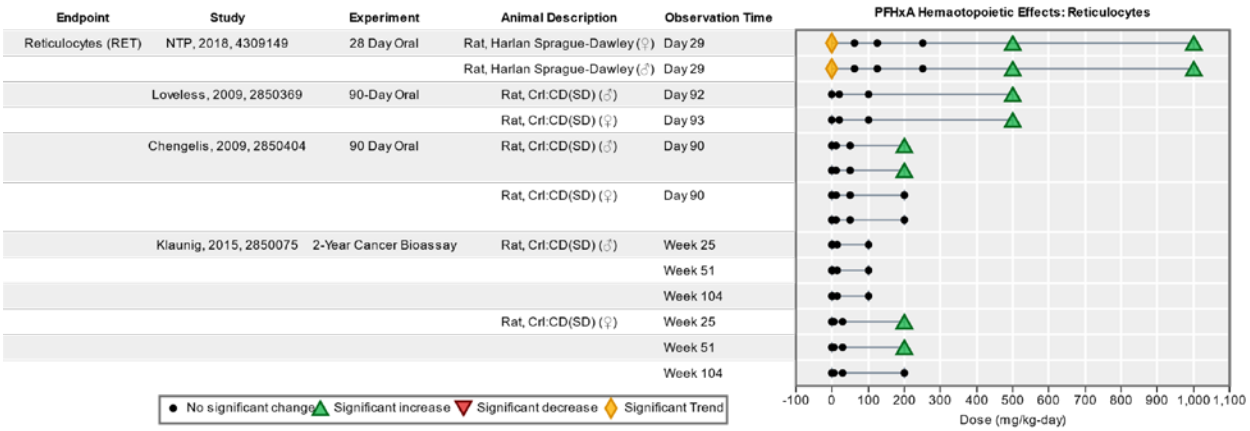


Figure 3-11. Hematological findings (reticulocytes) in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Table 3-22. Percent change in reticulocytes due to PFHxA exposure in short-term, subchronic, and chronic oral toxicity studies

Reference	Dose (mg/kg-d)													
	2.5	5	10	15	20	30	50	62.5	100	125	200	250	500	1,000
28-day, female rat (NTP, 2018)								−5		−15		15	**152**	**356**
28-day, male rat (NTP, 2018)								0		−2		20	**89**	**223**
90-day, female rat (Chengelis et al., 2009b)			−7				−13				**80**			
90-day, male rat (Chengelis et al., 2009b)			−5				−13				**59**			
90-day, female rat (Loveless et al., 2009)					7				13				**181**	
90-day, male rat (Loveless et al., 2009)					−14				−4				**210**	
2-year, female rat (Klaunig et al., 2015), Week 25		−5				11					26			
2-year, male rat (Klaunig et al., 2015), Week 25	−5			0					15					
2-year, female rat (Klaunig et al., 2015), Week 51		−25				−6					56			
2-year, male rat (Klaunig et al., 2015), Week 51	21			71					43					
2-year, female rat (Klaunig et al., 2015), Week 104		6				19					26			
2-year, male rat (Klaunig et al., 2015), Week 104	21			−6					29					

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Hemostasis

Hemostasis findings included platelet counts, prothrombin time, and activated partial thromboplastin time. Clotting times measured by Chengelis et al. (2009b) and Klaunig et al. (2015) could be complicated because blood samples were collected from the retro-orbital sinus, a technique not recommended because it leads to prolonged clotting times that might not be reliable; thus, these particular endpoints were considered uninformative and are not discussed further. Findings of statistically significant increased ($p < 0.05$) platelets were observed in the short-term (NTP, 2018) and subchronic (Chengelis et al., 2009b; Loveless et al., 2009) studies in males and females dosed with 500 mg/kg-day dose (Figure 3-16). Other hemostasis measures that included activated partial thromboplastin time (APTT) and prothrombin time (PT, a functional measure of a subset of clotting factors that contribute to APTT) were decreased inconsistently across sexes in one subchronic study (Loveless et al., 2009). PT was decreased in male dose groups receiving ≥20 mg/kg-day, whereas APTT was decreased in the 500 mg/kg-day female rat dose group. The observed increase in platelets and decreased clotting time (along with increased reticulocytes) were coherent changes indicative of an erythropoietic response in the bone marrow, suggesting bone marrow was not adversely affected by PFHxA exposure.

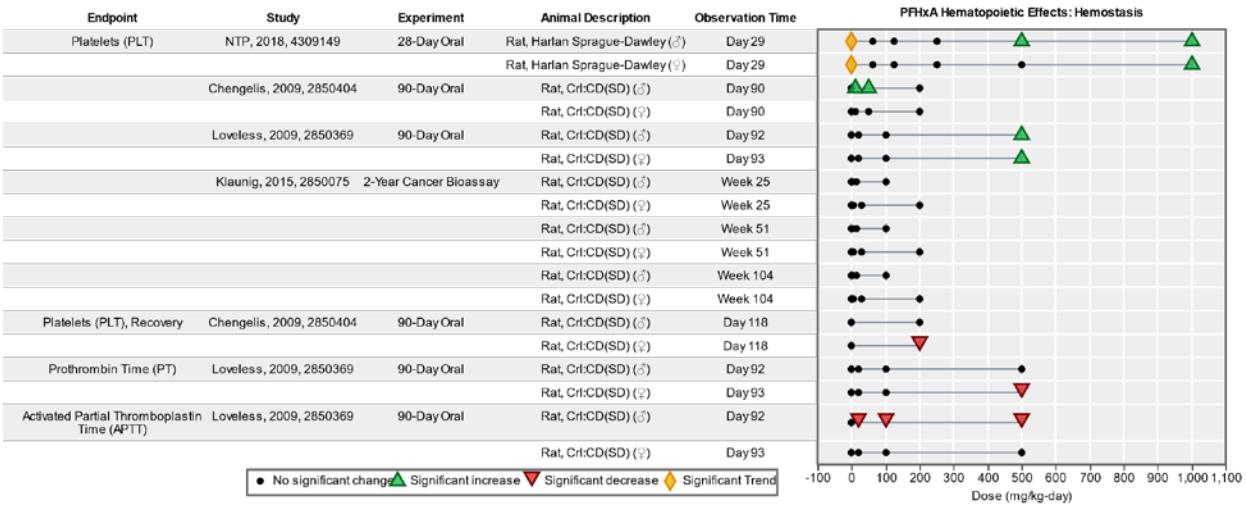


Figure 3-12. Hemostasis findings in rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Evidence Integration

The only available human study examining potential hematopoietic effects was considered *uninformative*; therefore, there is *indeterminate* human evidence of hematopoietic effects.

Collectively, the animal toxicological information provided coherent evidence indicative of macrocytic anemia (characterized by low hemoglobin and large red blood cells) that is consistent across multiple laboratories and experimental designs. Findings informing the overall judgment included consistent observations of decreased red blood cells, hematocrit, and hemoglobin at doses as low as 200 mg/kg-day generally in both sexes of Sprague-Dawley rat. This finding was considered an adverse response to PFHxA exposure and correlated with a compensatory increase in reticulocytes, an indicator of erythroid cell regeneration supported by histological findings of splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia. These collective erythroid responses provide evidence for PFHxA treatment-related effects on erythropoiesis. Blood loss could have been secondary to gastral erosion or ulceration (Klaunig et al., 2015) (summary level details are available in the Tableau link) but Klaunig et al. (2015) reported gastral erosion and ulceration were likely due to mechanical dosing errors ruling out treatment-related effects on blood loss.

Based on these data, there is *moderate* animal evidence of hematopoietic effects. Effects on red blood cell parameters including decreased hemoglobin and red blood cells, and decreased reticulocytes are consistent across both subchronic and chronic studies in the 200 mg/kg-day dose groups. Overall, the currently available ***evidence indicates*** that PFHxA likely causes hematopoietic effects in humans under relevant exposure circumstances. This conclusion is based on four *high* confidence studies in rats showing consistent (across durations and study types), dose-related, and coherent effects (across various outcome measures of hematopoietic function) at ≥500 mg/kg-day following short-term (28-day), subchronic (90-day), or chronic (2-year) exposures.

Table 3-23. Evidence profile table for hematopoietic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊕⊕⊙ ***Evidence indicates* (likely)** *Primary basis:* Four high confidence studies in rats ranging from short term to chronic exposure durations, in both sexes, generally at ≥200 mg/kg-day *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and lifestages:* No evidence to inform
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
• There were no informative human studies available from the PFHxA evidence base.				⊙⊙⊙ *Indeterminate*	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Hematology *4 high* confidence studies in adult rats: • 28-day • 90-day (2 studies) • 2-year	• *Consistent* changes (decreases in hematocrit, hemoglobin, red blood cells, and MCHC and increases in reticulocytes, MCV, and MCH) across studies • *Coherence* of red blood cells, HCT, and HGB and reticulocytes • *Large magnitude of effect* as high as 356% for reticulocytes • High confidence studies	• *Lack of dose-response gradient* across studies • *Lack of coherence* across sexes	• Decreased red blood cells, hematocrit, and hemoglobin at ≥62.5 mg/kg-d; both sexes • Increased MCH and MCV at ≥250; males more sensitive • Increased reticulocytes at ≥200 mg/kg-d; both sexes, all studies • Coherence of red blood cells and reticulocytes with splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia	⊕⊕⊙ *Moderate* Findings considered adverse based on coherent evidence that was consistent across multiple laboratories and experimental designs. Consistent findings of decreased red blood cells, hematocrit, and hemoglobin at ≥200 mg/kg-day correlated with a	

Evidence stream summary and interpretation					Evidence integration summary judgment
Hemostasis *4 high* confidence studies in adult rats: • 28-day • 90-day (2 studies) • 2-year	• *Consistent* treatment related effect on platelet levels • *Consistency* across study designs • *High* confidence studies	• *Lack of coherence* across sexes • *Lack of dose-response gradient*	• Increased platelet levels ≥10 mg/kg-d; both sexes, 1 28-day, 2 90-day studies • Decreased activated partial thromboplastin (APTT) at ≥20 mg/kg-d; males only, 1 90-day study • Decreased prothrombin (PT) time at 500 mg/kg-day; males only, 1 90-day study	compensatory findings of erythroid cell regeneration	
Mechanistic evidence and supplemental information					
Biological events or pathways	**Species or model systems**	**Key findings, limitations, and interpretation**		**Evidence stream summary**	
• No informative studies identified.					

3.2.5. Endocrine Effects

Human

Thyroid Hormones

Two studies examined the association between PFHxA exposure and thyroid hormones in humans (Figure 3-17). One was considered *uninformative* due to critical deficiencies in confounding and statistical analysis (Seo et al., 2018). The other study was a cross-sectional study of the general population in China and was considered *low* confidence (Li et al., 2017) due to concerns around participant selection, outcome measures, consideration of confounding, and decreased sensitivity. Regarding the latter concern, the exposure levels and range in Li et al. (2017) were low (median [range]: 0.01 [<LOD–1.1]) and 47% of samples were below the LOD, which precluded a meaningful analysis of associations with health outcomes. Among participants without thyroid disease inverse associations with free T3 and thyroid stimulating hormone (TSH) were reported, with the TSH being statistically significant (Pearson correlation coefficient =−0.27, $p < 0.01$). No association was observed with free T4. Total T4 and T3 were not measured in this study.

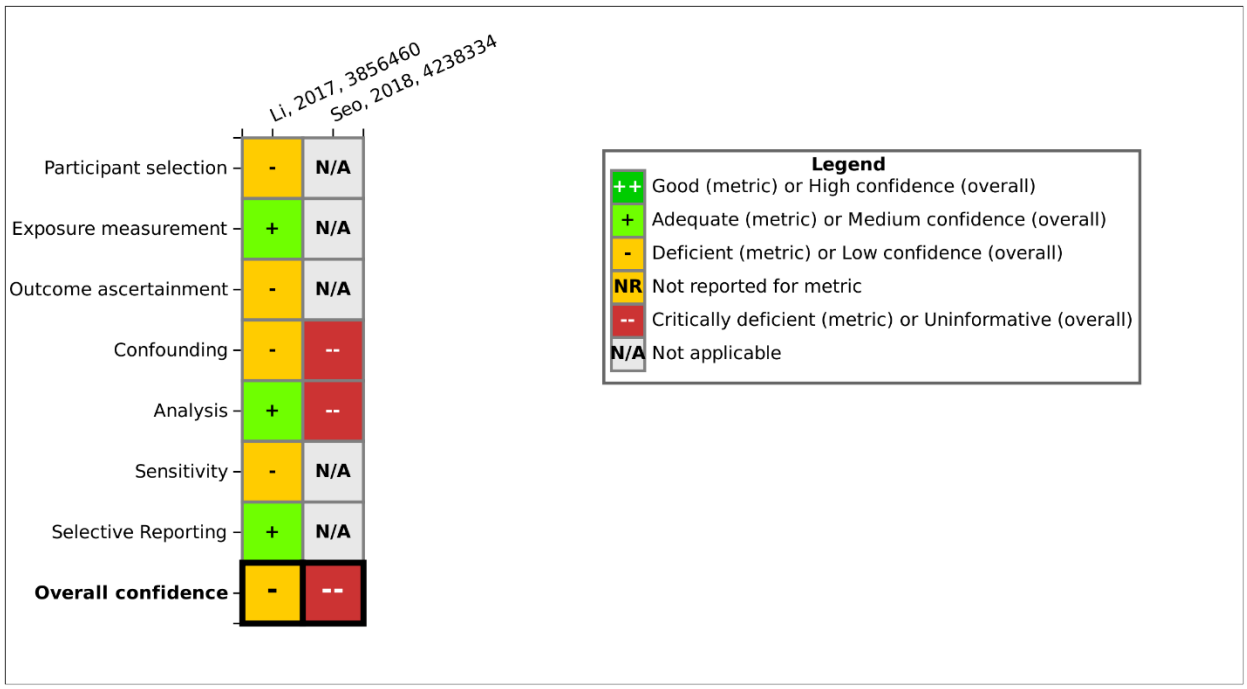


Figure 3-13. Study evaluation for human epidemiologic studies reporting toxicity findings from PFHxA exposures (HAWC: PFHxA – Human Toxicity Endocrine Effects link).

Animal

Four short-term (28-day), subchronic, and chronic animal studies evaluated potential endocrine effects of PFHxA or PFHxA sodium salt in rats. Most of the outcome-specific study ratings were rated *high* confidence. Histopathology for Chengelis et al. (2009b) was rated *low* confidence because of issues related to observational bias, concerns about endpoint sensitivity and specificity, and results presentation. A summary of the studies and the interpretations of confidence in the results for the different outcomes based on the individual study evaluations is presented in Table 3-24, and details are available by clicking the HAWC link.

Table 3-24. Endocrine endpoints for PFHxA and associated confidence scores from repeated-dose animal toxicity studies

Author (year)	Species, strain (sex)	Exposure design	Exposure route and dose range	Organ weight	Histopathology	Thyroid hormones
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	++	++
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	++	-	NM
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	NM
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	++	NM

Study evaluation for animal toxicological endocrine endpoints reported from studies with male and female rats receiving PFHxA[a] or PFHxA sodium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.
++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Thyroid Hormones

A single study evaluated potential PFHxA effects on endocrine function specific to thyroid hormones in rats exposed for 28 days (NTP, 2018). Specifically, males showed statistically significant, dose-dependent decreases in thyroid hormones. These outcomes showed a clear dose-dependent pattern of effect with treated animals showing reductions of 25–73% or 20–58% for free or total T4, respectively. Smaller decreases in T3 in males also were also observed (18–29%), although the dose-dependence of this effect was less clear. No treatment-related changes were

observed for T3 or T4 in females or for TSH in either sex (NTP, 2018). Results are summarized in Figure 3-18 and Table 3-25.

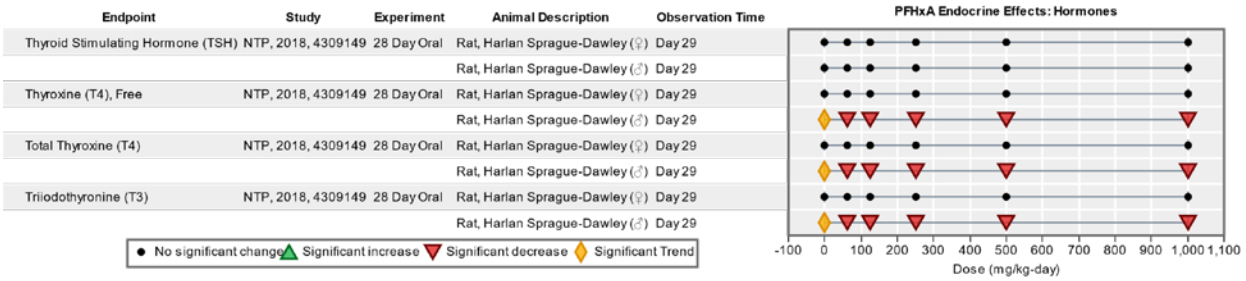


Figure 3-14. Thyroid hormone measures from the serum of rats exposed by gavage to PFHxA or PFHxA sodium salt (full details available by clicking the HAWC link).

Table 3-25. Percent change in thyroid hormone levels following PFHxA exposure in a 28-day oral toxicity study

Reference	Hormone	Dose (mg/kg-d)				
		62.5	125	250	500	1,000
28-day, female rat (NTP, 2018)	Free T4	−1	−4	9	−4	−19
28-day, male rat (NTP, 2018)		**−25**	**−38**	**−39**	**−55**	**−73**
28-day, female rat (NTP, 2018)	Total T4	−7	−11	−5	−9	−19
28-day, male rat (NTP, 2018)		**−20**	**−31**	**−32**	**−44**	**−58**
28-day, female rat (NTP, 2018)	T3	−1	−6	3	14	−3
28-day, male rat (NTP, 2018)		**−18**	**−26**	**−15**	**−16**	**−29**
28-day, female rat (NTP, 2018)	TSH	−15	−8	−9	40	−9
28-day, male rat (NTP, 2018)		9	5	6	9	−21

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors.

Histopathology

Four studies evaluated histopathological changes in endocrine tissues, including the thyroid, pituitary, and pancreas, in rodents exposed to PFHxA (Chengelis et al., 2009b; Klaunig et al., 2015; NTP, 2018) or PFHxA sodium salt (Loveless et al., 2009). Of these, Loveless et al. (2009) reported thyroid follicular cell hypertrophy in both male and female rats exposed to PFHxA sodium salt for 90 days. The hypertrophy persisted after the exposure ceased, with females showing an increase at the 30-day (but not 90-day) recovery whereas, in males this effect was observed at the 90-day recovery time point. NTP (2018) reported this outcome was not affected by PFHxA following a 28-day exposure at doses as high as 1,000 mg/kg-day. The remaining two studies

reported no treatment-related effects on thyroid histopathology at doses as high as 200 mg/kg-d following subchronic (90-day) or chronic (2-year) exposure to PFHxA. Notably, Chengelis et al. (2009b) did not specify what outcomes were evaluated. Therefore, whether thyroid follicular cell hypertrophy was measured is unclear. No other treatment-related histopathological effects were noted in the PFHxA evidence base. Results are summarized in Table 3-26.

Table 3-26. Incidence of thyroid follicular epithelial cell hypertrophy following PFHxA ammonium salt exposure in a 90-day oral toxicity study

Reference	Time point	Dose (mg/kg-d)			
		0	20	100	500
90-day, female rat (Loveless et al., 2009)	Exposure, Day 90	0/10	0/10	0/11	**4/10**
90-day, male rat (Loveless et al., 2009)		0/10	0/10	**1/10**	**2/10**
90-day, female rat (Loveless et al., 2009)	Recovery Day 30	0/10			**6/10**
90-day, male rat (Loveless et al., 2009)		0/10			**3/10**
90-day, female rat (Loveless et al., 2009)	Recovery, Day 90	0/10	0/10	0/9	0/10
90-day, male rat (Loveless et al., 2009)		0/10	0/10	0/10	**2/10**

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies.

Organ Weights

Three studies evaluated effects on thyroid and adrenal weights (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). Although no effects on relative thyroid weight were observed at the end of the 90-day exposure period in either subchronic study, Loveless et al. (2009) qualitatively reported a statistically significant increase in relative thyroid weight for female rats at the highest tested dose (500 mg/kg-day) of PFHxA sodium salt at the 30-day recovery. NTP (2018) observed a trend ($p < 0.05$) for decreased absolute adrenal gland weight in male rats exposed to 500 mg/kg-day. No other treatment-related effects on endocrine organ weights were observed (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018).

Evidence Integration

A single *low* confidence study provided some evidence of an association between PFHxA exposure and decreased T3 and TSH in humans, although methodological concerns reduce the reliability of these findings. Based on these results, there is *indeterminate* human evidence of endocrine effects.

Evidence supporting potential endocrine effects of PFHxA exposure is largely based on two *high* confidence rat studies showing decreases in serum thyroid hormone levels and increased thyroid epithelial cell hypertrophy in rats, but interpretation of these results is complex. The only available animal study that evaluated thyroid hormone levels showed a large magnitude of change in T4 (up to 73% decrease) and T3 (up to 20% decrease) with a clear dose-response for T4 (free

and total), but these effects were observed only in males (NTP, 2018). A second study found increased incidence of thyroid epithelial cell hypertrophy following a 90-day exposure to PFHxA sodium salt (Loveless et al., 2009). For the histopathological findings, treatment-related changes were reported for both males and females administered 500 mg/kg-day PFHxA sodium salt. The incidence of thyroid hypertrophy was higher in females than in males, although effects in males persisted longer after exposures had ceased. Also, no clear dose-response was found, with effects generally observed only at the highest dose tested. Three other studies evaluated thyroid histopathology but found no effects in either sex following a wide range of PFHxA exposure durations (28 d to 2 years) and doses (up to 1,000 mg/kg-day) (Chengelis et al., 2009b; Klaunig et al., 2015; NTP, 2018). No clear pattern of treatment-related effects were reported for endocrine organ weights.

Although the only available study examining thyroid hormones showed strong effects on T4 and T3 after short-term exposure, no effects were observed on TSH; however, a pattern of decreased T4 without changes in TSH is consistent with hypothyroxinemia and has been observed for other PFAS with more detailed studies of endocrine function, including PFBA and PFBS. During pregnancy and early development, perturbations in thyroid function can have impacts on normal growth and neurodevelopment in the offspring. Given the potential consistency of these findings with those observed for other PFAS, the availability of only one short-term study of thyroid hormones represents a significant data gap for PFHxA.

The small number of studies and inconsistent findings for endpoints reported across study designs reduces the strength of the available evidence; however, some of these inconsistencies could be explained by differences in the test article (i.e., PFHxA vs. PFHxA salts), dose levels examined (i.e., high dose ranged from 100 to 1,000 mg/kg-day), and exposure duration (i.e., short-term, subchronic, and chronic exposures). Evidence suggests sex-specific differences in the pharmacokinetics of PFHxA, with plasma concentrations measured 2–3 times higher in male rats when compared to females (Chang et al., 2008; Lau et al., 2004; Lau et al., 2006). Differences in pharmacokinetics might explain why effects on thyroid hormones were observed only in male rats, but why a similar sex-specific pattern was not observed for the reported thyroid histopathological effects is unclear. Furthermore, that the observed changes in thyroid histopathology are secondary to hepatic effects is possible. In rats, increases in thyroid epithelial cell hypertrophy are associated with induction of microsomal liver enzymes and hepatocellular hypertrophy (Cesta et al., 2014). Based on the results, there is *slight* animal evidence of endocrine effects.

Overall, the currently available ***evidence suggests***, but is not sufficient to infer, that PFHxA could cause endocrine effects in humans under relevant exposure circumstances. This conclusion is based on four animal studies generally rated *high* confidence that reported treatment-related changes in thyroid hormone levels and thyroid histopathology after exposure to PFHxA at ≥62.5 mg/kg-day (Table 3-27).

Table 3-27. Evidence profile table for endocrine effects

Evidence stream summary and interpretation					Evidence integration summary judgement
Evidence from studies of exposed humans					⊕⊙⊙ ***Evidence suggests but is not sufficient to infer*** *Primary basis:* Four animal studies generally rated high confidence that reported treatment related changes in thyroid hormone levels, thyroid histopathology after exposure to PFHxA at ≥ 63.5 mg/kg-day. *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans. *Cross-stream coherence:* Decreases in T3 were observed in both animal and human studies, although results in humans were of low confidence. *Susceptible populations and lifestages:*
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgments**	
Thyroid Hormones *1 low* confidence study	• No factors noted	• *Lack of coherence* across related thyroid hormone measures • *Low* confidence study	• Inverse associations between free T3 and TSH and PFHxA in a single *low* confidence study	⊙⊙⊙ ***Indeterminate***	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgments**	
Thyroid Hormones *1 high* confidence study in adult rats: • 28-day	• *High* confidence study. • *Dose-response* gradient observed for free and total T4 • Large *effect magnitude*; up to 73%	• No factors noted	• Decreased T4 (free and total) and T3 observed in males only at ≥ 62.5 mg/kg-day	⊕⊙⊙ ***Slight*** Some evidence of thyroid effects based on hormone and histopathological changes in two rat studies; however, the data is limited, lacking consistency across studies, and histopathological changes may be explained by non-thyroid related effects	
Histopathology *3 high* confidence studies in adult rats: • 28-day • 90-day • 2-year	• *High* confidence studies	• *Unexplained inconsistency* across studies	• Increased incidence of thyroid epithelial cell hypertrophy at ≥100 mg/kg-day for 90 days; persisted up to 90 days after exposure		

Evidence stream summary and interpretation					Evidence integration summary judgement
1 low confidence study in adult rats: • 90-day					No evidence to inform *Other inferences:* No mechanistic data or supplemental information on this health effect were identified to inform a potential MOA for the observed effects, although the pattern of the limited findings for PFHxA are consistent with hypothyroxinemia seen for other PFAS
Organ Weight *High* confidence: *3 high* confidence studies in adult rats: • 28-day • 90-day (2 studies)	• *High* confidence studies	• *Unexplained inconsistency* across studies	• Relative thyroid weights were increased only in females 30 days after exposure • Right adrenal weights decreased but no other adrenal effects were reported		
Mechanistic evidence and supplemental information					
Species or model systems	**Key findings, limitations, and interpretation**	**Evidence stream summary**		**Species or model systems**	
• No informative studies identified					

3.2.6. Male Reproductive Effects

Human

Sperm Parameters

One *low* confidence study (Song et al., 2018) examined the association between PFHxA exposure and semen parameters and reported no decrease in concentration or motility with higher levels of PFHxA in serum (Figure 3-19). A significant negative correlation between PFHxA levels in semen and sperm motility was found in this study (correlation coefficient = −0.35, $p < 0.01$), but analytical measurement of PFAS in semen is less established than in blood and the relevance to exposure is unclear. Still, exposure levels in the study were fairly high (median: 29 ng/mL, 5th–95th percentile: 11–70), so the study had reasonable sensitivity to detect an effect.

Reproductive Hormones

A single study rated *low* confidence due to low sensitivity and high potential for confounding (Figure 3-19) found some support for associations between PFHxA and reproductive hormones in a population of adolescent (13–15 years old) males in Taiwan (Zhou et al., 2016). Overall, authors reported no significant associations between PFHxA and serum testosterone and estradiol; however, when the data were stratified by sex, a significant negative association between testosterone and PFHxA exposure level (β = −0.31, 95% CI: −0.59, −0.02) was found in boys. The exposure levels in this study were low and the range narrow (median: 0.2, IQR 0.1–0.3), which might have reduced study sensitivity. The presence of an association despite reduced sensitivity could be due to either high potency of the exposure to cause these effects or potential confounding by other correlated PFAS, including PFOS, PFDA and PFNA.

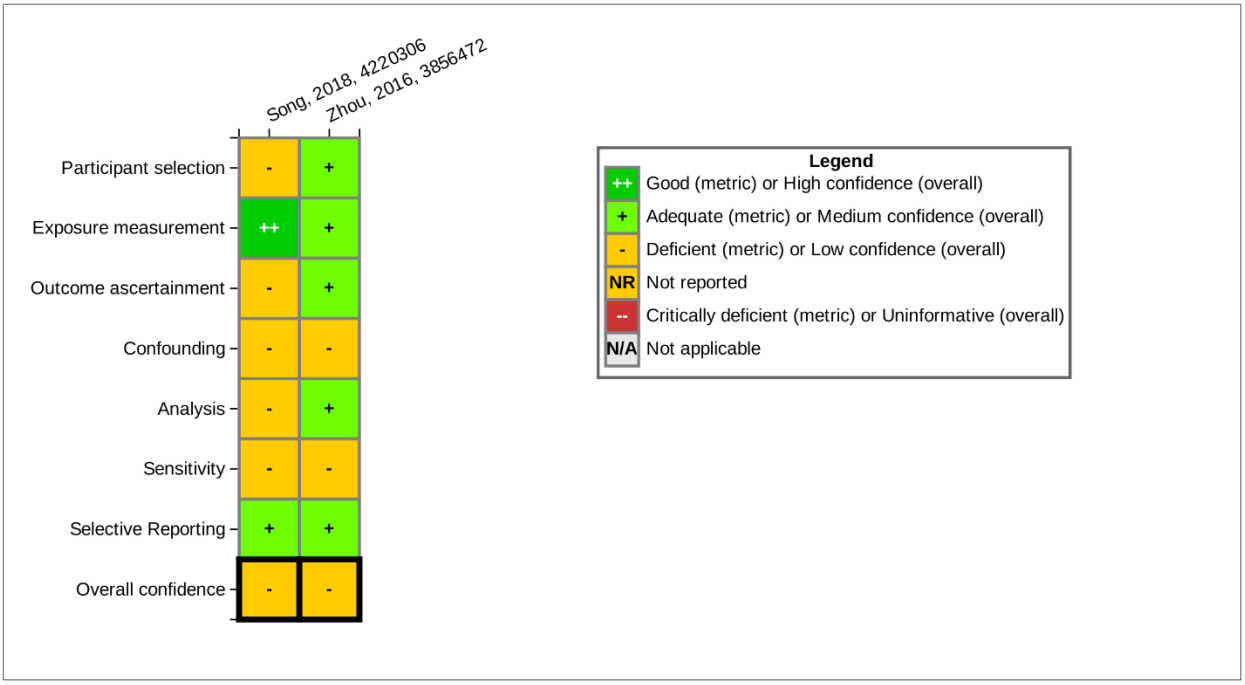


Figure 3-15. Study evaluation for human epidemiological studies reporting male reproductive findings from PFHxA exposures (HAWC: PFHxA – Human Toxicity Male Reproductive Effects link).

Animal

Several short-term (28-day), subchronic, and chronic animal studies evaluated sperm parameters, reproductive organ weights, and other reproductive male outcomes in rats receiving oral exposures of PFHxA and PFHxA sodium salt. Most outcome-specific study ratings were rated *high* confidence; however, some specific concerns were identified that resulted in *low* confidence ratings. Although generally a well-conducted study, NTP (2018) was rated *low* confidence for sperm parameters due to issues related to exposure duration and concerns for potential insensitivity. Histopathological results for Chengelis et al. (2009b) were rated *low* confidence because of issues related to observational bias, concerns about endpoint sensitivity and specificity, and results presentation. The results of the outcome-specific study evaluations are presented in Table 3-28, and details are available by clicking the HAWC link.

Table 3-28. Study design, exposure characteristics, and individual outcome ratings

Study	Species, strain (sex)	Exposure design	Exposure route and dose	Sperm parameters	Organ weight	Histopathology	Hormone levels	Reproductive system development
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	-	++	++	++	NM
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	NM	++	-	NM	NM
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days) One-generation reproductive: P0 females dosed 70 d prior to cohabitation, through gestation and lactation (126 days); P0 males dosed for 110 days Developmental: Gestation Days 6–20	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++	NM	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	NM	++	++	NM
Iwai and Hoberman (2014)[c]	Mouse, Crl: CD1(ICR); Charles River Laboratories, Inc.	Gestation Days 6–18	Gavage[d] Phase 1: 0, 100, 350, 500 mg/kg-d Phase 2: 0, 7, 35, 175 mg/kg-d	NM	NM	NM	NM	++

Study evaluation for animal toxicological endpoints reported from male reproductive studies with rats receiving PFHxA,[a] PFHxA sodium salt,[b] or PFHxA ammonium salt[d] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

[c]Phase 1 was a range-finding study used to determine the appropriate dose range for identification of a NOAEL in Phase 2.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Sperm Parameters

Evidence from a 28-day (NTP, 2018) and one-generation reproductive study (Loveless et al., 2009) included sperm parameters useful in evaluating potential male reproductive effects (Figure 3-20). In male rats receiving PFHxA daily by gavage for 28 days, a trend ($p < 0.05$) for decreased sperm count in the cauda epididymis was identified with a significant (25% change from control) decrease in the 1,000 mg/kg-day dose group. Animals in this dose group showed a significant decrease in body weight (13% change from control) at the end of the study but no other overt toxicity was indicated (e.g., mortalities or significant clinical observations) (NTP, 2018). Notably, these effects were observed despite concerns about sensitivity due to the short exposure duration of the study by NTP (2018). In the one-generation reproductive study, Loveless et al. (2009) found no treatment-related effects for sperm parameters following a 10-week premating exposure in male rats to PFHxA sodium salt at doses up to 500 mg/kg-day. Results are summarized in Figure 3-20.

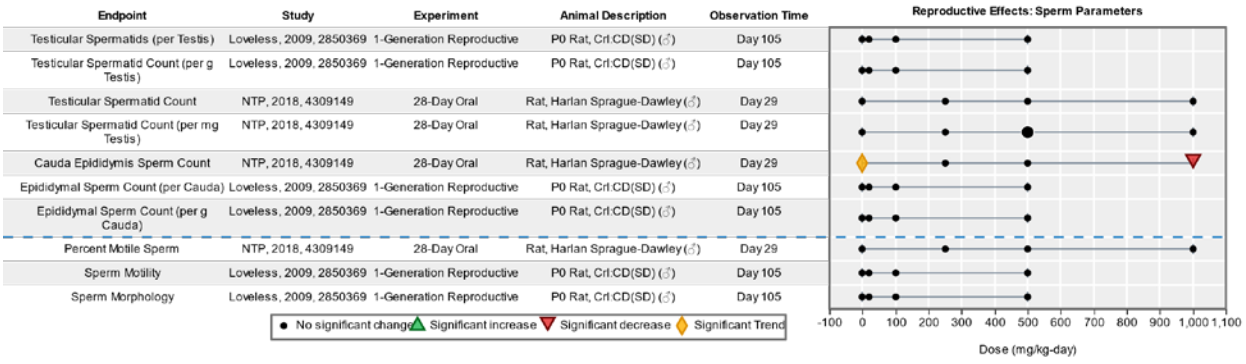


Figure 3-16. Male reproductive effects on sperm parameters in male rats exposed to PFHxA or sodium salt for 28 or 90 days (HAWC: PFHxA – Animal Toxicity Male Reproductive Effects link).

Reproductive Organ Weights

Reproductive studies commonly report both absolute and relative organ weights; however, for the testes, absolute weights are considered most informative for hazard evaluation (Bailey et al., 2004). Three studies (28- or 90-day exposure durations) reported data on the effects of PFHxA or PFHxA sodium salt exposure on male reproductive organ weights (i.e., testes, epididymis) in rats (Figure 3-21) (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). Two studies reported a modest but statistically significant ($p < 0.05$; 13–16% change from control) increase in relative, but not absolute, testis weight in rats exposed to 1,000 mg/kg-day for 28 days (NTP, 2018) or 500 mg/kg-day for 90 days (Loveless et al., 2009). No treatment-related effects on male reproductive organ weights were reported by Chengelis et al. (2009b).

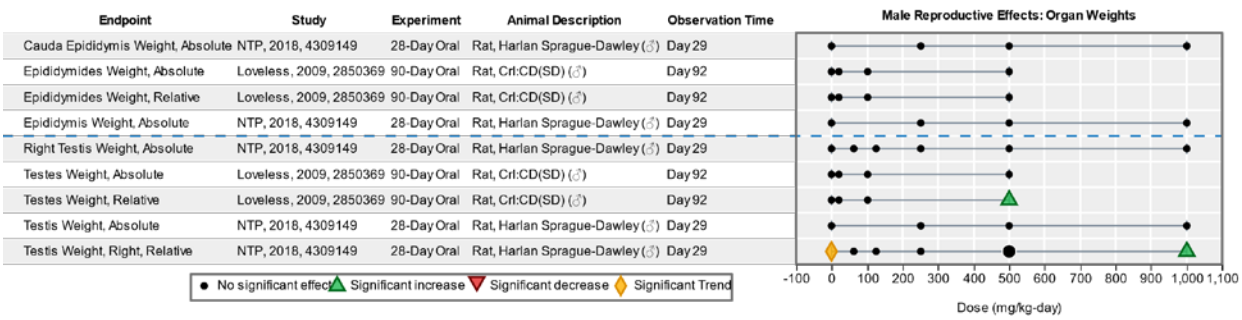


Figure 3-17. Male reproductive effects on epididymis and testis weight in rats exposed to PFHxA or PFHxA sodium salt (HAWC: PFHxA – Animal Toxicity Male Reproductive Effects link).

Reproductive Hormones

Two studies measured hormone levels (i.e., testosterone, estradiol, and luteinizing hormone) following exposure to PFHxA (Klaunig et al., 2015; NTP, 2018). Klaunig et al. (2015) reported a small, transient decrease in testosterone and luteinizing hormone in males at the 26-week time point. Effects were not dose dependent and were not significantly different from controls at doses up to 100 mg/kg-day PFHxA. This pattern was not observed at the 52-week time point. A short-term study found no effects on testosterone following exposure of up to 1,000 mg/kg-day for 28 days (NTP, 2018). Klaunig et al. (2015) also measured estradiol but found no treatment-related changes.

Histopathology

Four studies evaluated effects of PFHxA or PFHxA sodium salt on histopathology of the testes and epididymites and reported no treatment-related changes (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). One study was rated *low* confidence for this outcome (Chengelis et al., 2009b).

Male Reproductive System Development

Two studies examined outcomes related to male reproductive system development following developmental exposure to PFHxA ammonium or sodium salts (Iwai and Hoberman, 2014; Loveless et al., 2009). No treatment-related effects were reported on the age at preputial separation, a marker of puberty onset.

Evidence Integration

The available evidence informing the potential for an effect of PFHxA exposure on male reproduction in humans was limited to two *low* confidence studies that provided some indication of an association between PFHxA exposure and sperm motility (Song et al., 2018) and reproductive hormone levels (Zhou et al., 2016). These results are difficult to interpret, however, based on the

availability of a single study for each outcome and the high risk for bias in these evaluations. Based on these results, there is *indeterminate* human evidence of male reproductive effects.

In animals, the evidence supporting potential effects of PFHxA exposure on male reproduction was primarily limited to decreased sperm count (NTP, 2018) and increased relative testis weights (Loveless et al., 2009; NTP, 2018) at the highest tested doses in these studies (1,000 and 500 mg/kg-day, respectively). Decreased sperm count reported by NTP (2018) was considered *low* confidence due to the 28-day exposure duration and concerns that such short exposures would not capture the full spermatogenic cycle. Although finding effects in the presence of an insensitive exposure duration could indicate a sensitive window for chemical-specific perturbations, similar results were not observed in a *high* confidence subchronic study performed in the same rat strain (Loveless et al., 2009), albeit the highest tested dose was 500 as compared to 1,000 mg/kg-day in the NTP study. In addition, evidence of overt toxicity (i.e., reductions in body weight) was found in the 1,000 mg/kg-day males in the NTP (2018) study.

Two studies reported increased relative testis weight; however, the preferred metric of absolute testis weight did not change in either study and no changes in organ weight were observed in a second subchronic study (Chengelis et al., 2009b). Reproductive hormone (i.e., testosterone and luteinizing hormone) levels were reduced in the only chronic study; however, the effect was small in magnitude, was not dose-dependent, and was observed only at the 26-week time point (Klaunig et al., 2015). Similar results on testosterone were not reported in the short-term *high* confidence study (NTP, 2018). No other coherent findings (i.e., reproductive histopathology and male reproductive system development) supporting reproductive toxicity were identified in the animal evidence base. Based on these results there is *indeterminate* animal evidence of male reproductive effects.

Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA might cause male reproductive effects in humans under relevant exposure circumstances (Table 3-29).

Table 3-29. Evidence profile table for male reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊙⊙⊙ ***Evidence inadequate*** *Primary Basis:* Evidence is *low* confidence or largely null *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans *Cross stream coherence:* N/A (human evidence indeterminate) *Susceptible population and lifestages:* No evidence to inform
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Sperm Parameters *1 low* confidence study	• No factors noted	• *Low* confidence study.	• Association between PFHxA levels in semen and decreased sperm motility	⊙⊙⊙ ***Indeterminate***	
Reproductive Hormones *1 low* confidence study	• No factors noted	• *Low* confidence study	• Significant inverse association between PFHxA exposure and testosterone despite poor sensitivity		
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	⊙⊙⊙ ***Indeterminate*** The data are largely null. Some evidence of reproductive effects but limited by unexplained inconsistency and low sensitivity.	
Sperm Parameters *1 high* confidence study in adult rats: • 90-day *1 low* confidence in adult rats • 28-day	• No factors noted	• High dose elicited overt toxicity (i.e., decreased body weight) • *Unexplained inconsistency* across studies	• Decreased sperm count in the cauda epididymis at 1,000 mg/kg-day		
Organ Weights *3 high* confidence studies in adult rats: • 28-day • 90-day (2 studies)	• *High* confidence studies • *Dose-response* with longer exposure duration	• No factors noted	• Increased relative testis weight at ≥500 mg/kg-day; no change in absolute testis weights (preferred metric)		

Evidence stream summary and interpretation					Evidence integration summary judgment
Reproductive Hormones *2 high* confidence studies in adult rats: • 28-day • 2-year	• *High* confidence studies	• No factors noted	• Transient decrease of small magnitude in luteinizing hormone and testosterone		
Histopathology and Male Reproductive System Development *4 high* confidence studies in rats and mice: • 28-day (rat) • 90-day (rat) • GD 6–18 (mouse) • 2-year (rat) *1 low* confidence study in adult rats: • 90-day	• *High* confidence studies	• *Low sensitivity*.	• No treatment related effects reported at ≤1,000 mg/kg-day		
Mechanistic evidence and supplemental information					
Biological events of pathways	**Biological events of pathways**	**Biological events of pathways**		**Biological events of pathways**	
• No studies identified					

3.2.7. Female Reproductive Effects

Human

Reproductive Hormones

A single *low* confidence study (Figure 3-22) evaluated associations between PFHxA and reproductive hormones in a population of Taiwanese adolescents (13–15 years old) (Zhou et al., 2016). Overall, the authors reported nonsignificant inverse associations between PFHxA and serum testosterone and estradiol in females when the data were stratified by sex. Exposure levels to PFHxA were low, which might have reduced study sensitivity, as described above in the male reproductive section.

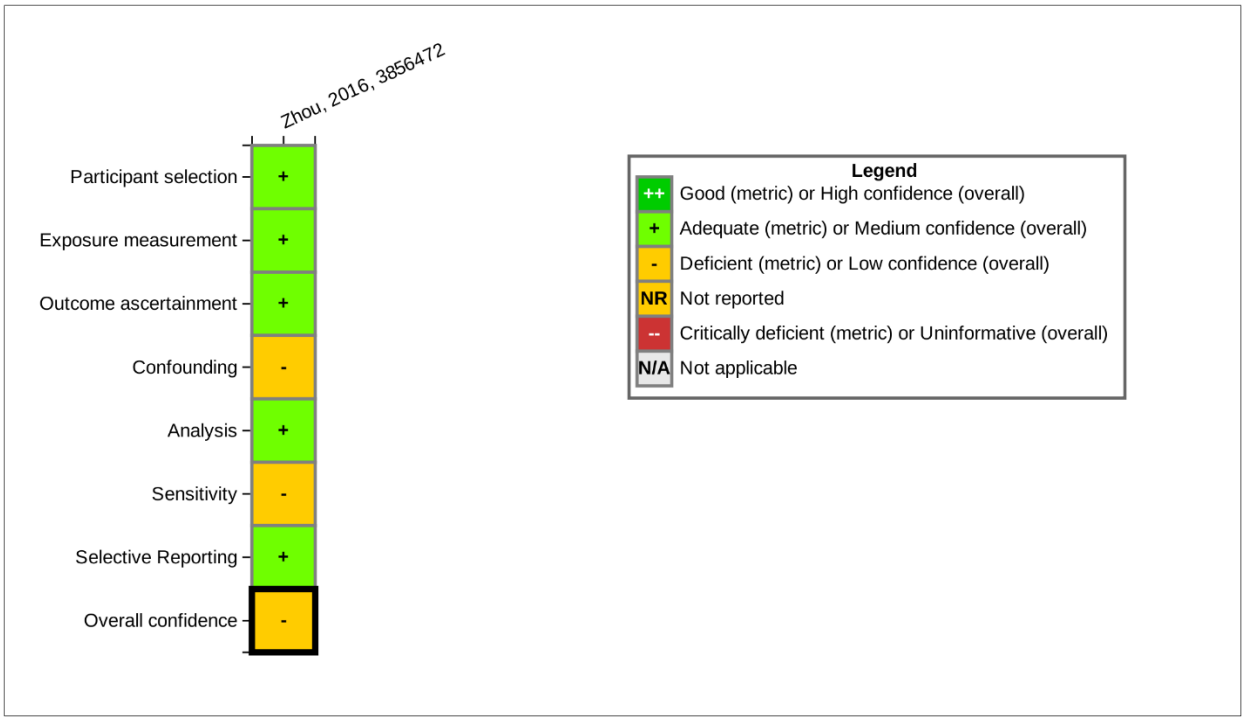


Figure 3-18. Study evaluation for human epidemiological studies reporting female reproductive findings from PFHxA exposures (HAWC: PFHxA – Human Toxicity Female Reproductive link).

Animal

Five animal studies evaluated outcomes related to female reproduction in rats and mice receiving PFHxA via gavage, PFHxA sodium salt, or PFHxA ammonium salt. Study designs included short-term (28-day), subchronic (90-day), and chronic (2-year) one-generation reproductive and developmental exposures. In general, the outcome-specific study ratings were *high* confidence. One study was rated *low* confidence for histopathology due to concerns about observational bias, endpoint sensitivity and specificity, and results presentation (Chengelis et al., 2009b). The results

of study evaluation for female reproductive outcomes are presented in Table 3-30 and details are available by clicking the HAWC link.

Table 3-30. Study design characteristics

Study	Species, strain (sex)	Exposure design	Exposure route and dose	Fertility and pregnancy	Organ weight	Histopathology	Reproductive hormones	Reproductive system development
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	++	++	++	NM
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	NM	++	-	NM	NM
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days) One-generation reproductive: P0 females dosed 70 d prior to cohabitation, through gestation and lactation (126 days); P0 males dosed for 110 days Developmental: Gestation Days 6–20	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++	NM	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	NM	++	++	NM
Iwai and Hoberman (2014)[c]	Mouse, Crl: CD1(ICR; Charles River Laboratories, Inc.	Gestation Days 6–18	Gavage[d] Phase 1: 0, 100, 350, 500 mg/kg-d Phase 2: 0, 7, 35, 175 mg/kg-d	++	NM	++	NM	++

Study evaluation for animal toxicological endpoints reported from female reproductive studies with rats receiving PFHxA,[a] PFHxA sodium salt,[b] or PFHxA ammonium salt[d] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

[c]Phase 1 was a range-finding study used to determine the appropriate dose range for identification of a NOAEL in Phase 2.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Fertility and Pregnancy Outcomes

Three studies evaluated outcomes related to fertility and pregnancy following exposure by gavage with PFHxA or PFHxA salts in rats or mice (Iwai and Hoberman, 2014; Loveless et al., 2009; NTP, 2018). Loveless et al. (2009) provided evidence for decreased maternal body weight gains (31% change from control) in rats exposed to 500 mg/kg-d from both developmental and one-generation reproductive experiments. Dams from the developmental exposure (GD 6–20) showed a statistically significant decrease in weight gain and in terminal body weight on GD 21. Deficits remained when correcting for gravid uterine weight, indicating that reductions were being driven by effects in the dams rather than by the number or size of fetuses. In the one-generation reproductive, dams continuously exposed from premating through lactation showed a decrease in weight gain during early gestation (GD 0–7), which was not significant over the entire gestational period (GD 0–21). These animals showed a statistically significant increase in body weight gains during lactation. No change in maternal body weights were identified in mice (Iwai and Hoberman, 2014). Results are presented in Figure 3-23. No effects on mating, pregnancy incidence, gestation length, number of implantations, or litter size occurred in either study that evaluated these outcomes (Iwai and Hoberman, 2014; Loveless et al., 2009). Estrous cyclicity in rats exposed as adults or during gestation was also unaffected in two studies (Loveless et al., 2009; NTP, 2018).

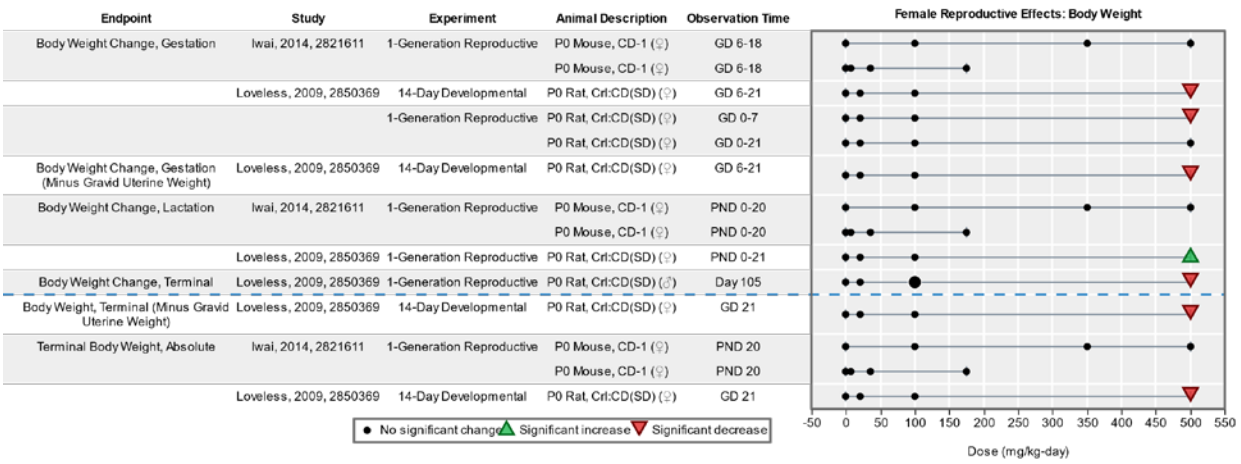


Figure 3-19. Effects on body weight in female rats and mice exposed to PFHxA or PFHxA ammonium salt in reproductive studies (HAWC: PFHxA – Animal Toxicity Female Reproductive Supporting Table).

Histopathology

Four studies evaluated effects on histopathology of reproductive organs (i.e., uterus and ovaries) in rodents following exposure to PFHxA (Figure 3-24) (Chengelis et al., 2009b; Klaunig et al., 2015; NTP, 2018) or PFHxA sodium salt (Loveless et al., 2009). Only NTP (2018) reported an effect of exposure, with females showing a statistically significant increase in the incidence of bilateral uterine horn dilation in all but the vehicle controls and highest dose group. Whereas the

control and high-dose group had 10 animals per group, however, groups showing a statistically significant increase had only 1–3 animals per group, complicating interpretation of these findings. The total incidence ranges from 1 to 3 animals/treatment group, regardless of sample size or PFHxA dose (Figure 3-24). The biological significance of these results is unclear. Uterine horn dilation can indicate an estrogenic effect, but no coherent changes in serum estradiol or estrous cyclicity were observed in this study. Similarly, no other treatment-related effects on female reproductive histopathology were reported (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018).

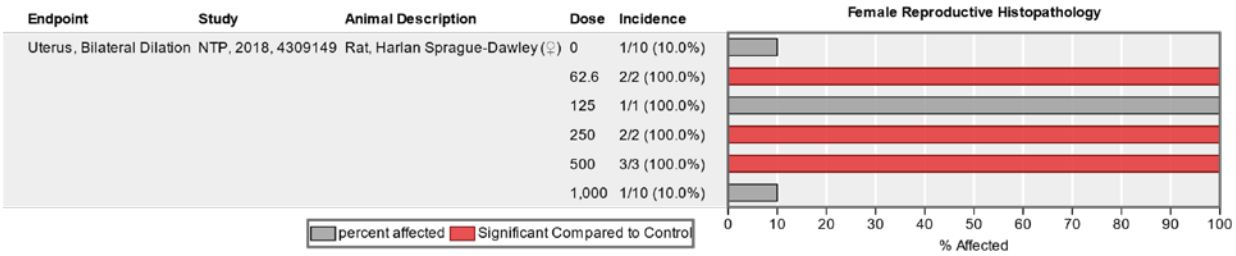


Figure 3-20. Female reproductive effects on uterine horn dilation in rats exposed to PFHxA for 28 days (HAWC: PFHxA – Animal Toxicity Female Reproductive link).

Organ Weights

Three studies evaluated effects of PFHxA exposure on uterine and ovarian weights (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). Authors reported no treatment-related effects for these outcomes.

Reproductive Hormones

Two studies measured effects of PFHxA or PFHxA ammonium salt on testosterone (Klaunig et al., 2015; NTP, 2018), estradiol, and luteinizing hormone (Klaunig et al., 2015). No treatment-related effects were reported in either study.

Female Reproductive System Development

Two studies evaluated the potential for reproductive development effects following developmental exposure to PFHxA ammonium or sodium salts. Iwai and Hoberman (2014) and Loveless et al. (2009) found no effects on age at vaginal opening, a measure of puberty onset.

Evidence Integration

A single *low* confidence human study reported a weak inverse association between PFHxA exposure measures and serum levels of reproductive hormone levels in adolescents (Zhou et al., 2016). Based on these results, there is *indeterminate* human evidence of female reproductive effects.

In animals, evidence supporting effects of PFHxA exposure female reproduction was largely limited to effects on maternal weight gain during gestation in rats (Loveless et al., 2009). Effects on maternal weight gain, however, were not consistent across studies. The observed uterine horn dilation appears influenced by differences in sample sizes, as the total incidence is similar across controls and all dosing groups. Furthermore, this latter finding is generally associated with estrogenic effects, but no coherent changes were observed that would be indicative of estrogenic changes in females. No treatment-related changes were reported for other female reproductive outcomes (Chengelis et al., 2009b; Iwai and Hoberman, 2014; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Based on these results, there is *indeterminate* animal evidence of female reproductive effects.

Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA might cause female reproductive effects in humans under relevant exposure circumstances (Table 3-31).

Table 3-31. Evidence profile table for female reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊙⊙⊙ ***Evidence inadequate***
Studies and confidence	**Factors that increase strength**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Reproductive Hormones *1 low* confidence study	• No factors noted	• *Low* confidence study	• Nonsignificant inverse association between PFHxA exposure and testosterone and estradiol	⊙⊙⊙ ***Indeterminate***	
Evidence from animal studies					
Studies and confidence	**Factors that increase strength**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	*Primary Basis:* Evidence is *low* confidence or largely null. *Human relevance:* • In the absence of evidence to the contrary, the evidence in rodents is presumed to be relevant to humans based on similarities in the anatomy and physiology of the reproductive systems across these two species. *Cross stream coherence:* • The strength of the evidence is neither increased nor decreased due to a lack of
Fertility and Pregnancy Outcomes *3 high* confidence studies in rats and mice: • 28-day (rat) • 90-day (rat) • GD 6–18 (mouse)	• *High* confidence studies	• *Unexplained inconsistency* across studies	• Decreases in maternal weight gain during gestation at 500 mg/kg-day	⊙⊙⊙ ***Indeterminate*** The animal evidence is largely null. Some evidence of female reproductive effects but results were not dose-dependent, and there was no coherent evidence supporting the biological significance of the findings	
Histopathology *4 high* confidence studies in rats and mice: • 28-day (rat) • 90-day (rat)	• *High* confidence studies	• *Lack of dose-response gradient* for uterine horn dilation	• Increase in bilateral uterus dilation reported for all groups except the highest dose		

Evidence stream summary and interpretation					Evidence integration summary judgment
• 2-year (rat) • GD 6–18 (mouse) *1 low* confidence study in adult rats: • 90-day		• *Unexplained inconsistency* across studies • *Lack of expected coherence* with other estrogen related outcomes			coherence across evidence streams. *Susceptible populations:* • None identified
Organ Weights, Reproductive Hormones, Reproductive System Development *6 high* confidence studies in rats and mice: • 28-day (rat) • 90-day (rat, 2 studies) • 2-year (rat) • GD 6–18 (mouse) • GD 6–20 (rat)	• *High* confidence studies	• No factors noted	• No treatment-related effects were reported at ≤1,000 mg/kg-day		
Mechanistic evidence and supplemental information					
Biological events of pathways	**Biological events of pathways**	**Biological events of pathways**		**Biological events of pathways**	
• No studies Identified					

3.2.8. Immune Effects

Human

Asthma

One *medium* confidence case-control study in Taiwan reported in three publications (Dong et al., 2013; Qin et al., 2017; Zhou et al., 2017) examined the potential association between PFHxA exposure and asthma, asthma symptoms, pulmonary function, and related immune markers (Figure 3-25). The only finding of note was a nonmonotonic positive association between incident asthma (i.e., diagnosis in the previous year) and PFHxA exposure (odds ratio [95% CI] for Q2: 1.2 [0.7, 2.1], Q3: 0.9 [0.5, 1.6], Q4: 1.6 [0.9, 2.9]) that was not statistically significant. No clear association was found with asthma severity or control of asthma symptoms (Dong et al., 2013), pulmonary function measured with spirometry (Qin et al., 2017), or immune markers (Dong et al., 2013) among children with asthma. The exposure levels in this study were low (median [IQR]: 0.2 [0.1–0.3]), which likely reduced study sensitivity.

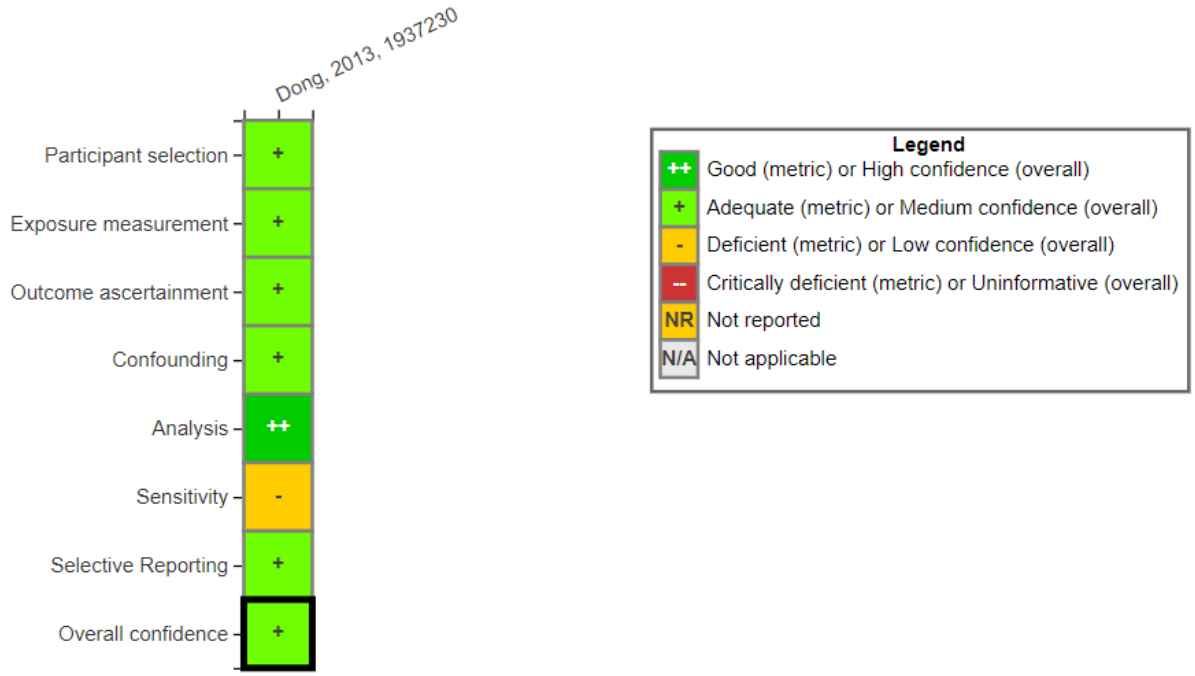


Figure 3-21. Study evaluation for human epidemiological studies reporting findings from PFHxA exposures (HAWC: PFHxA – Human Toxicity Immune Effects link).

The evaluation of Dong et al. (2013) encompasses all publications related to this study.

Animal

Several short-term (28-day), subchronic, and chronic animal studies evaluated toxicological findings of immune effects in rats receiving oral exposures of PFHxA and PFHxA sodium salt. Most of the outcome-specific study ratings were considered *high* confidence; however, some specific concerns were identified that resulted in a *low* confidence rating. Histopathology for Chengelis et al. (2009b) was rated *low* confidence because of issues related to observational bias, concerns about endpoint sensitivity and specificity, and results presentation. The results of the outcome-specific study evaluations are presented in Table 3-32 and details are available by clicking the HAWC link.

Table 3-32. Study design characteristics and individual outcome ratings for immune endpoints

Study	Species, strain (sex)	Exposure design	Exposure route and dose	Organ weight	Histopathology	Immune cell counts
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	++	++
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	++	-	++
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	++	++

Study evaluation for animal toxicological immune endpoints reported from studies with male and female rats receiving PFHxA[a] or PFHxA sodium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Organ Weights

Three studies evaluated effects on spleen and thymus weights in response to PFHxA (Chengelis et al., 2009b; NTP, 2018) or PFHxA sodium salt (Loveless et al., 2009) exposure.

The available evidence identified, in general, decreased absolute or relative thymus weights. Statistically significant decreases in absolute weights were found in males exposed to 500 mg/kg-day PFHxA sodium salt for 90 days (Loveless et al., 2009), and downward trends in both

relative and absolute organ weights were reported in males and females receiving PFHxA in the 28-day study (NTP, 2018).

Spleen weights did not show a clear pattern of effect across studies. In the 28-day study, a trend of increased weights in males and females receiving PFHxA (NTP, 2018) was observed, whereas spleen weights were decreased in males receiving PFHxA sodium salt in the 90-day study by Loveless et al. (2009). Chengelis et al. (2009b) qualitatively reported no treatment-related effects on spleen or thymus weights after exposure to ≤200 mg/kg-day PFHxA for 90 days. Results are summarized in Figure 3-26.

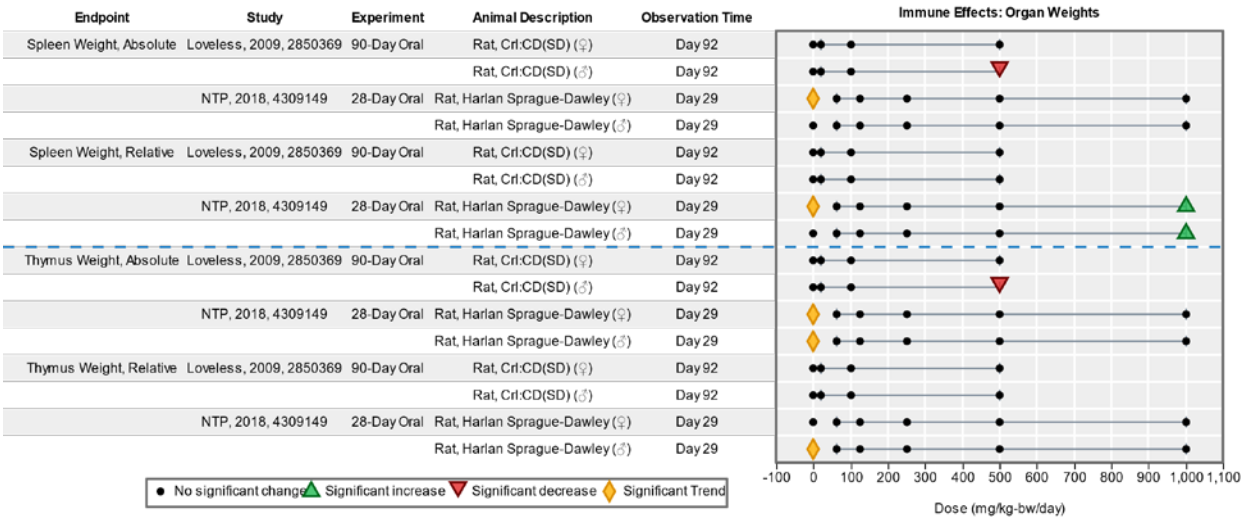


Figure 3-22. Immune organ weights in rats exposed by gavage to PFHxA or PFHxA sodium salt (HAWC: PFHxA – Animal Toxicity Immune Effects link).

Histopathology

Four studies examined spleen, thymus, lymph nodes, or bone marrow for histopathological changes (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Some evidence of effects in the spleen from two of these studies was found. NTP (2018) reported an increased incidence of extramedullary hematopoiesis in the spleens of males and females at 1,000 mg/kg-day after a 28-day exposure. Minimal to mild extramedullary hematopoiesis also was found in the spleens of male rats receiving 500 mg/kg-day PFHxA sodium salt (Loveless et al., 2009). This effect was coincident with erythroid hyperplasia of the bone marrow of males and females and might be related to the effects on red blood cells (discussed in "Hemostasis" of Section 3.2.4) rather than an immune-specific effect. These changes did not persist after the 30-day recovery and specific incidence data were not reported (Loveless et al., 2009). Spleen histopathological findings were null in the 90-day PFHxA subchronic study that tested doses up to 200 mg/kg-day (Chengelis et al., 2009b). All studies reported null results for histopathological examinations of the thymus, lymph node, and bone marrow (Chengelis et al., 2009b; Klaunig et al., 2015; NTP, 2018).

Immune Cell Counts

Four animal studies had evidence of hematological indicators of immunotoxicity (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Of these studies, NTP (2018) and Loveless et al. (2009) reported increased neutrophils at doses as low as 20 mg/kg-day and decreased basophils in males receiving ≥250 and 500 mg/kg-day PFHxA or PFHxA sodium salt, respectively. No effects were observed on basophils or neutrophils in the other two subchronic and rat studies (90 days and 2 years) at exposures to PFHxA as high as 200 mg/kg-day (Chengelis et al., 2009b; Klaunig et al., 2015). Eosinophils were decreased only in males exposed to PFHxA sodium salt for 90 days (Loveless et al., 2009). No other treatment-related effects were reported for specific white blood cell populations or total white blood cell counts following PFHxA or PFHxA sodium salt exposures in rats (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). Results are summarized in Figure 3-27.

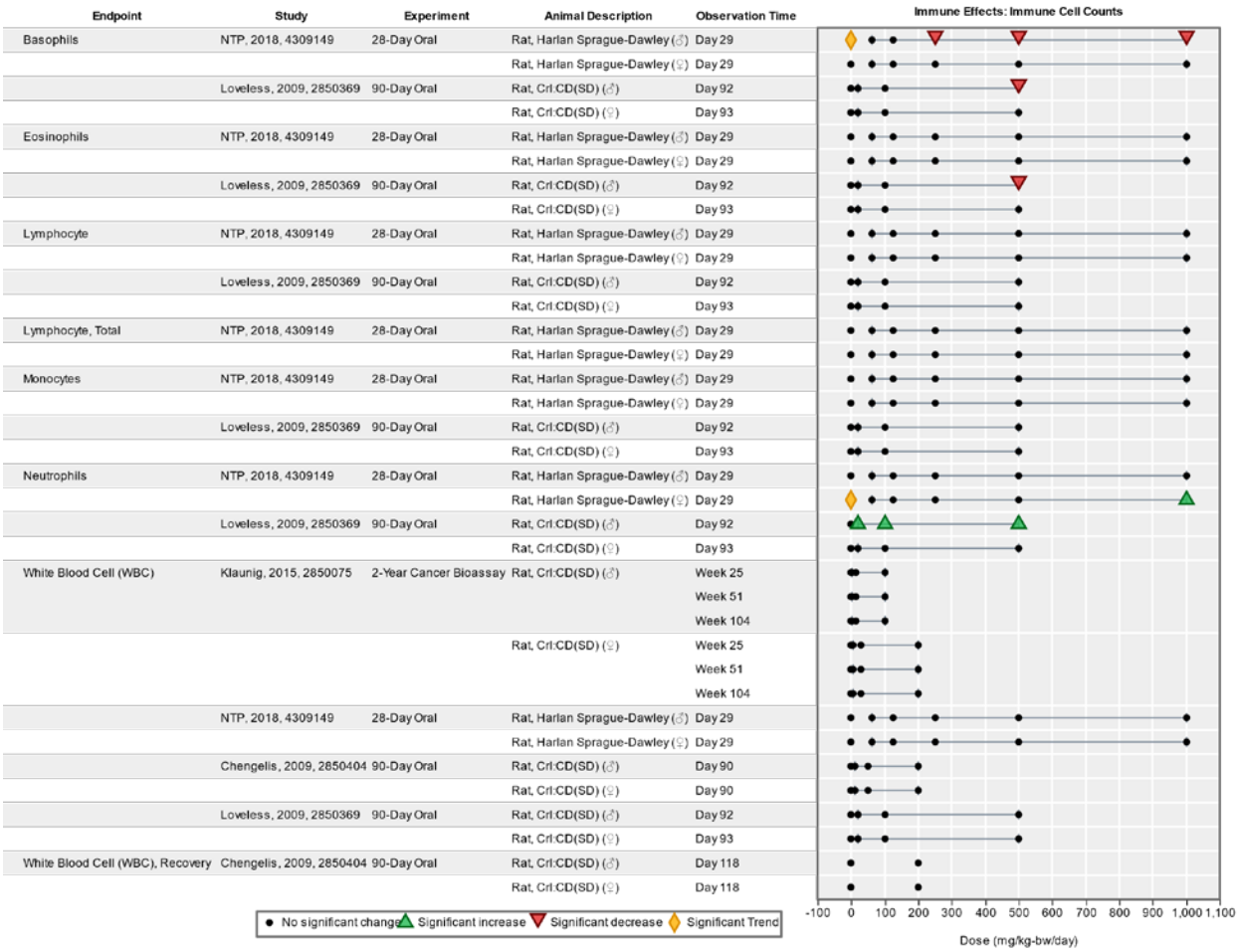


Figure 3-23. Immune cell counts in rats exposed by gavage to PFHxA or PFHxA sodium salt (HAWC: PFHxA – Animal Toxicity Immune Effects link).

Evidence Integration

The human evidence was limited to one *medium* confidence study that showed no clear association between PFHxA exposure and immune-related health outcomes, although the authors did observe a nonsignificant trend toward an association with asthma diagnosis in the previous year. Based on these results, there is *indeterminate* human evidence of immune effects.

With the exception of changes in thymus weight, the available animal toxicological evidence did not show a clear pattern of effect across studies. Specifically, two studies reported treatment-related changes in thymus and spleen weights in rats, but the direction of effect on spleen weights was not consistent across studies. Extramedullary hematopoiesis was the only histopathological finding of note, but this is interpreted as possibly secondary to the effects on red blood cells rather than an immune-specific effect and is discussed in that context in Section 3.2.4. Increases in neutrophils and decreases in basophils showed a consistent direction of effect across two studies (of the four available). Eosinophils also were decreased, but only in males in a single study. No other treatment-related changes were observed for immune cell counts (i.e., specific cell populations or total white blood cells), and discerning the biological significance of this pattern is difficult in isolation.

The evidence supporting the potential immunotoxicity to humans is limited by several factors, including the lack of consistency across studies for several of the affected outcomes. Furthermore, the evaluated outcomes are limited to changes in the structural components of the immune system, which are less predictive indicators of immunotoxicity (WHO, 2012). Additional studies, particularly those that evaluate changes in immune function (e.g., in response to foreign challenge) would be beneficial for understanding the potential for adverse effects of PFHxA exposure on the immune system. Based on these results, there is *indeterminate* animal evidence of immune effects.

Overall, the currently available evidence is ***inadequate*** to determine whether PFHxA exposure might cause immune system effects in humans under relevant exposure conditions (Table 3-33).

Table 3-33. Evidence profile table for immune effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊙⊙⊙ ***Inadequate*** *Primary basis:* Evidence is *low* confidence or limited *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and lifestages:* • No evidence to inform
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Asthma *1 medium* confidence study	• No factors noted	• Potential for residual confounding (e.g., with other PFAS) • Imprecision • Lack of internally coherent findings (no associations with other measures of pulmonary function)	• Nonsignificant association with asthma diagnosis, but other asthma-related outcomes were not affected	⊙⊙⊙ ***Indeterminate***	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Evidence stream judgment**	
Histopathology *3* high confidence studies in adult rats • 28-day • 90-day • 2-year *1* low confidence study in rats*:* • 90-day	• *High* confidence studies • *Consistency* across studies for extramedullary hematopoiesis	Lack of biological gradient/ dose-response.	• Increased splenic extramedullary hematopoiesis was observed male and female rats at 500 mg/kg-day; coincident with minimal erythroid hyperplasia of the bone marrow	⊙⊙⊙ ***Indeterminate*** Some evidence of immune system but limited by low sensitivity (observational outcomes less predictive of immune system toxicity), lack of coherence, and potential for non-immune related causes	
Immune Cell Counts *4* high confidence studies in rats: • 28-day	• *High* confidence studies • Consistency–studies for neutrophils and basophils	• Lack of biological gradient/ dose-response gradient	• Decreased basophil counts and increased neutrophil cell counts at ≥20 mg/kg-day		

Evidence stream summary and interpretation					Evidence integration summary judgment
• 90-day (2 studies) • 2-year		• Lack of coherence with other immune markers		[see Section 3.2.4 for additional discussion]	
Organ Weight *3 high* confidence studies in rats: • 28-day • 90-day (2 studies)	• *High* confidence studies	• Unexplained inconsistency across studies for spleen weights	• Thymus weights decreased at 500 mg/kg-day in short-term and subchronic studies • Changes in spleen weight were inconsistent in the direction of effect across studies		
Mechanistic evidence and supplemental information					
Biological events of pathways	**Biological events of pathways**	**Biological events of pathways**		**Biological events of pathways**	
• No studies Identified					

3.2.9. Nervous System Effects

Human

No studies were identified that evaluated the effects of PFHxA on the nervous system in humans.

Animal

Four short-term (28-day), subchronic, and chronic animal studies evaluated the effects of PFHxA or PFHxA sodium salt in rats. Most outcome-specific study ratings were *high* or *medium* confidence. One study was rated *low* confidence for histopathology due to concerns about observational bias, endpoint sensitivity and specificity, and data presentation (Chengelis et al., 2009b). A summary of the studies and the interpretations of confidence in the results for the different outcomes based on the individual study evaluations is presented in Table 3-34, and details are available by clicking the HAWC link.

Table 3-34. Nervous system endpoints for PFHxA and associated confidence scores from repeated-dose animal toxicity studies

Author (year)	Species, strain (sex)	Exposure design	Exposure route and dose range	Brain weight	Histopathology	Behavior
NTP (2018)	Rat, Harlan Sprague-Dawley (male and female)	Short term (28 days)	Gavage[a] Male and female: 0, 62.5, 125, 250, 500, 1,000 mg/kg-d	++	++	NM
Chengelis et al. (2009b)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[a] Male and female: 0, 10, 50, 200 mg/kg-d	++	-	+
Loveless et al. (2009)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	Subchronic (90 days)	Gavage[b] Male and female: 0, 20, 100, 500 mg/kg-d	++	++	++
Klaunig et al. (2015)	Rat, Crl:CD(SD) Sprague-Dawley (male and female)	2-year cancer bioassay	Gavage[a] Male: 0, 2.5, 15, 100 mg/kg-d Female: 0, 5, 30, 200 mg/kg-d	NM	++	++

Study evaluation for animal toxicological nervous system endpoints reported from studies with male and female rats receiving PFHxA[a] or PFHxA sodium salt[b] by gavage. Study evaluation details for all outcomes are available by clicking the HAWC link.

++ Outcome rating of *high* confidence; + outcome rating of *medium* confidence; - outcome rating of *low* confidence; -- outcome rating of *uninformative*; NR, outcome not reported; NM, outcome not measured.

Brain Weight

Three studies evaluated effects of PFHxA or PFHxA sodium salt on the nervous system in animals (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). Two studies reported increases in relative but not absolute brain weights after exposure to PFHxA or PFHxA sodium salt for 28 or 90 days, respectively (Chengelis et al., 2009b; Loveless et al., 2009). These effects were observed at the highest dose tested (200 or 500 mg/kg-day) and affected only males in one study (Loveless et al., 2009) and only females in the other (Chengelis et al., 2009b). Notably, relative weights are not considered appropriate for brain weight measurements because this measure is not typically affected by fluctuations in body weight(U.S. EPA, 1998); therefore, absolute brain weights are preferred.

Other Nervous System Effects

No treatment-related effects were observed on other nervous system outcomes, including behavior (i.e., open field locomotor activity, functional observational battery) and histopathology (Chengelis et al., 2009b; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018).

Evidence Integration

No human studies were identified to inform the potential nervous system effects of PFHxA or PFHxA salts, therefore there is *indeterminate* human evidence of nervous system effects.

In animals, the only available evidence to support an effect of PFHxA or PFHxA salts the nervous system stems from increase in relative brain weights, which is not considered a reliable measure of neurotoxicity (U.S. EPA, 1998). No treatment-related effects were reported for other nervous system outcomes.

Although the available animal toxicity data are largely null and derived from low risk of bias studies, some uncertainties and data gaps remain. The results are limited to a small number of studies in adult animals, and the evidence base is lacking studies that could inform potential for nervous system effects when exposure occurs during development. This lifestage is a known critical window of sensitivity for nervous system effects (U.S. EPA, 1998) and has been identified as a research area of potential concern for other PFAS known to affect thyroid function. No mechanistic data were identified to inform this potential health effect. Based on these results, there is *indeterminate* animal evidence of nervous system effects.

Overall, the currently available ***evidence is inadequate*** to assess whether PFHxA might cause nervous system effects in humans under relevant exposure circumstances.

Table 3-35. Evidence profile table for nervous system effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans					⊙⊙⊙ ***Inadequate*** *Primary Basis:* No evidence in humans and animal evidence is largely null or lacking biological relevance. *Human relevance:* Without evidence to the contrary, effects in rats are considered relevant to humans *Cross stream coherence:* N/A (human evidence *indeterminate*). *Susceptible populations and lifestages:* No evidence to inform.
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Strength of evidence**	
• No studies identified				⊙⊙⊙ ***Indeterminate***	
Evidence from animal studies					
Studies and confidence	**Factors that increase certainty**	**Factors that decrease certainty**	**Summary and key findings**	**Strength of evidence summary**	
Brain Weight *3 high* confidence studies in adult rats: • 28-day • 90-day (2 studies)	• *High* confidence studies	• No factors noted	• Increased relative brain weights in animals at ≥200 mg/kg-day; absolute brain weight unaffected	⊙⊙⊙ ***Indeterminate*** Evidence is largely null. The only evidence of nervous system effects was relative brain weight increases, which is not considered to be appropriate for evaluating nervous system toxicity.	
Histopathology *3 high* confidence studies in adult rats: • 28-day • 90-day • 2-year 1 *low* confidence study in adult rats: • 90-day	• *High* confidence studies	• No factors noted	• No treatment-related effects reported		
Behavior *2 high* confidence studies in adult rats: • 90-day • 2-year	• *High* and *medium* confidence studies	• No factors noted	• No treatment-related effects reported		

Evidence stream summary and interpretation					Evidence integration summary judgment
1 medium confidence study in adult rats: • 90-day					
Mechanistic evidence and supplemental information					
Biological events of pathways	**Biological events of pathways**	**Biological events of pathways**		**Biological events of pathways**	
• No studies Identified					

3.3. CARCINOGENICITY

3.3.1. Cancer

Human Studies

No human studies or studies of human cells were available.

Animal Studies

A *high* confidence cancer bioassay conducted in rats evaluated neoplastic and non-neoplastic lesions in the lungs, kidney, stomach, and liver of male rats dosed with 0, 2.5, 15, or 100 mg/kg-day and in female rats dosed with 0, 5, 30, or 200 mg/kg-day (Klaunig et al., 2015). Findings for non-neoplastic and neoplastic lesions were reported as null and are summarized in HAWC and in PFHxA Tableau.

Genotoxicity

Genotoxic, mutagenic, and clastogenic effects of PFHxA have been tested in several mammalian and prokaryotic cell systems in vitro (Table 3-36) (Eriksen et al., 2010; Lau, 2015; Loveless et al., 2009; Nobels et al., 2010). Sodium perfluorohexanoate (NaPFHx) was negative for mutagenicity in *Escherichia coli* strain WP2*uvr*A and *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in both the presence and absence of exogenous S9 metabolic activation (Loveless et al., 2009). Nobels et al. (2010) examined the ability of PFHxA to induce the expression of 14 prokaryotic stress response genes after exposure of the *E. coli* K-12 derivative SF_1 to 0.0156–1 mM PFHxA. The results of this study demonstrated that PFHxA did not significantly induce the expression of regulatory elements critical for the prokaryotic gene expression response to oxidative stress (KatG, Zwf, Soi28, and Nfo), membrane damage (MicF and OsmY), general cell lesions (UspA and ClpB), heavy metal stress (MerR), and DNA damage (Nfo, RecA, UmuDC, Ada, SfiA, and DinD). In mammalian cells in vitro, PFHxA did not generate reactive oxygen species (ROS) or oxidative deoxyribonucleic acid damage in the human hepatoma cell line, HepG2 (Eriksen et al., 2010). Lastly, NaPFHx failed to induce chromosomal aberrations in human peripheral blood lymphocytes in the presence and absence of exogenous metabolic activation, suggesting a lack of clastogenic activity (Loveless et al., 2009).

Evidence Integration

One study (Klaunig et al., 2015) evaluated the potential carcinogenicity of oral PFHxA exposure via histological evaluation of the lung, kidney, stomach, and liver of male rats, and did not observe significant treatment-related effects, and the few studies examining markers of potential genotoxicity were largely null (see Section 3.3). No studies of potential carcinogenicity in exposed humans or via other exposure routes were identified. As discussed above, given the sparse evidence base, and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA,

2005) EPA concluded there is ***inadequate information to assess carcinogenic potential*** for PFHxA for any route of exposure.

Table 3-36. Summary of PFHxA genotoxicity studies

PFHxA genotoxicity						
			Results[a]			
Endpoint	Test system	Doses/ Concentrations tested	Without exogenous activation	With exogenous activation	Comments	References
Genotoxicity						
ROS production	HepG2 (human hepatoma cell line)	0.4, 4, 40, 200, 400, 1,000, 2,000 µM	–	NA	Intracellular reactive oxygen species (ROS) production was measured using 2',7'-dichlorofluorescein diacetate. ROS production was measured every 15 min for 3 h. No clear concentration-response relationship was observed for PFHxA, whereas exposure to H_2O_2 (positive control) generated ROS in a concentration dependent manner.	Eriksen et al. (2010)
DNA damage	HepG2 (human hepatoma cell line)	100, 400 µM	–	NA	Comet assay to detect the formation of DNA strand breaks (including alkali-labile sites) and formamidopyrimidine-DNA-glycosylase sensitive sites after 24-h exposure. Cytotoxicity was monitored by measuring lactate dehydrogenase (LDH) activity to ensure observed DNA damage was not secondary to cytotoxicity.	Eriksen et al. (2010)
Cell stress-dependent gene expression	*Escherichia coli* K-12 derivative SF_1	0.0156, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1 mM	–	NA	Promoters of 14 prokaryotic DNA-damage responsive genes were fused to *lacZ* cassettes and expressed in *E. coli*. Activation of gene expression was measured after 90 min of exposure by β-galactosidase reduction capacity and spectrophotometrically at 420 nm. Genes involved in prokaryotic DNA damage and repair (*UmuDc* and *Ada*) were upregulated at approximately ≥1.4-fold but did not reach statistical significance at any dose. Study authors did not provide complete data for analysis.	Nobels et al. (2010)

PFHxA genotoxicity						
			Results[a]			
Endpoint	**Test system**	**Doses/ Concentrations tested**	**Without exogenous activation**	**With exogenous activation**	**Comments**	**References**
Mutation (Ames assay)	*Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537	333, 667, 1,000, 3,333, 5,000 µg/plate sodium perfluorohexanoate (NaPFHx)	–	–	Assay performed according to OECD Guideline 471. No positive mutagenic responses were observed at any dose level or with any tester strain in the presence or absence of S9 metabolic activation.	Loveless et al. (2009)
Mutation	*E. coli* WP2uvrA	333, 667, 1,000, 3,333, 5,000 µg/plate sodium perfluorohexanoate (NaPFHx)	–	–	Assay performed according to OECD Guideline 471. No positive mutagenic responses were observed at any dose level or with any tester strain in the presence or absence of S9 metabolic activation.	Loveless et al. (2009)
Chromosomal aberration	Human peripheral blood lymphocytes	4h (nonactivated): 2,000, 3,000, 3,860 µg/mL sodium perfluorohexanoate (NaPFHx) 4 h (activated) and 22 h (nonactivated): 250, 500, 1,000 µg/mL sodium perfluorohexanoate (NaPFHx)	–	–	Assay performed according to OECD Guideline 473. Percentage of cells with structural or numerical aberrations was not significantly increased above that of the vehicle control at any concentration. Aroclor-induced rat liver S9 was used for exogenous metabolic activation. Mitomycin C and cyclophosphamide were used as positive controls. Substantial toxicity (defined as a reduction in the mitotic index of >50% in the NaPFHx treated cell culture as compared to vehicle control) was observed in all test conditions.	Loveless et al. (2009)

[a]+ = positive; – = negative; NA = not applicable.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

For all noncancer health effects, limited or no human epidemiological evidence was available. Therefore, conclusions were based primarily on animal toxicological studies. The animal evidence base consists of short-term (NTP, 2018), subchronic (Chengelis et al., 2009b; Loveless et al., 2009), and chronic (Klaunig et al., 2015) studies in adult male and female Sprague-Dawley rats with exposure durations spanning 28 days to 2 years and with oral doses of 2.5–1,000 mg/kg-day PFHxA, PFHxA sodium salt, or PFHxA ammonium salt. Two developmental studies (Iwai and Hoberman, 2014; Loveless et al., 2009) with gestational exposure durations that represent a critical lifestage with maternal oral doses between 7–500 mg/kg-day also were available. The outcome-specific ratings for these studies were generally *high* confidence.

As described in detail in Section 3, the available ***evidence indicates*** that PFHxA exposure is likely to cause hepatic (Section 3.2.1), developmental (Section 3.2.2), and hematopoietic effects (Section 3.2.4) in humans, given relevant exposure circumstances.

The evidence for PFHxA-mediated adverse hepatic effects was based primarily on a set of consistent and coherent findings in animal studies, including hepatocellular hypertrophy and increased relative liver weight. Both effects could be adaptive changes to PFHxA exposure; however, these findings were considered adverse on the basis of their consistent effect between sexes and across studies. The effects also persisted during the recovery period and correlate with other endpoints (increased ALT and decreased serum globulins) collectively considered adverse. Available mechanistic evidence suggests increased peroxisomal beta oxidation and the involvement of both PPARα-dependent and -independent pathways in response to PFHxA exposure.

The data from the animal toxicological studies that supported identifying developmental effects as a potential human hazard included effects from two studies that reported consistent, dose-responsive, and substantial effects of PFHxA exposure on offspring body weights and mortality. Delayed eye opening was also reported, but only at doses associated with frank effects in the offspring (i.e., mortality). Effects on offspring body weight were observed in two species (rats and mice) exposed to different PFHxA salts (sodium and ammonium) using different exposure scenarios, although effects on mortality were observed only in the mouse study.

The primary support for hematopoietic effects included consistent decreases in red blood cells, hematocrit, and hemoglobin across study designs and exposure durations in male and female adult rats (Chengelis et al., 2009b; Loveless et al., 2009; NTP, 2018). These hematological findings correlate with increases in reticulocytes, an indicator of erythroid cell regeneration supported by

pathological findings in the spleen and bone marrow (Loveless et al., 2009). The decreases in hemoglobin were consistent with the decreased mean corpuscular hemoglobin concentration observed in both sexes (Loveless et al., 2009; NTP, 2018). When combined, increased mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) are indicators of anemia. Several of the hematological findings were significant at the highest dose tested in the subchronic studies and returned to control levels after 30- or 90-day recovery periods (or both) (Chengelis et al., 2009b; Loveless et al., 2009). Findings from females in the chronic study (e.g., HGB, RBC, and reticulocytes) were significant at the highest administered dose (200 mg/kg-day), whereas no effects were observed in males that received half (100 mg/kg-day) the female dose. Together, the subchronic and chronic evidence from males and female rats suggest PFHxA-mediated hematopoietic effects are dependent on both dose and duration.

The current ***evidence suggests***, but is not sufficient to infer, that PFHxA exposure might cause endocrine effects in humans. This judgment is based on evidence in animals showing decreases in thyroid hormone levels in male (but not female) rats exposed for 28 days and increased incidence of thyroid epithelial cell hypertrophy in male and female rats in one subchronic study (Section 3.2.5).

For all other health effects described in Section 3 (i.e., renal, male and female reproductive, immune, and nervous system) the ***evidence is inadequate*** to assess whether PFHxA might cause effects in human. The summary level findings from the animal toxicological studies that examined exposure to PFHxA can be viewed by clicking the PFHxA Tableau link, selecting the "Study Findings" tab, and filtering for the relevant health system.

The relevant exposure conditions that might lead to these health effects are further characterized in Section 5.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

Evidence is insufficient to make a judgment on whether PFHxA exposure might affect the development of any specific cancers. Consistent with EPA guidance (U.S. EPA, 2005) to apply a standard descriptor as part of the hazard narrative and to express a conclusion regarding the weight of evidence for the carcinogenic hazard potential, a descriptor of ***inadequate information to assess carcinogenic potential*** is applied for PFHxA.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES

No human studies were available to inform the potential for PFHxA exposure to affect sensitive subpopulations or lifestages.

In adult rats exposed to PFHxA for 28 days to 2 years, toxicological findings were either consistently observed at lower dose levels in males than females or the findings were observed only in males. The reason for this sex dependence is possibly due to sex-dependent PFHxA elimination

caused by sex-specific differences in the expression (mRNA and protein) of the renal organic anion transporting polypeptide (Oatp) 1a1 (Kudo et al., 2001) as discussed in Section 3.1.4. Currently, whether this sex-specific difference might also exist in humans is unclear.

Additionally, given the effects seen in the developing organism (i.e., perinatal mortality, reduced body weights, and delays in time to eye opening), the prenatal and early postnatal window represents a potentially sensitive lifestage for PFHxA exposure.

5. DERIVATION OF TOXICITY VALUES

5.1. HEALTH EFFECT CATEGORIES CONSIDERED (CANCER AND NONCANCER)

Multiple noncancer health effects were examined following oral PFHxA exposures in five animal toxicological studies (Chengelis et al., 2009b; Iwai and Hoberman, 2014; Klaunig et al., 2015; Loveless et al., 2009; NTP, 2018). These studies were generally rated *high* confidence in outcome-specific study evaluations. Based on these studies, it was determined that the ***evidence indicates*** PFHxA likely causes hepatic, developmental, and hematopoietic effects in humans under relevant exposure circumstances. These health effects were considered for derivation of toxicity values. The dose levels associated with these hazards are further characterized below (Section 5.2.1).

For endocrine effects, the currently available ***evidence suggests, but is not sufficient to infer*** that PFHxA may cause effects in humans. Although there was some evidence of effects on thyroid system function in rats (i.e., thyroid hormone levels and thyroid epithelial cell hypertrophy) the results lacked consistency and some of the observed changes could be explained by nonendocrine-related effects. Based on the limitations of the current evidence base, endocrine effects were not considered for derivation of toxicity values. For all other health effects (i.e., renal, male and female reproductive, immune, and nervous system), the ***evidence is inadequate*** to assess potential health effects, thus these were not considered for derivation of toxicity values.

No studies of inhalation exposure were identified, thus an RfC was not estimated (Section 5.2.2). Similarly, the evidence base related to potential carcinogenicity was determined to contain "***inadequate information to assess carcinogenic potential***"; therefore, no cancer toxicity values were estimated for any exposure route (Section 5.3).

5.2. NONCANCER TOXICITY VALUES

A reference dose (RfD) is the daily oral exposure to the human population (including sensitive subpopulations) that is likely without appreciable risk of deleterious effects during a lifetime. In addition to developing an RfD designed to protect against lifetime exposure (Section 5.2.1.1), a less-than-lifetime toxicity value (referred to as a "subchronic RfD"; see Section 5.2.1.2) is estimated. These subchronic toxicity values are presented as they might be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures). Both RfD and subchronic RfD derivations include organ/system-specific RfDs (osRfDs) associated with each health effect considered for point of departure (POD) derivation. Subsequent decisions related to dosimetric extrapolation, application of uncertainty factors, and confidence in toxicity values are discussed below.

As noted above, reference concentration (RfC) or subchronic RfC could not be developed.

5.2.1. Oral Reference Dose (RfD) Derivation

Study and Endpoint Selection

The following general considerations were used to identify studies for estimating points of departure (PODs) for potential use in toxicity value derivation. As described in Sections 2 and 3, the available epidemiological studies of PFHxA exposure are primarily low confidence and therefore were not further considered for dose-response analyses of PFHxA exposure. Within the available animal toxicological studies, preference was given to *medium* or *high* confidence subchronic, chronic, or developmental studies testing multiple dose levels, including doses near the lower end of the doses tested across the evidence base. These types of studies increase the confidence in the resultant RfD because they represent data with lower risk of bias and minimize the need for low-dose and exposure duration extrapolation (Appendix A, Section 11.1).

A summary of endpoints and rationales considered for toxicity value derivation is presented in Table 5-1.

Table 5-1. Endpoints considered for dose-response modeling and derivation of points of departure

Endpoint	Reference/ Confidence	Exposure duration	Strain/ Species	Sexes studied	POD derivation	Rationale
Hepatic Effects						
Relative liver weight	Chengelis et al. (2009b) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	Increases in relative liver weight were considered an adaptive change in response to PFHxA exposure
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	
Hepatocellular hypertrophy	Chengelis et al. (2009b) *Low* confidence	Subchronic	Crl:CD(SD) rat	Female	No	Increases in hepatocellular hypertrophy in combination with increased liver weight, increased serum enzymes, and decreased blood proteins were judged "likely" and considered adverse toxic effects to PFHxA exposure. The evidence was strengthened by consistency of the
	Chengelis et al. (2009b) *Low* confidence	Subchronic	Crl:CD(SD) rat	Male	Yes	
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	Yes	

Endpoint	Reference/ Confidence	Exposure duration	Strain/ Species	Sexes studied	POD derivation	Rationale
						effect across species and study designs, with the effect persisting into recovery periods. Although many pathways can lead to hypertrophy there was evidence for increased peroxisomal beta oxidation activity. Increased hepatocellular hypertrophy was considered the toxic effect altering homeostasis. Male-specific effects in Chengelis et al. (2009b) at 200 mg/kg-day, both sexes affected in Loveless et al. (2009).
Hepatocellular necrosis	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Female	No	Necrosis was considered an adverse effect downstream of hepatocellular hypertrophy, a response to toxic effects on homeostasis. Necrosis observed in females (at the highest dose of 200 mg/kg-day) was not replicated in other studies or sexes, and the less overt/more predictive indicator of hepatic toxicity (hepatocellular hypertrophy) was available.
Blood proteins (total	Chengelis et al. (2009b) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	Increases in blood protein findings were considered an

Endpoint	Reference/ Confidence	Exposure duration	Strain/ Species	Sexes studied	POD derivation	Rationale
protein and globulin)	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	adaptive change to PFHxA exposure.
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Both	No	
Hematopoietic Effects						
Hematocrit	Chengelis et al. (2009b) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	More direct measurements of red blood cells and hemoglobin are available.
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No	
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Both	No	
Hemoglobin	Chengelis et al. (2009b) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	Yes	Decreases considered similar in sensitivity to decreases in red blood cell counts. Hemoglobin reflects the oxygen carrying capacity of those cells. In Klaunig et al. (2015), the effects were specific to females.
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	Yes	
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Female	Yes	
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Male	No	No treatment-related effects were observed in males up to the high dose of 100 mg/kg-day.
Red blood cells	Chengelis et al. (2009b) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	Yes	Finding was more sensitive than other hematological findings (other than hematocrit) and consistent across study designs and exposure durations.
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	Yes	
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Both	Yes	
Reticulocytes	Chengelis et al. (2009b)	Subchronic	Crl:CD(SD) rat	Both	No	Increases were considered to reflect

Endpoint	Reference/ Confidence	Exposure duration	Strain/ Species	Sexes studied	POD derivation		Rationale
	High confidence						a compensatory response to decreased red blood cells and therefore not prioritized for derivation of toxicity values.
	Loveless et al. (2009) *High* confidence	Subchronic	Crl:CD(SD) rat	Both	No		
	Klaunig et al. (2015) *High* confidence	Chronic	Crl:CD(SD) rat	Both	No		
Developmental Effects							
Postnatal (F_1) pup body weight	Loveless et al. (2009) *High* confidence	One-generation reproductive; measured on PND 0, 4, 7, 14, 21	Crl:CD(SD) rat	Combined	Yes, PND 0	Yes, PND 0	Decrements in body weights were observed at doses that were not associated with frank effects and showed strong dose-response. Effects on body weight were strongest during the early postnatal period so these timepoints were prioritized.
	Iwai and Hoberman (2014) *High* confidence	Developmental (GD 6–18); measured on PND 0, 7, 14, 21	CD-1 mouse, F_1	Combined	Yes, PNDs 0 and 4		
F_1 fetal body weight	Loveless et al. (2009) *High* confidence	Developmental (GD 6–20); measured on GD 21	Crl:CD(SD) rat	Combined	No		Statistically nonsignificant 9% decrease at high dose was associated with maternal toxicity (i.e., reduced weight gain).
Perinatal mortality	Iwai and Hoberman (2014) *High* confidence	Developmental (GD 6–18); measured on PND 0–21, including stillbirths	CD-1 mouse, F_1	Combined	Yes		In mice, perinatal mortality (still birth and postnatal deaths from PND 0–21) showed a clear dose-response across two experimental cohorts of animals with overlapping dose ranges. Effects were observed at doses not associated with maternal toxicity.

Endpoint	Reference/ Confidence	Exposure duration	Strain/ Species	Sexes studied	POD derivation	Rationale
Eye opening	Iwai and Hoberman (2014) *High* confidence	Develop-mental (GD 6–18); measured on PND 10–17	CD-1 mouse, F_1	Combine d	No	Delays observed at a dose that elicited frank effects (i.e., increased offspring mortality.).

Estimation or Selection of Points of Departure (PODs) for RfD Derivation

The outcomes determined most relevant to the identified noncancer hazards from the animal studies advanced for dose-response (Table 5-1) were modeled using approaches consistent with EPA's *Benchmark Dose (BMD) Technical Guidance* document (U.S. EPA, 2012a). Specifically, the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change. BMD modeling of continuous data was conducted on the Health Assessment Workspace Collaborative (HAWC) website using EPA's Benchmark Dose Software (BMDS, Version 3.2).

Ideally, the selected BMR is based on data that support the biological relevance of the outcome being evaluated; however, in some cases there is no clear scientific understanding to support a biologically based BMR. In these instances, the BMD guidance provides some BMRs that can be applied to the data. For data drawn from toxicological studies, a suggested BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data can be used to estimate the BMD and BMDL. The selection of these BMRs, as indicated in Table 5-2, is based on BMD guidance stating that in the absence of information regarding the level of change considered biologically significant, these BMRs can be used (U.S. EPA, 2012a). For effects on offspring body weights, a BMR of 5% relative deviation (RD) from the control mean is used for continuous data to account for effects occurring in a sensitive lifestage (U.S. EPA, 2012a).

Table 5-2. Benchmark response levels selected for BMD modeling of PFHxA health outcomes

Endpoint	BMR	Rationale
Hepatic effects		
Hepatocellular hypertrophy	10% ER	For hepatic toxicity, 10% ER is considered a minimally biologically significant response level for this endpoint (U.S. EPA, 2012a).
Developmental effects		
Postnatal (F_1) body weight	5% RD	A 5% RD in markers of growth/development in gestational studies (e.g., fetal weight) has generally been considered a minimally biologically significant response level and has been used as the BMR

Endpoint	BMR	Rationale
		for benchmark dose modeling in prior IRIS assessments (U.S. EPA, 2003, 2004, 2012b).
Offspring mortality	1% ER	Although 5% ER is generally supported for developmental and reproductive outcomes (U.S. EPA, 2012a), a lower BMR of 1% ER was considered appropriate for modeling offspring mortality in light of the severity of the frank effect.
Hematopoietic effects		
Red blood cells	1 SD	No biological information is readily available that allows for determining a minimally biological significant response for these outcomes. The BMD Technical Guidance (U.S. EPA, 2012a) recommends a BMR based on 1 standard deviation (SD) in such a situation.
Hemoglobin		

An adequate fit is judged on the basis of $\chi 2$ goodness-of-fit p-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate on the basis of the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected (i.e., Test 2; $p < 0.05$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.05$), the data set is considered unsuitable for BMD modeling. Among all models providing adequate fit for a given endpoint, the benchmark dose lower confidence limit (BMDL) from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within three-fold). Otherwise, the lowest BMDL was selected as a potential POD for each endpoint.

Where modeling was feasible, the estimated BMDLs were used as PODs. Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Supplemental Information, Appendix B. The benchmark dose approach involving modeling to obtain the BMDL is preferred, but it involves modeling dose levels corresponding to BMR levels near the low end of the observable range of the data and is not always feasible. When data sets were not amenable to BMD modeling, no-observed-adverse-effect level (NOAEL) or lowest-observed-adverse-effect level (LOAEL) values were selected and used as the POD on the basis of expert judgment, considering the study design features (e.g., severity and rarity of the outcome; biological significance, considering the magnitude of change at the NOAEL or LOAEL; statistical significance and power; exposure and outcome ascertainment methods).

For the study by Iwai and Hoberman (2014), the experiment was conducted in two phases. With the exception of differences in the dose levels, the design and conduct were the same across the two phases. Specifically, in addition to concurrent control groups for each phase, animals were exposed to 100, 350, or 500 mg/kg-day in Phase 1 and 7, 35 or 175 mg/kg-day in Phase 2. When possible, the two phases were combined for modeling to provide a more robust dose range. If the combined data set did not result in adequate model fit, the phases were modeled separately and the results for the individual phases were presented.

Approach for Animal-Human Extrapolation of PFHxA Dosimetry

The IRIS PFAS protocol (Supplemental Information document, Appendix A) recommends the use of physiologically based pharmacokinetic (PBPK) models as the preferred approach for dosimetry extrapolation from animals to humans, while allowing for the consideration of data-informed extrapolations (such as the ratio of serum clearance values) for PFAS that lack a PBPK model. If chemical-specific information is not available, the protocol then recommends that doses be scaled allometrically using body weight $BW^{3/4}$ methods. This hierarchy of recommended approaches for cross-species dosimetry extrapolation is consistent with EPA's guidance on using allometric scaling for the derivation of oral reference doses (U.S. EPA, 2011). It also prioritizes the order of relative uncertainty associated with each approach as follows:

- A PBPK model that is well grounded in multiple data sets (including physiological data, in vitro distribution data, and in vivo PK data) has the least uncertainty.
- A data-informed extrapolation, based on empirical PK data in the species of interest, has intermediate uncertainty because it is based on direct observation of the internal dose (i.e., serum concentration) in experimental animals and humans, typically.
- $BW^{3/4}$ scaling has the greatest uncertainty, relative to the two above approaches, because it is based on a general assumption about the relative rate of clearance in humans vs. animals and makes use of no chemical-specific data. Further, as described in Section 3.1, a comparison of $BW^{3/4}$ scaling to the available PK data in rats and humans indicates that use of $BW^{3/4}$ would overpredict human clearance, and hence underpredict risk, by 1–2 orders of magnitude. Thus, $BW^{3/4}$ scaling was not considered appropriate for this assessment.

As discussed in Section 3.1.5, no PBPK model is available for PFHxA in rats, mice, or monkeys. Although a PBPK model for humans was described by Fàbrega et al. (2015), it was not considered sufficiently reliable for use in an IRIS Toxicological Review.

On the other hand, when PK data for PFHxA exist in relevant animal species (rats, mice, and monkeys) or humans, a data-informed extrapolation approach for estimating the dosimetric adjustment factor (DAF) can be used. Various PK analyses can be performed to extract meaningful information from PK data. Because PK data for various PFAS are available, including for PFBA (Chang et al., 2008), PFBS (Olsen et al., 2009), PFHxA (Dzierlenga et al., 2019), PFHxS (Sundström et al., 2012), PFNA (Tatum-Gibbs et al., 2011), and PFOA and PFOS (Kim et al., 2016b), that show a

clear biphasic elimination pattern indicative of distinct distribution and elimination phases, EPA chose to use a two-compartment PK model, similar to the analysis of (Fujii et al., 2015). The EPA model is characterized by equation 5-1:

$$C(t) = A \cdot \exp(-\alpha \cdot t) + B \cdot \exp(-\beta \cdot t) - flag_{oral} \cdot (A+B) \cdot \exp(-k_a \cdot t), \quad 5\text{-}1$$

where α and β are first-order rate constants (units of time^{-1}) representing the rate of distribution and elimination, respectively, k_a is a rate constant (units of time^{-1}) for oral absorption, and $flag_{oral}$ is set to zero when analyzing intravenous dose data or one for oral data. Details of the model fitting are provided in Appendix B. The model assumes that oral bioavailability is 100%, consistent with PK data from Dzierlenga et al. (2019) and other studies and that internal dosimetry and elimination are linear with dose. This is implicitly a two-compartment PK model represented by the model, for which the rate of elimination corresponds to β. It is presumed that the total concentration from several consecutive doses would be obtained by simply adding the individual concentration curves, given the distinct dose times.

This PK model assumes the parameters are independent of time and dose. As discussed in the "Elimination" section, PK studies that measured tissue concentrations after multiple days of exposure are consistent with simple PK models parameterized from one-day exposure and support the assumption that the model parameters are independent of time. Although PK data at lower doses do not show any trend consistent with dose-dependence, data for the highest dose indicate that elimination can be reduced [Dzierlenga et al. (2019); the opposite of what is predicted based on the hypothesis of saturable resorption]. A systematic deviation from this assumption has not been observed in the other relevant data (Chengelis et al., 2009a; Gannon et al., 2011; Iwabuchi et al., 2017). Further, because PFHxA is not metabolized, nonlinearity in its internal dose is not expected due to that mechanism. Parameter estimation, however, was performed both including and excluding the highest dose data. Had the resulting estimate of β been significantly different when the high-dose data were included, this would have indicated a dose dependence. The results of the alternative analyses did not indicate such a difference, however, leading to the conclusion that PFHxA PK is not dose dependent and that the assumption of nonvarying parameters in the PK model equation is appropriate. Further details are provided in Appendix C.

Given the fit of this model to a specific data set, the AUC from the time of exposure to infinity is:

$$AUC_{inf} = A/\alpha + B/\beta - flag_{oral} \cdot (A+B)/k_a \quad 5\text{-}2$$

AUC is the integral of the chemical concentration in blood or serum over time, with units of mass × time / volume (e.g., mg-h/L), and is considered an appropriate measure of internal dose when the chemical has an accumulative effect over time.

By definition, the clearance (CL) of a compound is the effective volume of blood cleared of the compound per unit time (units of volume/time). Mathematically, given the PK model described

above, CL = dose/AUC_{inf}. If one assumes that risk increases in proportion to AUC, the ratio of clearance in animal to that in the human, CL_A:CL_H, can then be used to convert an oral dose-rate in animals (mg/kg-day) to a human equivalent dose (HED) rate. A similar approach using the ratio of the beta-phase half-lives can be used and is outlined in the Supplementary Information, Appendix C, but that approach ignores differences in the absorption rate and alpha-phase distribution rate that impact AUC and is, therefore, considered to produce a more uncertain outcome. Effectively, using the half-life ratio assumes that another pharmacokinetic parameter, the volume of distribution, is the same between species (this is contrary to available data, see below).

To avoid assuming the volume of distribution is equal between rats and humans, the HED can be calculated using clearance:

$$HED = (CL_H/CL_{A[s]}) \times POD \quad 5\text{-}3$$

Given the PK model and definition of clearance above, the resulting HED is the dose that results in the same AUC in humans as is predicted in animals exposed at the POD, provided that one can obtain a value of CL_H (see below).

In the term $CL_{A[s]}$, the [s] in the subscript refers to the sex-specific value available for animals but not humans in the case of PFHxA. Because there are sex-specific values (significant differences between males and females) in clearance among mice and rats, the CL values for female rodents would be used to extrapolate health effects in female rodents and the CL values for male rodents would be used to extrapolate male rodent health effects. This choice simply ensures that an observed effect in male rats, for example, is extrapolated using the expected internal dose for male rats. When endpoints from both male and female animals are analyzed (i.e., separate dose-response analyses are conducted for results in males vs. females) resulting in sex-specific PODs, the corresponding male and female human HEDs would be calculated, using ($CL_H/CL_{A[s]}$).

The volume of distribution in the beta phase (i.e., after the chemical has distributed into the body as a whole) given the two-compartment model above is:

$$V_{d,\beta} = CL/\beta = dose/[\beta \times (A/\alpha + B/\beta - flag_{oral} \times (A+B)/k_a)] \quad 5\text{-}4$$

With the exception of the i.v. dose data from Dzierlenga et al. (2019), the V_d for rats for all other experiments and studies for male and female rats were between 0.9 and 1.7 L/kg and the averages for males and females were virtually indistinguishable: 1.37 and 1.35 L/kg, respectively. For the i.v. dose data from Dzierlenga et al. (2019), $V_{d,\beta}$ was 5.2 L/kg in male rats and 18.7 L/kg in female rats. In contrast, $V_{d,\beta}$ for the i.v. dose data from Chengelis et al. (2009a) was 0.93 L/kg for both male and female rats. Thus, excluding those specific i.v. experiments, $V_{d,\beta}$ in rats does not appear to be sex specific and an overall average of 1.36 L/kg appears appropriate for that species.

For male and female mice, the corresponding V_d was 0.75 and 0.78 L/kg, respectively, based on data from Gannon et al. (2011), again not indicating a significant sex difference, although the value is somewhat lower than in rats.

For male and female monkeys, Chengelis et al. (2009a) reported V_d = 0.99 ± 0.58 L/kg and 0.47 ± 0.35 L/kg, respectively. Although these indicate a possible sex difference, only three animals of each sex were used and the estimated ranges (0.39–1.5 vs. 0.23–0.87 L/kg) significantly overlap. Hence, some caution in interpreting these data is required. The overall average V_d for monkeys, 0.73 L/kg, is similar to the value for mice, although also lower than the value in rats.

Because the volume of distribution (V_d) has not been determined in humans, but an estimate for the human half-life ($t_{1/2}$) is available, three options for estimating a clearance in humans can be considered, although this might be viewed as extreme for the purpose of predicting HED values. The observed $t_{1/2}$ in humans is presumed to represent the beta or clearance phase, given the PFHxA study participant evaluation occurred over months after primary exposure to PFHxA had ended (Nilsson et al., 2010). Hence it is presumed that $t_{1/2} = \ln(2)/\beta$. Rearranging the two equations, $CL = V_{d,\beta} \times \beta = V_{d,\beta} \times \ln(2)/ t_{1/2}$. Three options were considered, as follows:

1) The V_d for humans is equal to that determined in the next closest species biologically, monkeys. This assumes the biological and biochemical factors that determine the tissue:serum concentration ratio and the relative proportion (fraction of BW) for various tissues is similar in humans and monkeys. This assumption presumes the relative binding of PFHxA in human serum relative to various other tissues in the body is like that in monkeys but leads to a conclusion that renal clearance in humans is significantly slower than in other species.

2) Use the clearance values estimated for mice, rats, and monkeys to estimate the clearance in humans via allometric scaling. The results for mice, rats, and monkeys in Table 5-3 show almost no trend with increasing species BW, but can be fitted with a power function to obtain $CL = 0.152 \cdot BW^{-0.023}$ (L/kg), assuming standard BW values of 0.03 and 0.25 kg for mice and rats, respectively, and the reported BW of monkeys used by Chengelis et al. (2009a). For a standard human BW of 70 kg, the resulting predicted clearance in humans is 0.138 L/h-kg[3]. If this is the actual clearance in humans, but $t_{1/2}$ = 768 h, human $V_{d,\beta} = CL \times t_{1/2}/\ln(2)$ = 153 L/kg. Note human participants were exposed to PFHxA for a months, which could have allowed them to accumulate a deep tissue dose, while the monkey PK study involved only a single i.v. administration. Thus, a much higher V_d might have been estimated in monkeys had they been subject to repeated doses.

3) The apparent human half-life reported by Russell et al. (2013) might be an artifact of significant ongoing exposure to PFHxA during the period of observation. Pérez et al. (2013) detected PFHxA levels in human tissues higher than other PFAS and other observational studies regularly detect PFHxA in human serum demonstrating widespread human exposure to the general population. Thus, there is no reason to believe the subjects of Nilsson et al. (2010) did not also have some level of ongoing exposure; the question is whether such exposure was significant relative to the body burden accumulated from exposure as ski-wax technicians. If the value of CL estimated in (2) (0.138 L/h-kg) is an accurate prediction for humans *and* the V_d is equal to the average estimated for monkeys (0.73 L/kg), the half-life in humans should be $t_{1/2} = \ln(2) \times V_d / CL = \ln(2) \times 0.73$

[3]If a BW of 80 kg is used for humans (U.S. EPA, 2019d), the result is 0.137 L/h-kg. The calculation was performed using 70 kg for comparability with previous assessments.

(L/kg)/(0.138 L/h-kg) = 5.3 h. If this were the case, human serum levels would fall to 96% in a single day, while the data of Nilsson et al. (2010) show that for such a decline to occur takes at least two months. If this were the case, even after a day or two off work, a technician's serum concentration would be near zero. Further, the serum concentrations reported (Nilsson et al., 2010) do decline to near or below the limit of detection by late spring or early summer of each year, indicating that other ongoing sources of exposure were not significant for that population. Thus, this third option seems extremely unlikely and will not be evaluated further.

The two options for human CL estimated in points (1) and (2) above are provided in Table 5-3.

Table 5-3. Summary of serum half-lives and estimated clearance for PFHxA

Species/Sex	Study design	Elimination half-life ($t_{1/2}$) (h)	Clearance (CL) (L/h-kg)	Volume of distribution (V_d) (L/kg)	References/Data sources
Rat, female	Oral and i.v.	2.7 (0.5–11.2)	0.383 (0.259–0.574)[a]	1.48 (0.27–4.42)[a]	Dzierlenga et al. (2019); Chengelis et al. (2009a); Gannon et al. (2011)
Rat, male	Oral and i.v.	5.4 (1.6–19.5)	0.163 (0.112–0.228)[a]	1.31 (0.37–4.4)[a]	Dzierlenga et al. (2019); Chengelis et al. (2009a); Iwabuchi et al. (2017); Gannon et al. (2011)
Mouse, female	Oral	7.9 (2.8–23)	0.206 (0.137–0.308)[a]	2.46 (0.82–6.82)[a]	Gannon et al. (2011); Daikin Industries (2010)
Mouse, male	Oral	10.6 (2.3–29)	0.0894 (0.053–0.153)[a]	1.38 (0.31–3.73)[a]	Gannon et al. (2011)
Monkey, female	i.v.	2.4	0.136	0.474 ± 0.349[b]	Chengelis et al. (2009a)
Monkey, male	i.v.	5.3	0.122	0.989 ± 0.579[b]	Chengelis et al. (2009a)
Human, male and female	Ecological	337	1.5×10^{-3} [(c)] 0.152[d]	0.73[c] 74[d]	Russell et al. (2013)

[a]For each experiment (study/route/dose), a separate distribution of CL = dose/AUC_{inf} and $V_{d\beta}$ = CL/β was generated. Median, 5th, and 95th percentiles of each distribution were calculated and are available on request. Results across experiments/dose levels were pooled, and the values presented here are statistics for the pooled results, 50th (5th–95th) percentiles for each species/sex.
[b]Reported mean ± SD from 3 male or female monkeys.
[c]CL = $V_d \times \ln(2)/t_{1/2}$ with V_d assumed as the average of the estimated values for male and female monkeys.
[d]Human CL estimated by allometric scaling from values estimated for mice, rats, and monkeys; human V_d = CL × $t_{1/2}$/ln(2).

Thus, two alternative values of the DAF, CL_H:$CL_{A[s]}$—which is the ratio of clearance values—can be obtained (Table 5-4).

Table 5-4. Two options for rat, mouse, and human clearance values and data-informed dosimetric adjustment factor (DAF)

Sex	Species	Animal clearance (L/h-kg)[a]	Human clearance (L/h-kg)	DAF (CL_H:$CL_{A[s]}$)
Male	Rat	0.163	$1.50\ (0.90–2.48) \times 10^{-3}$ [(b)] (mean, 90% CI, using preferred [data-driven] approach)	9.2×10^{-3}
	Mouse	0.0894		1.7×10^{-2}
Female	Rat	0.383		3.9×10^{-3}
	Mouse	0.206		7.3×10^{-3}
Male	Rat	0.163	0.152[c] (alternative approach)	0.93
	Mouse	0.0894		1.7
Female	Rat	0.383		0.40
	Mouse	0.206		0.74

Shaded values were applied to derive the POD_{HED}.
[a]Species/sex-specific CL values (Supplementary Information, Appendix C).
[b]Calculated from human $t_{1/2}$ value, obtained by Bayesian PK analysis and average volume of distribution for male and female monkeys (Table 5-3).
[c]Calculated from allometric scaling of CL using results in Table 5-3.

To evaluate whether it is more reasonable to expect CL or V_d to be similar in humans as in experimental animals, values of CL were examined directly in humans for PFHxS, PFNA, and PFDA by Zhang et al. (2013b) and can be compared to those for experimental animals. Briefly, Zhang et al. (2013b) measured PFAS concentrations in serum and matched 24-hour urine samples to directly measure urinary clearance. To avoid the complicating issue of losses from menstrual blood, results for men and women over the age of 50 years are evaluated here. Median urinary CL values reported by Zhang et al. (2013b) were 0.015, 0.094, and 0.035 mL/kg-day for PFHxS, PFNA, and PFDA, respectively.

Kim et al. (2016b) reported renal PFHxS clearance of 0.76 mL/kg-day while Kim et al. (2016b) and Sundström et al. (2012) reported *total* clearance of 7–9 mL/kg-day. Sundström et al. (2012) also reported total clearance of PFHxS of 3–5 mL/kg-day in male mice and 1.3–1.9 mL/kg-day in monkeys. Thus, these results for PFHxS show significantly slower clearance in humans than in mice, rats, and monkeys.

The reported dose/AUC can be used to derive clearance values for PFNA from the results of Tatum-Gibbs et al. (2011). The estimated CL in rats is highly variable across the studies evaluated but ranged from 2 to 66 mL/kg-day in males and from 4 to 106 mL/kg-day in females (Benskin et al., 2009; De Silva et al., 2009; Ohmori et al., 2003; Tatum-Gibbs et al., 2011). CL in male and female mice reported by Tatum-Gibbs et al. (2011) ranged from 3 to 10 mL/kg-day. Although the wide range for rats indicates a degree of uncertainty, these results indicate that clearance in mice and rats is similar and much larger than the corresponding human value (0.094 mL/kg-day) (Zhang et al., 2013b).

Therefore, the top set of DAFs in Table 5-4—based on CL_{human} = 6.6 × 10⁻⁴ L/kg-h—are the preferred set because they are consistent with data for other PFAS, and the reasonable expectation, based on data from multiple chemicals, is the volume of distribution in humans does not substantially differ from that in experimental animals.

Representative calculations of the human equivalent dose (HED) for considered health effects follow, using the POD of 20 mg/kg-day for postnatal (F_1) body weight at PND 0 (Loveless et al., 2009) as an example and the female rat DAF of 3.9×10^{-3}, based on clearance:

$$HED = POD \left({}^{mg}/_{kg\text{-}day}\right) \times DAF$$

$$HED = 20 \left({}^{mg}/_{kg\text{-}day}\right) \times 3.9 \times 10^{-3} = 0.078 \left({}^{mg}/_{kg\text{-}day}\right) \quad \text{5-5}$$

In general, clearance captures the overall relationship between exposure and internal dose, specifically the average concentration of a substance in serum, while the half-life does not. In particular, use of half-life makes an intrinsic assumption that V_d is the same in the test species as in humans. From Table 5-3 one can see a significant difference between rats and monkeys, which leads one also to expect a difference between rats and humans.

HED based on clearance incorporates the observed differences in V_d among mice, rats, and primates, and is therefore, the preferred approach for dosimetry extrapolation from animals to humans.

Uncertainty in the application of the DAF based on clearance remains, given that neither V_d nor CL were measured or determined in humans. *To estimate CL in humans, the human V_d was assumed equal to the average value estimated in male and female monkeys, which seems less uncertain given the data*. The V_d of male and female mice was assumed the same as in male and female rats, respectively. Because the difference in V_d between male and female rats is small, using these sex-specific V_d values for mice will give similar results to using an average.

Uncertainty of animal-human extrapolation of PFHxA dosimetry

Although the variability between and even within some data sets for rats (~4-fold for males and ~6-fold variation for females between lowest and highest mean clearance values) is large, the number of studies provides confidence in the estimated average clearance values for both male and female rats, which is reflected by the modest 90% CI for rat CL in Table 5-3.

Only one PK study is available for mice, although with two dose levels (Gannon et al., 2011). Further, the data for the 100 mg/kg dose approach a plateau, as if clearance stopped when the concentration was around 0.5 µg/g, although such a plateau was not observed for the 2 mg/kg data. EPA concluded that the data, which used ^{14}C labeling, were not correctly adjusted for the background signal or LOD. EPA was able to analyze the two dose levels for male and female mice successfully, however, by focusing on the data above the concentration at which the plateau occurred. Because the data from Gannon et al. (2011) for rats is near the middle of the range for other rat studies and the methods described otherwise are appropriate, it is presumed that this study has good quality results, with the exception of the LOD correction of this dose in mice, is

presumed. Therefore, some uncertainty remains with the clearance value obtained for mice from this study.

The PK study of Chengelis et al. (2009a) is considered high quality, but the results for monkeys used only three males and three females.

Uncertainty in the application of the DAF based on clearance remains, given that neither V_d nor CL were measured or determined in humans. To estimate CL in humans, the human V_d was assumed equal to the average value estimated in male and female monkeys, which seems less uncertain given the data and analyses described above. The V_d of male and female mice was assumed the same as in male and female rats, respectively. Because the difference in V_d between male and female rats was small, using these sex-specific values for mice will give similar results to using an average.

One alternative approach to using clearance in mice or rats to estimate the average blood concentrations in those species for each bioassay might be to use the measured serum concentrations from toxicological studies as BMD modeling inputs and then the estimated human clearance value to calculate the HED. Three of the four studies being evaluated, however, did not measure PFHxA serum concentrations (Chengelis et al., 2009b; Iwai and Hoberman, 2014; Klaunig et al., 2015; Loveless et al., 2009). Although Iwai and Hoberman (2014) attempted to measure serum concentrations in mice, all serum measurements were below the LOQ. Therefore, this alternative approach cannot be applied in evaluating these dose-response data.

POD_{HED} for RfD derivation

Table 5-5 presents the estimated POD_{HED} (mg/kg-day) values or the hepatic, developmental, and hematopoietic toxicity endpoints considered for RfD derivation based on the endpoint selection justification in Table 5-1 and preferred DAF values in Table 5-4.

The last column in Table 5-5 includes normalization from the ammonium salt to the free acid using a molecular weight conversion [MW free acid/MW ammonium salt = 314/336 = 0.935 (Iwai and Hoberman, 2014)] and sodium salt to free acid [MW free acid/MW sodium salt = 314/331 = 0.949 (Loveless et al., 2009)]. The POD_{HED} for postnatal (F_1) body weights used the female HED, as exposures were to the dams and assumed equal clearance in a developing offspring as an adult.

The free acid of PFHxA is calculated using the ratio of molecular weights, as follows:

$$PFHxA\ (free\ acid) = \left(\frac{MW\ free\ acid}{MW\ ammonium\ salt}\right) = \left(\frac{314}{336}\right) = 0.935$$

$$PFHxA\ (free\ acid) = \left(\frac{MW\ free\ acid}{MW\ sodium\ salt}\right) = \left(\frac{314}{331}\right) = 0.949 \qquad 5\text{-}6$$

Table 5-5. PODs considered for the derivation of the RfD

Endpoint	Study/Confidence	Species, strain (sex)	POD type/model	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)
Hepatic effects					
↑Hepatocellular hypertrophy	Chengelis et al. (2009b) *Low* confidence	Rat, Crl:CD(SD) (male)	NOAEL[b] (0% response)	50	0.46
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{10ER}$ Multistage 1 NCV	10.66	0.093[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{10ER}$ Multistage 3 NCV	96.32	0.36[c]
Hematopoietic effects					
↓Hemoglobin	Klaunig et al. (2015) *High* confidence	Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Linear CV	122.77	0.48
	Chengelis et al. (2009b) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{1SD}$ Polynomial 3 CV	81.35	0.75
		Rat, Crl:CD(SD) (female)	NOAEL[d] (3% decrease)	50	0.19
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	NOAEL[d] (6% decrease)	50	0.44[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Polynomial 3 CV	127.61	0.47[c]
↓Red blood cell	Klaunig et al. (2015) *High* confidence	Rat, Crl:CD(SD) (male)	NOAEL[b] (4% decrease)	100	0.93
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Linear CV	109.15	0.43
	Chengelis et al. (2009b) *High* confidence	Rat, Crl:CD(SD) (male)	NOAEL[d] (no change)	50	0.46
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Exponential 5 CV	16.32	0.06
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{1SD}$ Linear NCV	44.57	0.39[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Linear CV	112.36	0.42[c]
Developmental effects					
↓Postnatal (F_1) body weight, PND 0	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD), F_1 (combined)	$BMDL_{5RD}$ Hill	10.62	0.039[c]
↓Postnatal (F_1) body weight, PND 0	Iwai and Hoberman (2014) *High* confidence	Mouse, CD-1, F_1 (combined)	$BMDL_{5RD}$ Polynomial 3 CV Phase 2	80.06	0.55[e]

Endpoint	Study/Confidence	Species, strain (sex)	POD type/model	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)
↓Postnatal (F_1) body weight, PND 4			$BMDL_{5RD}$ Exponential-M5 Phase 1 and 2 Polynomial 3 CV Phase 2	103.12 89.79	0.70[e] 0.61[e]
↑Perinatal (F_1) mortality (PND 0–21, including stillbirths)	Iwai and Hoberman (2014) *High* confidence	Mouse, CD-1, F_1 (combined)[g]	$BMDL_{1ER}$ Nested Logistic Phase 1 Model Average[h] Phase 2	98.61 102.65	0.67[e,f] 0.70[e,f]

CV = constant variance; NCV = nonconstant variance; SD = standard deviation.

[a]HED calculations based on the DAF, the ratio of human and animal clearance values (Table 5-4). DAF values for female rats and female mice were used for the respective developmental effects on combined male and female pups of each species.

[b]Response only at high dose with responses far above BMR level, data not modeled.

[c]POD_{HED} multiplied by normalization factor to convert from sodium salt to free acid (MW free acid/MW sodium salt = 314/331 = 0.949).

[d]No models provided adequate fit; therefore a NOAEL approach was selected.

[e]POD_{HED} multiplied by normalization factor to convert from ammonium salt to the free acid (MW free acid/MW ammonium salt = 314/336 = 0.935).

[f]The combined data set from phases 1 and 2 did not provide adequate fit for modeling, so the phases were modeled separately and both PODs are presented.

[g]Data sets were modeled using BMDS 2.7

[h]An average of BMDLs from NCTR (BMDL of 78.90 mg/kg-day) and Rai Van Ryzin (126.4 mg/kg-day) models with an identical AIC value is selected as the final BMDL (102.65 mg/kg/day)

Derivation of Candidate Toxicity Values for the RfD

The PODs calculated in Table 5-5 were narrowed, within a health effect, for derivation of candidate lifetime toxicity values based on the POD, certainty in the POD, and biological understanding (if any) of the mechanisms of potential PFHxA-mediated toxicity. The selection of the endpoints for which an RfD was determined (Table 5-6) was based on several factors, including whether the endpoint is protective of a lifetime exposure, whether an endpoint with less uncertainty or greater sensitivity exists, and whether the endpoint is protective of both sexes and all life stages. Based on these considerations, the endpoints in Table 5-5 were narrowed to the following: for hepatic endpoints to hepatocellular hypertrophy from a subchronic study (Loveless et al., 2009), for hematopoietic endpoints to RBCs and HGB from the chronic study (Klaunig et al., 2015), and for developmental endpoints to offspring body weight from (Loveless et al., 2009).

For the hepatic endpoint, hepatocellular hypertrophy was moved forward for POD determination. This was based on consistent evidence across studies and sexes for increased hepatocellular hypertrophy accompanied by increased relative liver weight, increased serum enzymes, and decreased proteins that when interpreted together indicate hepatic toxicity and altered homeostasis. This alteration in homeostasis is anticipated to lead to adverse toxic

responses including necrosis. The lowest effect level for hepatocellular hypertrophy was observed in the subchronic studies in the 100 mg/kg-day male dose group (Loveless et al., 2009). Males were more sensitive for this endpoint than females (the lowest effect level was 100 mg/kg-day in males vs. 500 mg/kg-day in females) although the effect persisted in both sexes 90 days after recovery (500 mg/kg-day). In the chronic study, the 200 mg/kg-day female dose group was sensitive for necrosis (note the highest administered dose in males was 100 mg/kg-day). Considering that hepatocellular hypertrophy likely precedes necrosis and the dose causing necrosis in the chronic study (Klaunig et al., 2015) was two times higher than the 100 mg/kg-day PFHxA dose causing hypertrophy in the subchronic study (Loveless et al., 2009), hypertrophy from male rats in the subchronic study (Loveless et al., 2009) was selected as the appropriate endpoint and advanced for RfD determination.

For developmental effects, decreased postnatal (F1) body weight was prioritized over offspring mortality (Table 5-6). This was based on the severity of the outcome and the lower POD_{HED} for fetal body weight, versus mortality, and is expected to be protective of all developmental effects. Of the two body weight data sets, the data from (Loveless et al., 2009) were advanced because the study design included a longer exposure that spanned fetal development through continuous maternal exposure, through gestation, and until the end of lactation) versus Iwai and Hoberman (2014) where offspring were exposed only through the study GD 6–18.

For hematopoietic effects, endpoints were available from both subchronic studies and the chronic study. Because these endpoints were available from the chronic study, their suitability for RfD determination was based on evaluating evidence for the magnitude of change, the deviation around the mean within a large cohort (7000 rats) of laboratory animals (Matsuzawa et al., 1993), and the sensitivity of the endpoint to respond to PFHxA exposure. The magnitude of change for RBCs (~8% decreased) or HGB (~5% decrease) was similar when comparisons were made between chronic and subchronic studies. RBCs and HGB were decreased in both males and females dosed with 200 mg/kg-day in the subchronic study (Chengelis et al., 2009b) and in females dosed with 200 mg/kg-day in the chronic study. The biological significance of the magnitude of change for both RBC and HGB in rats is uncertain, but the effect on red blood cell parameters had a slightly lower POD than HGB and was concurrent with increased reticulocyte levels, a compensatory response to anemia. Females were more sensitive in the chronic study when the magnitude of effect between males and females, at similar dose levels, were compared. Note, however, females received twice the maximum dose that male rats received, which might explain sex-specific differences in the chronic study. Therefore, the female RBC hematological endpoint from the chronic study was prioritized for RfD determination (Klaunig et al., 2015).

Table 5-6. Uncertainty factors for the development of the RfD for PFHxA

UF	Value	Justification
UF_A	3	A UF_A of 3 is applied to account for uncertainty in characterizing the PK and pharmacodynamic differences between species following oral NaPFHx/NH_4PFHxA/PFHxA exposure. Some aspects of the cross-species extrapolation of PK processes have been accounted for by calculating an HED through application of a DAF based on animal and human clearance; however, residual uncertainty related to potential pharmacodynamic differences remains. Therefore, a UF_A of 3 was selected for PFHxA; see text above for further discussion.
UF_H	10	A UF_H of 10 is applied for interindividual variability in humans in the absence of quantitative information on potential differences in PK and pharmacodynamics relating to NaPFHx/NH_4^+PFHxA/PFHxA exposure in humans.
UF_S	1 (developmental and hematopoietic endpoints)	A UF_S of 1 is applied to developmental endpoints from the one-generation reproductive study by Loveless et al. (2009) and Iwai and Hoberman (2014). The developmental period is recognized as a susceptible lifestage and studies using exposure designs capturing sensitive developmental windows (i.e., gestation or lactation) are more relevant for induction of developmental effects than lifetime exposures (U.S. EPA, 1991). Although effects on body weights are not unique to development and studies evaluating the body weight effects of postnatal exposure are lacking, the current evidence for PFHxA suggests this is a sensitive lifestage for body weight effects of PFHxA exposure based on effects being measured at lower doses than adults. A UF_S of 1 is also applied to hematopoietic endpoints in the study (Klaunig et al., 2015) as the 51 weeks of daily exposure represented more than 10% of a rodent life span and the incidence or severity of these outcomes is not anticipated to increase with increasing exposure duration.
	3 (hepatic)	A UF_S of 3 is applied to hepatocellular hypertrophy for the purpose of deriving a lifetime RfD. Although the endpoint was derived from a 90-day subchronic study (Loveless et al., 2009), the evidence supports a pathway where hepatocellular hypertrophy is the toxic effect altering homeostasis. The evidence suggests that hepatocellular hypertrophy is an adverse hepatic response to PFHxA exposure that worsens with longer exposure toxic effects such as necrosis.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL.
UF_D	3	A UF_D of 3 is applied because the evidence base for hepatic, hematopoietic, and developmental endpoints included two subchronic studies and one chronic study in Sprague-Dawley rats and developmental/reproductive studies in Sprague-Dawley rats and Crl:CD1 mice. Limitations, as described in U.S. EPA (2002c) were used as the basis for a UF_D = 3. These limitations included a lack of informative human studies for most outcomes, subchronic or chronic toxicity studies in more than one species, or a multigenerational study. For developmental outcomes, pups were indirectly exposed via the dam (i.e., via placental or lactational transfer); thus, the dose received by the pups is unclear and might be significantly less than that administered to the dams.
UF_C	See Table 5-7 and Table 5-11	Composite uncertainty factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$.

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002c), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFHxA. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated uncertainty factor (UF) to be applied to the candidate POD_{HED} values are listed in Table 5-6.

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002c), the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans, which inherently accounts for uncertainty regarding the PK and pharmacodynamic differences between species. The PK uncertainty is accounted for through the application of dosimetric approaches for estimation of human equivalent doses as described above. However, this leaves some residual uncertainty understanding dose to target sites of toxicity (PK) and how adverse effects occur when molecules reach the target sites (pharmacodynamics). For developmental and hematopoietic outcomes, the evidence base lacked chemical- and species-specific information that would have been useful for informing the UF_A; therefore, a UF_A of 3 was applied. For hepatic effects, mechanistic and supplemental information useful for further evaluating the interspecies uncertainty factor was available. This evidence was PPARα pathway rich, likely due to the known species specificity for PPARα -linked oncogenic pathways (Klaunig et al., 2003). PFHxA, however, was noncarcinogenic in Sprague-Dawley rats (Klaunig et al., 2015).

As described in Section 3.2.1, PFHxA activates several receptors, and multiple pathways lead to hepatocellular hypertrophy and increased liver weight. The pharmacodynamic relationship between these PFHxA receptor-mediated interactions is not clear from the available evidence, but there are pathways with which these receptors are involved. Although some prototypical PPARα activators exhibit an exaggerated activation (and downstream response) in rodent as compared to human receptors, some evidence from in vitro studies suggests that PFHxA might induce human PPARα at similar (or lower) concentrations to mouse PPARα. Interpretation of these results is limited, however, as the data are derived from two experiments from same group (Wolf et al., 2014; Wolf et al., 2008). Given the suggestion of similar sensitivities in PPARα activation by PFHxA across species and possible PPARα-independent contributions to the observed hepatic effects, the possibility that humans might exhibit pharmacodynamic sensitivity for hepatic effects different from rats cannot be ruled out. Thus, based on the residual uncertainty surrounding the interspecies differences in pharmacodynamics described above, a factor of 3 is applied to account for the pharmacodynamic uncertainty of the UF_A for all potential health effect consequences of PFHxA exposure.

The uncertainty factors described in Table 5-6 were applied and the resulting candidate values for use in estimating an RfD for lifetime exposure are shown in Table 5-7.

Table 5-7. Candidate values for PFHxA

Endpoint	Study/ Confidence	Species, strain (sex)	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)
↑Hepatocellular hypertrophy, 90 day	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	10.66	0.093[b]	3	10	3	1	3	300	3×10^{-4}
↓Red blood cells, 51 weeks	Klaunig et al. (2015) *High* confidence	Rat, Crl:CD(SD) (female)	109.15	0.43[b]	3	10	1	1	3	100	4×10^{-3}
↓F_1 body weight, PND 0	Loveless et al. (2009) *High* confidence	Rat, Sprague-Dawley, F_1 (combined)	10.62	0.039[b]	3	10	1	1	3	100	4×10^{-4}

[a]HED calculations based on DAF, the ratio of human and animal clearance values (Table 5-4). DAF values for female rats and female mice were used for the respective developmental effects on combined male and female pups of each species.

[b]POD_{HED} multiplied by normalization factor to convert from sodium salt to free acid (MW free acid/MW sodium salt = 314/331 = 0.949).

Selection of Lifetime Toxicity Value(s)

Selection of Organ- or System-Specific RfDs

Organ/system-specific (os)RfDs associated with each health effect are presented in Table 5-8 as they could be useful for certain decision purposes (i.e., site-specific risk assessments). The rationale for and application of osRfD are described in the Protocol, Appendix A. Confidence in each osRfD is described in Table 5-8 and is based on several factors, including confidence in the study, the evidence base supporting the hazard, and quantitative estimate for each osRfD.

Table 5-8. Confidence in the organ/system-specific RfDs for PFHxA

Confidence categories	Designation	Discussion
Hepatic osRfD = 3×10^{-4} mg/kg-d		
Confidence in the study used to derive osRfD	*High*	Confidence in the study (Loveless et al., 2009) is *high* based on the study evaluation results (i.e., rated high confidence overall) (HAWC link). The overall study size, design, and test species were considered relevant for deriving toxicity values.
Confidence in the evidence base for hepatic effects	*Medium*	Confidence in the oral toxicity evidence base for hepatic effects is *medium* based on consistent, dose-dependent, and biologically coherent effects on organ weight and histopathology observed in multiple *high* confidence subchronic and chronic studies. The available mechanistic evidence also supports biological plausibility of the observed effects. Limitations of the

Confidence categories	Designation	Discussion
		evidence base for hepatic effects is the lack of human studies, and measures that would have been useful to inform the pathways for hepatic effects leading to hepatocellular hypertrophy (e.g., specific stains for hepatic vacuole contents, specific histological for pathology).
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium*, given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those for monkeys and rats, respectively.
Overall confidence in the hepatic osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is primarily driven by *medium* confidence in the overall evidence base for hepatic effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD. *High* confidence in the study was not interpreted to warrant changing the overall confidence from *medium*.
Hematopoietic osRfD = 4×10^{-3} mg/kg-d		
Confidence in study	*High*	Confidence in the study (Klaunig et al., 2015) is *high* based on the study evaluation results (i.e., rated *high* confidence overall) (HAWC link) and characteristics that make it suitable for deriving toxicity values, including relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base for hematopoietic effects	*High*	Confidence in the evidence base for hematopoietic effects was *high* based on consistent and biologically coherent effects on red blood cells, hemoglobin, and other hematological parameters measured across multiple *high* confidence chronic and subchronic studies. The RBC and hemoglobin findings were correlative with an erythrogenic response indicated by increased reticulocytes and pathological findings of splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia. Limitations of the hematopoietic evidence base are lack of human studies and some hematological measures were observed only at the highest dose, limiting interpretation of dose-response.
Confidence in the quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those in monkeys and rats, respectively.
Confidence in hematopoietic osRfD	*High*	The overall confidence in the osRfD is *high* and is primarily driven by *high* confidence in the overall evidence base for hematopoietic effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD.

Confidence categories	Designation	Discussion
Developmental osRfD = 4×10^{-4} mg/kg-d		
Confidence in study	*High*	Confidence in the study (Loveless et al., 2009) is *high* based on study evaluation results (i.e., rated *high* confidence overall) (HAWC link) and characteristics that make it suitable for deriving toxicity values, including relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base for developmental effects	*Medium*	Confidence in evidence base for developmental effects is *medium* based on the availability of data from two studies in different species (i.e., rats and mice) that consistently observed decreases in offspring body weight and coherent increases in perinatal mortality. Areas of uncertainty included lack of human data and multigenerational animal toxicity studies. Also, data to inform other organ/system-specific hazards (e.g., thyroid, immune, nervous system) following a developmental exposure are lacking. Additionally, the actual dose received by the offspring is unclear because the pups were indirectly exposed via the dams. Together these present significant data gaps in the potential effects during this sensitive life stage.
Confidence in the quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those in monkeys and rats, respectively.
Confidence in developmental osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is primarily driven by *medium* confidence in the overall evidence base for developmental effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD. *High* confidence in the study was not interpreted to warrant changing the overall confidence from *medium*.

Selection of RfD and Confidence Statement

Organ/system-specific RfD values for PFHxA selected in the previous section are summarized in Table 5 9.

Table 5-9. Organ/system-specific RfD (osRfD) values for PFHxA

System	Basis	POD_{HED}	UF_C	osRfD (mg/kg-d)	Confidence
Hepatic	Increased hepatocellular hypertrophy in adult male Crl:CD Sprague-Dawley rats	0.093 mg/kg-d based on $BMDL_{10ER}$ and free salt normalization (Loveless et al., 2009)	300	3×10^{-4}	*Medium*

System	Basis	POD_{HED}	UF_C	osRfD (mg/kg-d)	Confidence
Hematopoietic	Decreased red blood cells in adult female Crl:CD Sprague-Dawley rats	0.43 mg/kg-d based on $BMDL_{1SD}$ (Klaunig et al., 2015)	100	4×10^{-3}	*High*
Developmental	Decreased postnatal (PND 0) body weight in F_1 Sprague-Dawley male and female rats, exposed throughout lactation and gestation	0.039 mg/kg-d based on $BMDL_{5RD}$ and free salt normalization (Loveless et al., 2009)	100	4×10^{-4}	*Medium*

From the identified human health effects of PFHxA and derived osRfDs for hepatic, hematopoietic, and developmental effects (Table 5-9), an ***RfD of 4 × 10⁻⁴ mg/kg-day based on decreased postnatal (F_1) body weight*** in rats was selected. As described in Table 5-8, confidence in the RfD is medium, based on medium confidence in the developmental osRfD. The decision to select the developmental osRfD was based on all available osRfDs in addition to overall confidence and composite uncertainty for those osRfDs. The confidence in the selected RfD is equivalent to that of the hepatic osRfDs but lower than the hematopoietic osRfD. The developmental endpoint decreased F1 body weight at PND 0 having the lowest overall PODHED of 0.039 mg/kg-d based on $BMDL_{5RD}$ and free salt normalization (Loveless et al., 2009) and UFc of 100. The developmental osRfD was considered protective across all lifestages, including developmental. The hepatic osRfD was slightly lower but was based on a higher PODHED (0.093 mg/kg-day) and UFC (300). The developmental osRfD, therefore, is based on the lowest PODHED and lowest UFC using a study considered high confidence. The developmental osRfD is expected to be protective across all life stages, including developmental.

Estimation or Selection of Points of Departure (PODs) for Subchronic RfD Derivation

In addition to providing an RfD for lifetime exposure in health systems, this document also provides an RfD for less-than-lifetime ("subchronic") exposures. These subchronic RfDs were based on the endpoints advanced for POD derivation in Table 5-1. Data to inform potential hepatic and hematopoietic effects from the *high* confidence subchronic studies by (Chengelis et al., 2009b; Loveless et al., 2009) were considered the most informative for developing candidate values. The *high* confidence developmental/reproductive studies (Iwai and Hoberman, 2014; Loveless et al., 2009) were also advanced for candidate value derivation. The *high* confidence short-term study (NTP, 2018) was not advanced based on the same rationale as described above for the lifetime RfD.

In general, the rationales for advancing these endpoints for subchronic value derivation are the same as described and summarized above in Table 5-1; however, for hematopoietic effects, subchronic data from Chengelis et al. (2009b) and Loveless et al. (2009) were prioritized over the data from the chronic study by Klaunig et al. (2015) for use in deriving a subchronic RfD.

The endpoints selected for dose-response were modeled using approaches consistent with EPA's *Benchmark Dose Technical Guidance* document (U.S. EPA, 2012a). The approach was the same as described above for derivation of lifetime toxicity values, the BMRs selected for dose-response modeling and the rationales for their selection (see Table 5-2), and the dosimetric adjustments using the ratio of the clearance in animal to that in the human and salt to free acid normalization. Table 5-10 presents the estimated POD_{HED} (mg/kg-day) values for the hepatic, developmental, and hematopoietic toxicity endpoints considered for subchronic RfD derivation.

Table 5-10. PODs considered for the derivation of the subchronic RfD

Endpoint	Study/Confidence	Species, strain (sex)	POD type/model	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)
Hepatic effects					
↑Hepatocellular hypertrophy	Chengelis et al. (2009b) *Low* confidence	Rat, Crl:CD(SD) (male)	NOAEL[b] (0% response)	50	0.46
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{10ER}$ Multistage 1 NCV	10.66	0.093[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{10ER}$ Multistage 3 NCV	96.32	0.36[c]
Hematopoietic effects					
↓Hemoglobin	Chengelis et al. (2009b) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{1SD}$ Polynomial 3 CV	81.35	0.75
		Rat, Crl:CD(SD) (female)	NOAEL[d] (3% decrease)	50	0.19
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	NOAEL[d] (6% decrease)	50	0.44[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Polynomial 3 CV	127.61	0.47[c]
↓Red blood cell	Chengelis et al. (2009b) *High* confidence	Rat, Crl:CD(SD) (male)	NOAEL[d] (no change)	50	0.46

Endpoint	Study/Confidence	Species, strain (sex)	POD type/model	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Exponential 5 CV	16.32	0.064
	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	$BMDL_{1SD}$ Linear NCV	44.57	0.39[c]
		Rat, Crl:CD(SD) (female)	$BMDL_{1SD}$ Linear CV	112.36	0.42[c]
Developmental Effects					
↓Postnatal (F_1) body weight, PND 0	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD), F_1 (combined)	$BMDL_{5RD}$ Hill	10.62	0.039[c]
↓Postnatal (F_1) body weight, PND 0	Iwai and Hoberman (2014) *High* confidence	Mouse, CD-1, F_1 (combined)	$BMDL_{5RD}$ Polynomial 3 CV Phase 2	80.06	0.55[e]
↓Postnatal (F_1) body weight, PND 4			$BMDL_{5RD}$ Exponential-M5 Phase 1 and 2 Polynomial 3 CV Phase 2	103.12 89.79	0.70[e] 0.61[e]
↑Perinatal Mortality	Iwai and Hoberman (2014) *High* confidence	Mouse, CD-1, F_1 (combined)[g]	$BMDL_{1ER}$ Nested Logistic Phase 1 Model Average[h] Phase 2	98.61 102.65	0.67[e,f] 0.70[e,f]

1SD = 1 standard deviation, CV = constant variance, NCV = nonconstant variance.

[a]HED calculations based on the DAF, the ratio of human and animal clearance values (Table 5-3). DAF values for female rats and female mice were used for the respective developmental effects on combined male and female pups of each species.

[b]Response only at high dose with responses far above BMR level, data not modeled.

[c]POD_{HED} multiplied by normalization factor to convert from sodium salt to free acid (MW free acid/MW sodium salt = 314/331 = 0.949).

[d]No models provided adequate fit therefore a NOAEL approach was selected.

[e]POD_{HED} multiplied by normalization factor to convert from sodium salt to free acid (MW free acid/MW sodium salt = 314/331 = 0.934).

[f]The combined data set from phases 1 and 2 did not provide adequate fit for modeling, so the phases were modeled separately and both PODs are presented.

[g]Data sets were modeled using BMDS 2.7

[h]An average of BMDLs from NCTR (BMDL of 78.90 mg/kg-day) and Rai Van Ryzin (126.4 mg/kg-day) models with an identical AIC value is selected as the final BMDL (102.65 mg/kg/day)

Derivation of Candidate Toxicity Values for the Subchronic RfD

The POD_{HED} values listed in Table 5-10 were further narrowed for subchronic osRfD derivation and subchronic RfD selection. RBCs were a more sensitive POD_{HED} for hematopoietic effects. Therefore, the red blood cell endpoint from female rats from Chengelis et al. (2009b)was advanced for subchronic RfD derivation over male endpoints for hematocrit and red blood cells based on female RBC being more sensitive and therefore expected to be protective of effects in both sexes. Applying the rationales described for the selection of the lifetime osRfDs, the same endpoints were advanced for derivation of the hepatic and developmental subchronic osRfDs: male hepatocellular hypertrophy and decreased F_1 body weight at PND 0 (Loveless et al., 2009).

As described above under "Derivation of Candidate Values for the RfD," and in U.S. EPA (2002c), five possible areas of uncertainty and variability were considered in deriving the candidate subchronic values for PFHxA. In general, the explanations for these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate POD_{HED} values are listed above and in Table 5-6, including the UF_D which remained at 3 due to data gaps (i.e., for most outcomes, a lack of: informative human studies, animal studies from multiple species or spanning multiple generations, studies of other organ/system-specific effects associated with other PFAS, including PFOA and PFOS, particularly following developmental exposure). The exception that a $UF_S = 1$ was applied for all endpoints since no subchronic to chronic extrapolation was required for the subchronic RfD. The resulting candidate values are shown in Table 5-11.

Table 5-11. Candidate values for deriving the subchronic RfD for PFHxA

Endpoint	Study/Confidence	Species, strain (sex)	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)
↑Hepatocellular hypertrophy, 90 day	Loveless et al. (2009) *High* confidence	Rat, Crl:CD(SD) (male)	10.66	0.093[b]	3	10	1	1	3	100	9×10^{-4}
↓Red blood cell, 90 day	Chengelis et al. (2009b) *High* confidence	Rat, Crl:CD(SD) (female)	16.32	0.064	3	10	1	1	3	100	6×10^{-4}
↓Postnatal (F_1) body weight, PND 0	Loveless et al. (2009) *High* confidence	Rat, Sprague-Dawley, F_1 (combined)	10.62	0.039[b]	3	10	1	1	3	100	4×10^{-4}

[a]The RfD for the free acid of PFHxA is calculated using the ratio of molecular weights as described above.
[b]POD_{HED} multiplied by normalization from the sodium salt to free acid (MW free acid/MW sodium salt = 314/331 = 0.949).

Selection of Subchronic Organ- or System-Specific RfDs

As described above, subchronic osRfDs associated with each health effect are presented as they may be useful for certain decision purposes (i.e., site-specific risk assessments with less-than-lifetime exposures). Confidence in each osRfD are described in Table 5-12 and consider confidence in the study used to derive the quantitative estimate, the overall health effect, specific evidence base, and quantitative estimate for each osRfD.

Table 5-12. Confidence in the subchronic organ/system-specific RfDs for PFHxA

Confidence categories	Designation[a]	Discussion
Hepatic subchronic osRfD = 9×10^{-4} mg/kg-d		
Confidence in the study used to derive the subchronic osRfD	*High*	Confidence in the study (Loveless et al., 2009) is *high* based on the study evaluation results (i.e., rated high confidence overall) HAWC link) and characteristics that make it suitable for deriving toxicity values, including relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in the evidence base for hepatic effects	*Medium*	Confidence in the oral toxicity evidence base for hepatic effects is *medium* based on consistent, dose-dependent, and biologically coherent effects on organ weight and histopathology observed in multiple *high* confidence subchronic and chronic studies. The available mechanistic evidence also supports biological plausibility of the observed effects. Limitations of the evidence base for hepatic effects is the lack of human studies, and measures that would have been useful to inform the pathways for hepatic effects leading to hepatocellular hypertrophy (e.g., specific stains for hepatic vacuole contents, specific histological for pathology).
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and subchronic osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those in monkeys and rats, respectively.
Overall confidence in the hepatic subchronic osRfD	*Medium*	The overall confidence in the subchronic osRfD is *medium* and is primarily driven by *medium* confidence in the overall evidence base for hepatic effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD. *High* confidence in the study was not interpreted to warrant changing the overall confidence from medium.
Hematopoietic subchronic osRfD = 6×10^{-4} mg/kg-d		
Confidence in study used to derive the subchronic osRfD	*High*	Confidence in the study (Chengelis et al., 2009b) is *high* based on the study evaluation results (i.e., rated high confidence overall) (HAWC link) and characteristics that make it suitable for deriving toxicity

Confidence categories	Designation[a]	Discussion
		values, including relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base for hematopoietic effects	*High*	Confidence in the evidence base for hematopoietic effects was *high* based on consistent and biologically coherent effects on red blood cells, hemoglobin, and other hematological parameters measured across multiple *high* confidence chronic and subchronic studies. The RBC and hemoglobin findings were correlative with an erythrogenic response indicated by increased reticulocytes and pathological findings of splenic extramedullary hematopoiesis and bone marrow erythroid hyperplasia. Limitations of the hematopoietic evidence base are lack of human studies and some hematological measures were observed only at the highest dose, limiting interpretation of dose-response.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and subchronic osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those in monkeys and rats, respectively.
Confidence in hematopoietic subchronic osRfD	*High*	The overall confidence in the subchronic osRfD is *high* and is primarily driven by *high* confidence in the overall evidence base for hematopoietic effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD.
Developmental subchronic osRfD = 4×10^{-4} mg/kg-d		
Confidence in study used to derive the subchronic osRfD	*High*	Confidence in the study (Loveless et al., 2009) is *high* based on the study evaluation results (i.e., rated high confidence overall) (HAWC link) and characteristics that make it suitable for deriving toxicity values, including relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base for developmental effects	*Medium*	Confidence in evidence base for developmental effects is *medium* based on the availability of data from two studies in different species (i.e., rats and mice) that consistently observed decreases in offspring body weight and coherent increases in mortality. One area of uncertainty is that there were no multigenerational studies available. Also, data to inform other organ/system-specific hazards (e.g., thyroid, immune, nervous system) following a developmental exposure is lacking. Additionally, the actual dose received by the offspring is unclear since the pups were indirectly exposed via the dams. Together these present significant data gaps in the potential effects during this sensitive life stage.
Confidence in the quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and subchronic osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment based on PFHxA-specific PK information. Some residual uncertainty in the application

Confidence categories	Designation[a]	Discussion
		of the dosimetric approach described above is that V_d and CL were not measured in humans or mice and were considered equivalent to those in monkeys and rats, respectively.
Confidence in developmental subchronic osRfD	*Medium*	The overall confidence in the subchronic osRfD is *medium* and is primarily driven by *medium* confidence in the overall evidence base for developmental effects, *high* confidence in the study, and *medium* confidence in quantitation of the POD. *High* confidence in the study was not interpreted to warrant changing the overall confidence from *medium*.

Selection of Subchronic RfD and Confidence Statement

Organ/system-specific subchronic RfD values for PFHxA selected in the previous section are summarized in Table 5-13.

Table 5-13. Subchronic osRfD values for PFHxA

System	Basis	POD_{HED}	UF_C	osRfD (mg/kg-d)	Confidence
Hepatic	Increased hepatocellular hypertrophy in adult male Crl:CD Sprague-Dawley rats	0.093 mg/kg-d based on $BMDL_{10ER}$ and free salt normalization (Loveless et al., 2009)	100	9×10^{-4}	*Medium*
Hematopoietic	Decreased red blood cells in adult female Crl:CD Sprague-Dawley rats	0.064 mg/kg-d based on $BMDL_{1SD}$ (Chengelis et al., 2009b)	100	6×10^{-4}	*High*
Developmental	Decreased postnatal (PND 0) body weight in F_1 Sprague-Dawley male and female rats, exposed throughout lactation and gestation	0.039 mg/kg-d based on $BMDL_{5RD}$ and free salt normalization (Loveless et al., 2009)	100	4×10^{-4}	*Medium*

From the identified targets of PFHxA toxicity and derived subchronic osRfDs (Table 5-13), an ***RfD of 4 × 10⁻⁴ mg/kg-day based on decreased postnatal body weight*** is selected for less-than-lifetime exposure. Confidence in the RfD is medium, based on medium confidence in the developmental osRfD, as described in Table 5-12. The confidence in the selected RfD is equivalent to that of the hepatic osRfDs but lower than the hematopoietic osRfD. The developmental osRfD is expected to be protective of all life stages, including developmental. The UFc (Table 5-13) is equivalent to the other osRfDs and the endpoint has the lowest POD_{HED} (0.039 mg/kg-day, Table 5-

11). The decision to select the developmental osRfD was based on all of the available osRfDs in addition to overall confidence and composite uncertainty for those osRfDs.

5.2.2. Inhalation Reference Concentration (RfC)

No published studies investigating the inhalation effects of subchronic, chronic, or gestational exposure to PFHxA in humans or animals have been identified. Therefore, an RfC is not derived.

5.3. CANCER TOXICITY VALUES

As discussed in Sections 3.3 and 4.2, given the sparse evidence base and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded that there is ***inadequate information to assess carcinogenic potential*** for PFHxA for any route of exposure. Therefore, consistent with the *Guidelines* and the lack of adequate data on the potential carcinogenicity of PFHxA, quantitative estimates for either oral (oral slope factor, OSF) or inhalation (inhalation unit risk; IUR) exposure were not derived.

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