EPA/635/R-20/131a
Interagency Review Draft
www.epa.gov/iris

Toxicological Review of Perfluorobutanoic Acid and Related Compound Ammonium Perfluorobutanoic Acid

[CASRN 375-22-4
CASRN 10495-86-0]

August 2020

Integrated Risk Information System
Center for Public Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is a preliminary draft for review purposes only. This information is distributed solely for the purpose of predissemination peer review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS

TABLES

FIGURES

ABBREVIATIONS AND ACRONYMS

AIC	Akaike's information criterion
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BUN	blood urea nitrogen
BW	body weight
CA	chromosomal aberration
CASRN	Chemical Abstracts Service registry number
CHO	Chinese hamster ovary (cell line cells)
CL	confidence limit
CL	clearance
CNS	central nervous system
CYP450	cytochrome P450
DAF	dosimetric adjustment factor
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
EPA	Environmental Protection Agency
ER	extra risk
FDA	Food and Drug Administration
FEV_1	forced expiratory volume of 1 second
GD	gestation day
GDH	glutamate dehydrogenase
GGT	γ-glutamyl transferase
GLP	good laboratory practices
GSH	glutathione
GST	glutathione-S-transferase
HBCD	hexabromocyclododecane
Hb/g-A	animal blood:gas partition coefficient
Hb/g-H	human blood:gas partition coefficient
HEC	human equivalent concentration
HED	human equivalent dose
HERO	Health and Environmental Research Online
i.p.	intraperitoneal
IRIS	Integrated Risk Information System
i.v.	intravenous
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LLOQ	lower limit of quantitation
LOAEL	lowest-observed-adverse-effect level
MBq	megabecquerel
MN	micronuclei
MNPCE	micronucleated polychromatic erythrocyte
MOA	mode of action
MTD	maximum tolerated dose
NCEA	National Center for Environmental Assessment
NCI	National Cancer Institute
NOAEL	no-observed-adverse-effect level
NTP	National Toxicology Program
NZW	New Zealand White (rabbit breed)
ORD	Office of Research and Development
P	partition coefficient
PBPK	physiologically based pharmacokinetic
PND	postnatal day
POD	point of departure
POD_{ADJ}	duration-adjusted POD
QSAR	quantitative structure-activity relationship
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RGDR	regional gas dose ratio
RNA	ribonucleic acid
SAR	structure activity relationship
SCE	sister chromatid exchange
SD	standard deviation
SDH	sorbitol dehydrogenase
SE	standard error
SGOT	serum glutamic oxaloacetic transaminase, also known as AST
SGPT	serum glutamic pyruvic transaminase, also known as ALT
TSCATS	Toxic Substances Control Act Test Submissions
TWA	time-weighted average
UF	uncertainty factor
UF_A	animal-to-human uncertainty factor
UF_C	composite uncertainty factor
UF_D	database deficiencies uncertainty factor
UF_H	human variation uncertainty factor
UF_L	LOAEL-to-NOAEL uncertainty factor
UF_S	subchronic-to-chronic uncertainty factor
V_d	volume of distribution
WOS	Web of Science

AUTHORS|CONTRIBUTORS|REVIEWERS

Assessment Managers (Lead Authors)

J. Allen Davis, MSPH	U.S. EPA/Office of Research and Development/Center for Public Health and Environmental Assessment
Michele M. Taylor, PhD	

Authors

Andrew Kraft, PhD (IRIS PFAS Lead)	U.S. EPA/Office of Research and Development/Center for Public Health and Environmental Assessment
Jason C. Lambert, PhD, DABT	
Elizabeth Radke, PhD	
Paul Schlosser, PhD	

Contributors

Michelle Angrish, PhD	U.S. EPA/Office of Research and Development/Center for Public Health and Environmental Assessment
Xabier Arzuaga, PhD	
Johanna Congleton, PhD	
Ingrid Druwe, PhD	
Kelly Garcia, BS	
Andrew Greenhalgh, BS	
Carolyn Gigot, BA	
Mary Gilbert, PhD	
Christopher Lau, PhD	
April Luke, MS	
Pam Noyes, PhD	
Katherine O'Shaughnessy, PhD	
Elizabeth Oesterling Owens, PhD	
Tammy Stoker, PhD	
Andre Weaver, PhD	
Amina Wilkins, MPH	
Michael Wright, ScD	
Jay Zhao, PhD	
Chris Corton, PhD	U.S. EPA/Office of Research and Development/Center for Computational Toxicology and Exposure

Production Team

Maureen Johnson	U.S. EPA
Ryan Jones	Office of Research and Development
Dahnish Shams	Center for Public Health and Environmental Assessment
Vicki Soto	
Samuel Thacker	

Executive Direction

Wayne Casio	CPHEA Center Director
Kay Holt	CPHEA Deputy Center Director
Samantha Jones	CPHEA Associate Director
Emma Lavoie	CPHEA Senior Science Advisor
Kristina Thayer*	CPAD Division Director
James Avery	CPAD Associate Division Director
Andrew Kraft	CPAD Senior Science Advisor, Integrated Risk Information System, IRIS PFAS Team Lead
Belinda Hawkins	CPAD Senior Science Advisor
Paul White*	CPAD Senior Science Advisor
Ravi Subramaniam*	CPHEA/CPAD/Toxic Effects Assessment (DC) Branch Chief
Janice Lee	CPHEA/CPAD/Toxic Effects Assessment (RTP) Branch Chief
Barbara Glenn	CPHEA/CPAD/Science Assessment Methods Branch Chief
Viktor Morozov	CPHEA/CPAD/Quantitative Assessment Branch Chief
Karen Hogan*	CPHEA/CPAD/Emeritus
Alan Stern*	NJDEP (retired), Contractor
	*Executive Review Committee

Internal Reviewers

NAME	Office of the Administrator/Office of Children's Health Protection
NAME	Office of Air and Radiation/Office of Air Quality Planning and Standards
NAME	Office of Air and Radiation/Office of Transportation and Air Quality
NAME	Office of Chemical Safety and Pollution Prevention/Office of Pesticide Programs
NAME	Office of Chemical Safety and Pollution Prevention/Office of Pollution Prevention and Toxics
NAME	Office of Land and Emergency Management
NAME	Office of Land and Emergency Management/Federal Facilities Forum
NAME	Office of Policy
NAME	Office of Water
NAME	Region 1, Boston
NAME	Region 2, New York
NAME	Region 3, Philadelphia
NAME	Region 4, Atlanta
NAME	Region 5, Chicago
NAME	Region 6, Dallas
NAME	Region 7, Kansas City
NAME	Region 8, Denver
NAME	Region 9, San Francisco
NAME	Region 10, Seattle

Reviewers

This assessment was provided for review to scientists in EPA's program and regional offices. Comments were submitted by:

Office of the Administrator/Office of Children's Health Protection
Office of Air and Radiation/Office of Air Quality Planning and Standards
Office of Air and Radiation/Office of Transportation and Air Quality
Office of Chemical Safety and Pollution Prevention/Office of Pesticide Programs
Office of Chemical Safety and Pollution Prevention/Office of Pollution Prevention and Toxics
Office of Land and Emergency Management
Office of Land and Emergency Management/Federal Facilities Forum
Office of Policy
Office of Water
Region 1, Boston
Region 2, New York
Region 3, Philadelphia
Region 4, Atlanta
Region 5, Chicago
Region 6, Dallas
Region 7, Kansas City
Region 8, Denver
Region 9, San Francisco
Region 10, Seattle

This assessment was provided for review to other federal agencies and the Executive Office of the President (EOP). A summary and EPA's disposition of major comments from the other federal agencies and EOP is available on the IRIS website. Comments were submitted by:

Department of Labor
The White House
- Office of Science and Technology Policy
- Office of Management and Budget
- Council on Environmental Quality

Department of Defense
Department of Energy
Department of Commerce
Department of Transportation
Nuclear Regulatory Commission
National Aeronautics and Space Administration
Department of Agriculture
Small Business Administration
Consumer Product Safety Commission
Department of Veteran Affairs
Department of Health and Human Services
- National Library of Medicine
- National Center for Environmental Health
- Food and Drug Administration
- Agency for Toxic Substances and Disease Registry
- National Institutes of Health
- National Institute of Occupational Safety and Health

Department of Homeland Security
Department of Interior

This assessment was released for public comment on [month] [day], [year] and comments were due on [month] [day], [year]. The public comments are available on Regulations.gov. A summary and EPA's disposition of the comments from the public are available in the revised external review draft assessment on the IRIS website. Comments were received from the following entities:

NAME	Affiliation
NAME	Affiliation
NAME	Affiliation
NAME	Affiliation

This assessment was peer reviewed by independent, expert scientists external to EPA convened by [specify]. A peer-review meeting was held on [month] [day], [year]. The report of the review of the EPA's Draft Toxicological Review of [specify chemical], dated [month] [day], [year], is available on the IRIS website. A summary and EPA's disposition of the comments received is included in Appendix [X].

NAME	Affiliation
NAME	Affiliation
NAME	Affiliation
NAME	Affiliation

The postexternal review draft of the assessment was provided for review to scientists in EPA's program and regional offices, and to other federal agencies and the Executive Office of the President (EOP). A summary and EPA's disposition of major comments from the other federal agencies and EOP is available on the IRIS website. Comments were submitted by:

NAME	Affiliation
NAME	Affiliation
NAME	Affiliation
NAME	Affiliation

EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Perfluorobutanoic acid (PFBA, CASRN 375-22-4) and its related salt (ammonium perfluorobutanoic acid [NH_4^+PFBA], CASRN 10495-86-0) are members of the group of per- and polyfluoroalkyl substances (PFAS). Concerns about PFBA 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 man-made 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. PFBA is a breakdown product of other PFAS that are used in stain-resistant fabrics, paper food packaging, and carpets; it was also used for manufacturing photographic film, and it is used as a substitute for longer chain perfluoroalkyl carboxylic acids (PFCAs) in consumer products. PFBA has been found to accumulate in agricultural crops and has been detected in household dust, soils, food products, and surface, ground, and drinking water. As such, exposure is possible via inhalation of indoor or outdoor air, ingestion of drinking water and food, and dermal contact with PFBA-containing products.

Human epidemiology studies have examined possible associations between PFBA exposure and health outcomes such as thyroid hormones or disease, hepatic enzymes, birth outcomes (e.g., birth weight, gestational duration), semen parameters, blood lipids, and blood pressure. The ability to draw conclusions regarding these associations is limited due to the methodological conduct of the studies (studies were generally considered *low* confidence for these outcomes), the small number of studies per health outcome, and the generally null findings coincident with notable sources of study insensitivity due to lack of detecting quantifiable levels of PFBA in blood samples or a narrow concentration ranged across exposure groups. No studies were identified that evaluated the association between PFBA exposure and carcinogenicity.

Animal studies of PFBA exposure have exclusively been through the oral route (i.e., no inhalation or dermal studies were identified during the literature search) and have examined noncancer endpoints only. The available rat and mouse toxicological studies provide ***sufficient evidence*** to support identification of developmental, thyroid, and liver effects as potential human health hazards following repeated PFBA exposures in utero and/or during adulthood.

Liver effects manifested as increased relative liver weight in adult animals and increased incidence of hepatocellular hypertrophy. Thyroid effects in adult exposed rats were expressed through decreases in free and total thyroxine (T4) and increased incidence of thyroid follicular hypertrophy and hyperplasia. Developmental effects in exposed animals were expressed as the loss of viable offspring (total litter resorption), and delays in developmental milestones: eye opening,

vaginal opening, and preputial separation. ***Insufficient evidence*** was available to determine whether reproductive effects might represent a potential human health hazard following PFBA exposure.

The few epidemiologic studies did not inform the potential for effects in the thyroid, liver, reproductive system, or developing offspring.

Table ES-1 below summarizes health effects with enough evidence available to synthesize and draw hazard conclusions as well as the toxicity values derived for those health effects.

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

Health system	Evidence integration judgment	Toxicity value[a]	Value (mg/kg-d)	Confidence	UF_C	Basis
		RfD	1×10^{-3}	*Medium*	1,000	Hepatic and thyroid effects
		Subchronic RfD	5×10^{-3}	*Medium-low*	100	Developmental effects
Hepatic	*Sufficient evidence for hazard*	osRfD	1×10^{-3}	*Medium*	1,000	Increased hepatocellular hypertrophy in adult rats
		Subchronic osRfD	1×10^{-2}	*Medium*	100	Increased hepatocellular hypertrophy in adult rats
Thyroid	*Sufficient evidence for hazard*	osRfD	1×10^{-3}	*Medium-low*	1,000	Decreased total T4 in adult rats
		Subchronic osRfD	1×10^{-2}	*Medium-low*	100	Decreased total T4 in adult rats
Developmental	*Sufficient evidence for hazard*	osRfD	5×10^{-3}	*Medium-low*	100	Developmental delays in mice[b]
		Subchronic osRfD	5×10^{-3}	*Medium-low*	100	Developmental delays in mice[b]
Reproductive	*Insufficient evidence*	osRfD	Not derived	NA	NA	NA
		Subchronic osRfD	Not derived	NA	NA	NA

RfD = reference dose (in mg/kg-day) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-day) for less-than-lifetime exposure; osRfD = organ-specific oral reference dose (in mg/kg-day); UF_C = composite uncertainty factor; NA = not applicable.

[a] All values presented in this table are for the ammonium salt of PFBA, methods to calculate RfDs for the free acid of PFBA are presented in Section 5

[b] POD represents three types of developmental delays observed in the same study.

Chronic Oral Reference Dose (RfD) for Noncancer Effects

From the identified human health hazards of potential concern for adults and developing offspring (liver, thyroid, developmental toxicity), increased liver hypertrophy and decreased T4 in adult male rats, as reported in Butenhoff et al. (2012), were selected as the basis for the oral reference dose (RfD). A benchmark dose lower confidence limit (BMDL) of 5.4 mg/kg-day was identified for increased liver hypertrophy, and a no-observed-adverse-effect level (NOAEL) of 6 mg/kg-day was identified for decreased T4 and used as the points of departure (PODs). The ratio of serum clearance values between rats and humans was used to account for toxicokinetic differences between species, resulting in the human equivalent doses (POD_{HED}) of 1.1 mg/kg-day and 1.3 mg/kg-day for increased liver hypertrophy and decreased T4, respectively. The RfD for PFBA was calculated by dividing the POD_{HED} values by a composite uncertainty factor (UF_C) of 1,000 to account for residual toxicokinetic and toxicodynamic uncertainty in the extrapolation from rats to humans (UF_A), interindividual differences in human susceptibility (UF_H), extrapolation from a subchronic-to-chronic duration (UF_S), and deficiencies in the toxicity database (UF_D). The selected RfD derived based on liver and thyroid effects is 1×10^{-3} mg/kg-day[1].

Confidence in the Oral Reference Dose (RfD)

The overall confidence in the RfD is *medium*. The subchronic toxicity exposure study conducted by Butenhoff et al. (2012) reported administration of NH_4^+PFBA by gavage to Sprague-Dawley (S-D) rats for 90 days. This study is rated as *high* confidence with adequate reporting and appropriate study design, methods and conduct (see study evaluation analysis in Health Assessment Workspace Collaborative [HAWC]). Confidence in the oral toxicity database for derivation of the RfD is *medium* because there are consistent and coherent effects within both individual organ systems used to support the RfD, although important uncertainties remain. Confidence in the quantification of the PODs supporting the RfD is *medium*, given the use of BMD modeling within the observed range of the data for liver effects, use of a NOAEL roughly equivalent with a decrease of 1 standard deviation for thyroid effects (suggesting that this POD may not be substantially more uncertain than a BMD-based POD, although one source of uncertainty impacting confidence is the observation of responses only in the high dose group), and dosimetric adjustments using PFBA-specific toxicokinetic information (see Table 5-8) .

Noncancer Effects Observed Following Inhalation Exposure

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

[1]The RfD for the ammonium salt of PFBA (1.00×10^{-3} mg/kg-day) and the free acid of PFBA (9.26×10^{-4} mg/kg-day) both round to a final value of 9×10^{-4} mg/kg-day.

Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the EPA concluded that there is *inadequate information to assess carcinogenic potential* for PFBA by either oral or inhalation routes of exposure. Therefore, the lack of data on the carcinogenicity of PFBA 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 organ/system-specific RfDs for lifetime exposures in multiple systems (see Table 5-9), less-than-lifetime (subchronic) RfDs were also derived (see Table 5-10). In the case of PFBA, all studies used to calculate the subchronic values were subchronic or gestational in duration. Therefore, the method to calculate the organ/system-specific subchronic RfDs is identical to that used for calculating the organ/system-specific RfDs, except in the application of the UF_S. (e.g., UF_S=1 rather than 10). Thus, the individual organs and systems for which specific subchronic RfD values were derived were the liver, thyroid, and the developing fetus. The value for the developing fetus was selected for the subchronic RfD. A BMDL of 3.8 mg/kg-day for increased time to vaginal opening in neonatal female mice was used as the basis for the POD (as for the RfD, the HED was based on the ratio of serum clearance values between mice and humans). The subchronic RfD for PFBA was calculated by dividing the POD_{HED} of 0.5 mg/kg-day by a composite uncertainty factor of 100 to account for extrapolation from rats to humans (UF_A), for interindividual differences in human susceptibility (UF_H), and deficiencies in the toxicity database (UF_D). The subchronic RfD derived from the effects on delayed time to delayed vaginal opening, as representative of general developmental delays, was 5×10^{-3} mg/kg-day.[2]

[2]The subchronic RfD for the ammonium salt of PFBA (5.00×10^{-3} mg/kg-day) and the free acid of PFBA (4.63×10^{-3} mg/kg-day) both round to a final value of 5×10^{-3} mg/kg-day.

1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS

A series of five PFAS assessments (PFBA, perfluorohexanoic acid [PFHxA], perfluorohexanesulfonate [PFHxS], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts; see December 2018 IRIS Outlook) is being developed by the Integrated Risk Information System (IRIS) Program at the request of the U.S. Environmental Protection Agency (EPA) national programs and regions. Appendix A is the systematic review protocol for these five PFAS assessments. The protocol outlines the scoping and problem formulation efforts relating to these assessments, including a summary of other federal and state reference values for PFBA. The protocol also lays out the systematic review and dose-response methods used to conduct this review (see also Section 1.2). This systematic review protocol was released for public comment in November 2019 and was subsequently updated based on those public comments. Appendix A includes the updated version of the protocol, including a summary of the updates in the protocol history section (see Appendix A.12).

1.1. BACKGROUND INFORMATION ON PERFLUOROBUTANOIC ACID (PFBA)

Section 1.1 provides a brief overview of aspects of the physiochemical properties, human exposure, and environmental fate characteristics of perfluorobutanoic acid (PFBA, CASRN 375-22-4) and its related salt (ammonium perfluorobutanoic acid [NH_4^+PFBA], CASRN 10495-86-0) that may provide useful context for this assessment. This overview is not intended to provide a comprehensive description of the available information on these topics. The reader is encouraged to refer to source materials cited below, more recent publications on these topics, and the assessment systematic review protocol (see Appendix A).

1.1.1. Physical and Chemical Properties

PFBA and its related salt (NH_4^+PFBA) are members of the group of per- and polyfluoroalkyl substances (PFAS). Concerns about PFBA and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment (Sundström et al., 2012). The specific chemical formula of PFBA is $C_4HF_7O_2$ and the chemical formula of NH_4^+PFBA is $C_4H_4F_7NO_2$. More specifically, these PFAS are classified as a perfluoroalkyl carboxylic acids [PFCAs; OECD (2018)]. Because PFBA and NH_4^+PFBA are PFCAs containing less than seven perfluorinated carbon groups, they are considered short-chain PFAS

(ATSDR, 2018a). The chemical structures of PFBA and NH_4^+PFBA are presented in Figure 1-1, and select physicochemical properties are provided in Table 1-1.

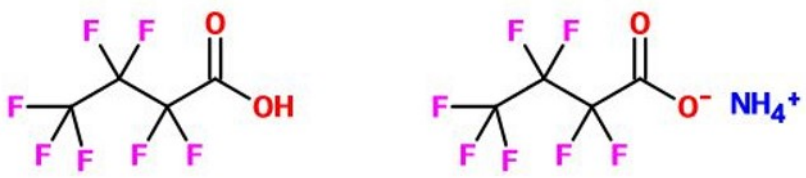


Figure 1-1. Chemical structures of perfluorobutanoic acid (PFBA) and ammonium perfluorobutanoic acid (NH_4^+PFBA).

Table 1-1. Predicted or experimental physicochemical properties of perfluorobutanoic acid (PFBA; CASRN 375-22-4) and ammonium perfluorobutanoic acid (NH_4^+PFBA; CASRN 10495-86-0)

Property (unit)	Value	
	PFBA (free acid)	NH_4^+PFBA
Molecular weight (g/mol)	214[a]	ND
Melting point (°C)	−17.5[a]	ND
Boiling point (°C)	121[a]	ND
Density (g/cm^3)	1.65[a]	ND
Vapor pressure (mm Hg)	6.37[a]	ND
Henry's law constant (atm-m^3/mole)	4.99×10^{-5}[a, b]	ND
Water solubility (mol/L)	2.09×10^{-3}[a]	ND
PKa	0.08[b, c]	ND
Log *P*: Octanol-Water	1.43[a]	ND
Soil adsorption coefficient (L/kg)	47.9[a, b]*	ND
Bioconcentration factor (BCF)	7.61[a]	ND

ND = no data.

[a]U.S. EPA (2018a) Chemicals Dashboard (PFBA DTXSID: 4059916): https://comptox.epa.gov/dashboard/dsstoxdb/results?utf8=%E2%9C%93&search=375-22-4. Median or average experimental values used where available; otherwise median or average predicted values used depending on which was available.
[b]Predicted.
[c]ATSDR (2018a).

1.1.2. Sources, Production, and Use

PFAS are not naturally occurring in the environment (ATSDR, 2018b). They are man-made compounds that are 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. PFBA is a breakdown product of other PFAS that are used in stain-resistant fabrics, paper food packaging, and carpets; it was also used for manufacturing photographic film (MDH, 2017). Shorter-chain PFAS like PFBA are also being used as substitutes for longer chain PFAS in consumer products (Liu et al., 2014). Kotthoff et al. (2015) analyzed a variety of consumer products for PFAS. PFBA was detected in nano- and impregnation-sprays, outdoor textiles, carpet gloves, paper-based food contact materials, ski wax, and leather.

The U.S. Environmental Protection Agency (EPA) has been working with companies in the fluorochemical industry since the early 2000s to phase out the production and use of PFAS [ATSDR (2018a); https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS]. However, the production and use of these chemicals have resulted in their release to the environment through various waste streams (NLM, 2016, 2013). Also, because products containing PFAS are still in use, they may continue to be a source of environmental contamination due to disposal or breakdown in the environment (Kim and Kannan, 2007).

No Chemical Reporting Data (CDR) on production volume for PFBA or its salt are available in EPA's ChemView (U.S. EPA, 2019a). Also, because there are no requirements to report on releases to the environment from facilities manufacturing, processing, or otherwise using PFAS, no quantitative information on PFBA is available in EPA's Toxic Release Inventory [TRI; U.S. EPA (2019a); ATSDR (2018b)].

Wang et al. (2014) estimated global emission estimates of PFBA from direct and indirect (i.e., formation degradation of precursors) sources between 1951 and 2030 to be between 15 and 915 metric tons. The lower estimate assumes that producers cease production and use of long-chain PFCAs and their precursors in line with global transition trends. The higher estimate assumes that the emission scenario in 2015 remains constant until 2030.

1.1.3. Environmental Fate and Transport

PFAS are stable and persistent in the environment (ATSDR, 2018b), and many are found worldwide in the air, soil, groundwater, surface water, and in the tissues of plants and animals (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS).

PFAS that are released to air, exist in the vapor phase in the atmosphere and resist photolysis, but particle-bound concentrations have also been measured (NLM, 2017, 2016, 2013; Kim and Kannan, 2007). Wet and dry deposition are potential removal processes for particle-bound PFAS in air (ATSDR, 2018b).

PFBA would be expected to be mobile in soil based on its soil adsorption coefficient (see Table 1-1). Zhao et al. (2016) observed that shorter chain PFAS like PFBA were transported more readily from the roots to the shoots of wheat plants than longer chain PFAS. Venkatesan and Halden (2014) analyzed archived samples from outdoor mesocosms to investigate the fate over 3 years of PFAS in agricultural soil amended with biosolids. The mean half-life for PFBA in these environmental samples was estimated to be 385 days.

The potential for PFAS to bioconcentrate in aquatic organisms is dependent on their bioconcentration factors (see Table 1-1), with longer chain PFAS accumulating to a greater degree. Thus, the potential for PFBA to bioaccumulate is low compared with other PFAS (bioconcentration factor of 7.61 vs. 789 and 752 for perfluorodecanoic acid [PFDA] and perfluorononanoic acid [PFNA], respectively). PFBA has been found to bioaccumulate in foods grown on PFAS-containing soil. Blaine et al. (2013) conducted a series of greenhouse and field experiments to investigate the potential for PFAS to be taken up by lettuce, tomatoes, and corn when grown in industrially impacted biosolids-amended soil and municipal biosolids-amended soil. PFBA was found to bioaccumulate more readily than other PFAS with bioaccumulation factors of 28.4–56.8 for lettuce, 12.2–18.2 for tomatoes, and 68.4 for corn.

PFBA has not been evaluated under the National Air Toxics Assessment program (https://www.epa.gov/national-air-toxics-assessment). Likewise, although the EPA conducted monitoring for several PFAS in drinking water as part of the third Unregulated Contaminant Monitoring Rule [UCMR; U.S. EPA (2019b)], PFBA was not among the 30 contaminants monitored.

However, PFBA can be detected in most dust samples obtained from U.S. homes and vehicles, and has been measured at higher levels in the soil and sediment surrounding perfluorochemical industrial facilities, at U.S. military facilities, and at training grounds where aqueous film-forming foam (AFFF) has been used for fire suppression (see Appendix A.2.1). PFBA has also been measured in the surface water and groundwater at military installations, AFFF training grounds, and industrial sites; although data are sparse. PFBA levels in water at these sites seem to exceed those identified in drinking water (see Appendix A.2.1).

PFBA can also be detected in food. PFBA has been found in fish at 16% of sites sampled in the U.S. Great Lakes (Stahl et al., 2014) and, while most of the available data are from samples from outside of the United States, PFBA has been detected in grocery items including dairy products, meats and seafood, fruits and vegetables, food packaging, and spices (see Appendix A.2.1).

Specifically regarding drinking water, PFBA concentrations ranged from 0.0855 to 2.04 µg/L in seven municipal wells in Oakdale, MN (U.S. EPA, 2019a). In New Jersey public water systems, only 3% of raw water samples contained PFBA, and did so at concentrations much less than those reported in Minnesota [range from nondetectable to 0.006 µg/L; (Post et al., 2013)]. Heo et al. (2014) detected PFBA in tap water and bottled water in Korea at mean concentrations of 2.02 and 0.039 ng/L, respectively. The concentrations of PFBA measured at National Priorities List (NPL) sites are provided in Table 1-2 (ATSDR, 2018b).

Table 1-2. Perfluorobutanoic acid (PFBA) levels in water, soil, and air at National Priority List (NPL) sites

Media	Value	Number of NPL sites
Water (ppb) Median Geometric mean	 2.15 1.03	3
Soil (ppb) Median Geometric mean	 1,600 1,600	2
Air (ppbv) Median Geometric mean	 ND ND	

ND = No data.
Source: ATSDR (2018b)

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

The general population may be exposed to PFAS via inhalation of indoor or outdoor air, ingestion of drinking water and food, and dermal contact with PFAS-containing products (ATSDR, 2018b; NLM, 2017, 2013). Exposure may also occur via hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018b). However, the oral route of exposure has been considered the most important one among the general population (Klaunig et al., 2015). Contaminated drinking water is likely to be a significant source of exposure. Due to the high water solubility and mobility of PFAS in groundwater (and lack of remediation technology at water treatment facilities), it is possible for populations consuming drinking water from any contaminated watershed to receive PFAS exposure (Sun et al., 2016). Gebbink et al. (2015) modelled exposure to PFBA among the adult general population based on a number of exposure scenarios based on the 5th, median, and 95th percentiles of all input exposure parameters. "Intermediate" exposure (i.e., based on median inputs for all exposure parameters) from direct and indirect (i.e., precursor) sources was estimated to be 19 pg/kg-day. Of the pathways evaluated (i.e., ingestion of dust, food, water; inhalation of air), direct intake of PFBA in water accounted for the largest portion (approximately 90–100%) of total exposure for all three exposure scenarios considered.

Several PFAS have been monitored in the human population as part of the National Health and Nutrition Examination Survey [NHANES; CDC (2019)], but PFBA was not among those measured.

Although PFBA-specific exposure information is sparse, populations that may experience exposures greater than those of the general population may include individuals in occupations that require frequent contact with materials containing PFAS that breakdown into PFBA such as

individuals working with stain-resistant fabrics, paper food packaging, ski wax, and carpets (see Section 1.1.2). For example, Nilsson et al. (2010) observed a significant correlation between the number of years individuals had worked as ski wax technicians and their blood levels of PFBA. Populations living near fluorochemical facilities where environmental contamination to PFAS that can breakdown into PFBA has occurred may also be more highly exposed (ATSDR, 2018b).

1.2. SUMMARY OF ASSESSMENT METHODS

Section 1.2 summarizes the methods used for developing this assessment. A more detailed description of the methods for each step of the assessment development process is provided in the systematic review protocol (see Appendix A). 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 (Table 1-3), are provided in Appendix A.4 and Appendix B, respectively. The results of the current literature search and screening efforts are documented below. Briefly, a literature search was first conducted in 2017 and regular updates are performed (the literature searches will continue to be updated until shortly before release of the document for public comment). The literature search queries the following databases (no date or language restrictions were applied):

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

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

- Review of studies cited in any PFBA PECO-relevant studies and published journal reviews; finalized or draft U.S. state, U.S. federal, and international assessments (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 the attention of EPA. For example, studies submitted to EPA by the manufacturers in support of requirements under the Toxic Substances Control Act (TSCA).

- Identification of studies during screening for other PFAS. For example, epidemiology studies relevant to PFBA were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the Integrated Risk Information System (IRIS) Program.

- Other gray literature (i.e., primary studies not indexed in typical databases, 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 the attention of EPA.

All literature is tracked in the U.S. EPA Health and Environmental Research Online (HERO) database (https://hero.epa.gov/hero/index.cfm/project/page/project_id/2632). The PECO criteria (Table 1-3) identify the evidence that addresses the specific aims of the assessment and to focus the literature screening, including study inclusion/exclusion.

Table 1-3. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	Human: Any population and lifestage (occupational or general population, including children and other sensitive populations). The following study designs will be included: controlled exposure, cohort, case control, and cross-sectional. (Note: Case reports and case series will be tracked as potential supplemental material.) Animal: Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Other: In vitro, in silico, or nonmammalian models of genotoxicity. (Note: Other in vitro, in silico, or nonmammalian models will be tracked as potential supplemental material.)
Exposures	Human: Studies providing quantitative estimates of PFBA exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational-setting measures (e.g., water levels or air concentrations, residential location and/or duration, job title, or work title). (Note: Studies that provide qualitative, but not quantitative, estimates of exposure will be tracked as supplemental material.) Animal: Oral or inhalation studies including quantified exposure to PFBA based on administered dose, dietary level, or concentration. (Note: Nonoral and noninhalation studies will be tracked as potential supplemental material.) PFBA mixture studies are included if they employ an experimental arm that involves exposure to a single PFBA.. (Note: Other PFBA mixture studies are tracked as potential supplemental material.) Studies must address exposure to following: PFBA (CASRN 375-22-4), or PFBA ammonium salt (CASRN 10495-86-0). [Note: Although PFBAs are not metabolized or transformed in the body, there are precursor compounds known to be bio-transformed to a PFAS of interest; e.g., 6:2 fluorotelomer alcohol is metabolized to PFHxA and PFBA (Russell et al., 2015). Thus, studies of precursor PFAS that identify and quantify PFBA will be tracked as potential supplemental material (e.g., for ADME analyses or interpretations).]
Comparators	Human: A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. Animal: Includes comparisons to historical controls or a concurrent control group that is unexposed, exposed to vehicle-only or air-only exposures. (Note: Experiments including exposure to PFBA across different durations or exposure levels without including one of these control groups will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)
Outcomes	All cancer and noncancer health outcomes. (Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing towards toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)

In addition to those studies meeting the PECO criteria and studies excluded as not relevant to the assessment, 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 (see Appendix A.2.4) and other potential 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 populations (e.g., nonmammalian models)
- In vitro and in silico models
- Absorption, distribution, metabolism, and excretion (ADME) and toxicokinetic studies (excluding models)[3]
- 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; https://distillercer.com/products/distillersr-systematic-review-software/). Literature inventories for PECO-relevant studies 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.

1.2.2. Evaluation of Individual Studies

The detailed approaches used for the evaluation of epidemiologic and animal toxicological studies used in the PFBA assessment are provided in the systematic review protocol (see Appendix A.6). The general approach for evaluating PECO-relevant health effect studies is the same for epidemiology 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 for the review of epidemiology and animal toxicological studies are potential bias (systematic errors or deviations from the truth related to internal validity that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the

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

ability of a study to detect a true effect; low sensitivity is a bias towards the null when an effect exists). In evaluating individual studies, two or more reviewers independently arrived at judgments regarding 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 Collaboration (HAWC). To develop these judgments, each reviewer assigned a category 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.6 for definitions) related to each evaluation domain representing the different characteristics of the study methods that were evaluated based on the criteria outlined in HAWC.

Once all evaluation domains were evaluated, the identified strengths and limitations were collectively considered 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 to be of a notable degree or to have a notable 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 than *high* or *medium* confidence results during evidence synthesis and integration (see Sections 1.2.4 and 1.2.5).
- *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 (see 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. Briefly, data extraction and content management was carried out using HAWC. Data extraction elements that were collected from epidemiological, controlled human exposure, animal toxicological, and in vitro

studies are described in HAWC (https://hawcprd.epa.gov/about/). Not all studies that meet the PECO criteria went through data extraction: studies evaluated as being *uninformative* were not considered further and therefore did not undergo data extraction, and outcomes determined to be less relevant during PECO refinement did not go through data extraction. The same was true for *low* confidence studies when *medium* and *high* confidence studies (e.g., on an outcome) were available. All findings are considered for extraction, regardless of the statistical significance of their findings. The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). For quality control, data extraction was performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction were resolved by discussion or consultation within 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). As described below in Section 2, 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 providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from Hill (1965). Mechanistic evidence is also synthesized as necessary to help inform key decisions regarding the human and animal evidence.

The syntheses of the human and animal health effects-evidence focuses on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies. The evidence synthesis is based primarily on studies of *high* and *medium* confidence. *Low* confidence studies may 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, then a careful examination of the study evaluation and sensitivity with potential effects on the evidence synthesis conclusions will be included in the narrative. When possible, results across studies are compared using graphs and charts or other data visualization strategies. The synthesis of mechanistic information informs the integration of health effects evidence for both hazard identification (e.g., biological plausibility or coherence 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 dose-response evaluation (e.g., selection of benchmark response levels, selection of uncertainty factors). Evaluations of mechanistic information typically differ from evaluations of phenotypic evidence (e.g., from routine toxicological studies). This is primarily because mechanistic data evaluations consider the support for and involvement of specific events or sets of events within the context of a

broader research question (e.g., support for a hypothesized mode of action; consistency with known biological processes), rather than evaluations of individual apical endpoints considered in relative isolation.

Following the synthesis of human and animal health effects data, and mechanistic data, integrated judgments are drawn across all lines of evidence for each assessed health effect. 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 are summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Sir Bradford Hill (Hill, 1965). This process is similar to that used by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Morgan et al., 2016; Guyatt et al., 2011; Schünemann et al., 2011), which arrives at an overall integration conclusion based on consideration of the body of evidence. 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 is 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 is sufficient (or insufficient) to indicate that PFAS exposure has the potential to be hazardous to humans.

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

1.2.5. Dose-Response Analysis

The details for the dose-response employed in this assessment can be found in Appendix A.11. Briefly, a dose-response assessment was performed for noncancer health hazards, following exposure to PFBA via the oral route, as supported by existing data. For oral noncancer hazards, oral reference doses (RfDs) are derived when possible. 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 to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002). The derivation of reference value, like the RfD,

depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer outcomes, a dose-response assessments was conducted when the evidence integration judgments indicate there is "sufficient evidence for hazard," with preference given to health effects with stronger evidence scenarios within that category and quantitative analyses were not attempted for "insufficient evidence."

Consistent with EPA practice, the PFBA 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, 2012a, 2005):

- Within the observed dose range, the preferred approach was to use dose-response modeling to incorporate as much of the data set as possible into the analysis. This modeling to derive a point of departure (POD) ideally includes an exposure level near the lower end of the range of observation, without significant extrapolation to lower exposure levels.

- As derivation of cancer risk estimates and reference values nearly always involves extrapolation to exposures lower than the POD; the approaches to be applied in these assessments are described in more detail in Section A.11.2.

When sufficient and appropriate human and laboratory animal data are available for the same outcome, human data are generally preferred for the dose-response assessment because use of human data eliminates the need to perform interspecies extrapolations. For reference values, this assessment will derive a candidate value from each suitable data set. Evaluation of these candidate values will yield a single organ/system-specific value for each organ/system under consideration from which a single overall reference value will be selected to cover all health outcomes across all organs/systems. While this overall reference value represents the focus of these dose-response assessments, the organ/system-specific values can be useful for subsequent cumulative risk assessments that consider the combined effect of multiple PFAS (or other agents) acting at a common organ/system. For noncancer toxicity values, uncertainties in these estimates are characterized and discussed.

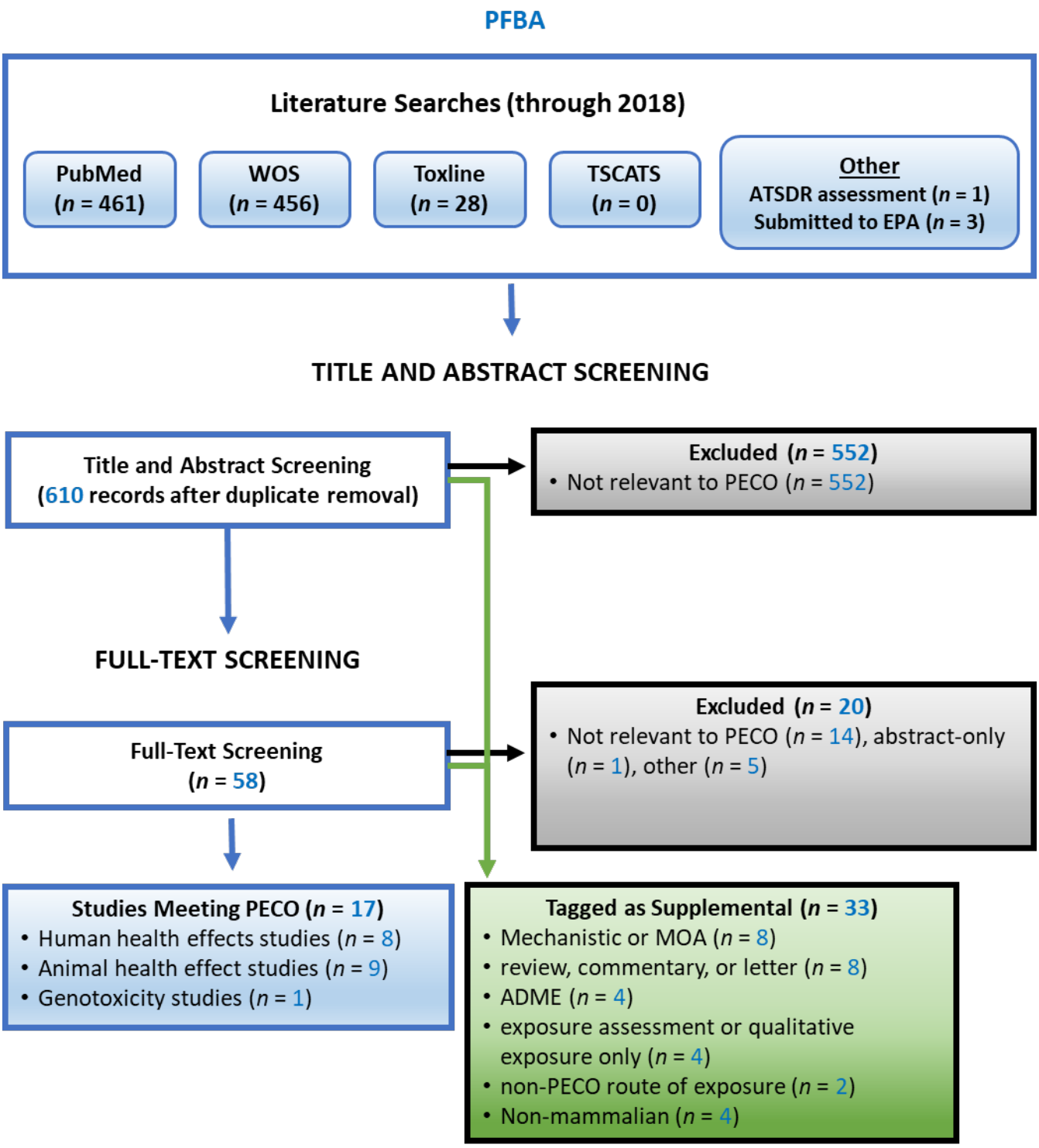
For dose-response purposes, EPA has developed a standard set of models (http://www.epa.gov/ncea/bmds) that can be applied to typical data sets, including those that are nonlinear. In situations where there are alternative models with significant biological support (e.g., toxicodynamic models), those models are included as alternatives in the assessment(s) along with a discussion of the models' strengths and uncertainties. EPA has developed 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)]. Additional judgment or alternative analyses are used if the procedure fails to yield reliable results; for

example, if the fit is poor, modeling may be restricted to the lower doses, especially if there is competing toxicity at higher doses. For each modeled response, a POD from the observed data was estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses. The POD is used as the starting point for subsequent extrapolations and analyses. For noncancer effects, the POD is used in calculating the RfD.

2. LITERATURE SEARCH AND STUDY EVALUATION RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 610 unique records, with 4 records identified from additional sources, such as Toxic Substances Control Act (TSCA) submissions, posted National Toxicology Program (NTP) study tables, and review of reference lists from other authoritative sources (ATSDR, 2018b) (see Figure 2-1). Of the 610 identified, 552 were excluded during title and abstract screening, and 58 were reviewed at the full-text level. Of the 58 screened at the full-text level, 17 were considered to meet the populations, exposures, comparators, and outcomes (PECO) eligibility criteria (see Table 8, Appendix A). The studies meeting PECO at the full-text level included six epidemiologic studies, nine animal studies, and one in vivo genotoxicity study. No high-throughput screening data on perfluorobutanoic acid (PFBA) are currently available from ToxCast or Tox21.



1

Figure 2-1. Literature search and screening flow diagram for perfluorobutanoic acid (PFBA).

2.2. STUDY EVALUATION RESULTS

Human and animal studies have evaluated potential effects to the thyroid, reproductive systems, developing fetus, liver, urinary, and other organ systems (e.g., hematological) following exposure to PFBA. The evidence base for these outcomes are presented in Sections 3.2.1–3.2.5.

The database of all repeated-dose oral toxicity studies for PFBA and the related compound ammonium perfluorobutanoic acid (NH_4^+PFBA) that are potentially relevant for deriving oral reference dose (RfD) values includes four short-term studies in rats and mice (Permadi et al., 1993; Permadi et al., 1992; Just et al., 1989; Ikeda et al., 1985), two 28-day studies in rats and mice (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a), one subchronic-duration study in rats (Butenhoff et al., 2012; van Otterdijk, 2007b), and one gestational exposure study in mice (Das et al., 2008). In addition, eight epidemiologic studies were identified that report on the association between PFBA and human health effects (Nian et al., 2019; Wang et al., 2019; Song et al., 2018; Bao et al., 2017; Li et al., 2017a; Li et al., 2017b; Kim et al., 2016; Fu et al., 2014). The available animal studies were generally well conducted and rigorous (i.e., *medium* or *high* confidence; see Figure 2-2); thus, specific study limitations identified during evaluation are primarily discussed for studies interpreted as *low* confidence, or when a limitation affects a specific inference for drawing conclusions (e.g., in relation to a specific assessed endpoint within the health effects synthesis sections below). No animal studies were considered *uninformative*. Thus, all animal studies meeting PECO criteria during literature screening are included in the evidence synthesis and dose-response analysis.

The study evaluations of the available epidemiology studies are summarized in Figure 2-3, and rationales for each domain and overall confidence rating are available in Health Assessment Workspace Collaborative (HAWC; see link in Figure 2-3). Based on the study evaluations, two human epidemiology studies were considered uninformative due to critical deficiencies in exposure measurement (Li et al., 2017b) and in participant selection, exposure measurement, and controlling for potential confounders (Kim et al., 2016); these studies are not discussed further in this assessment.

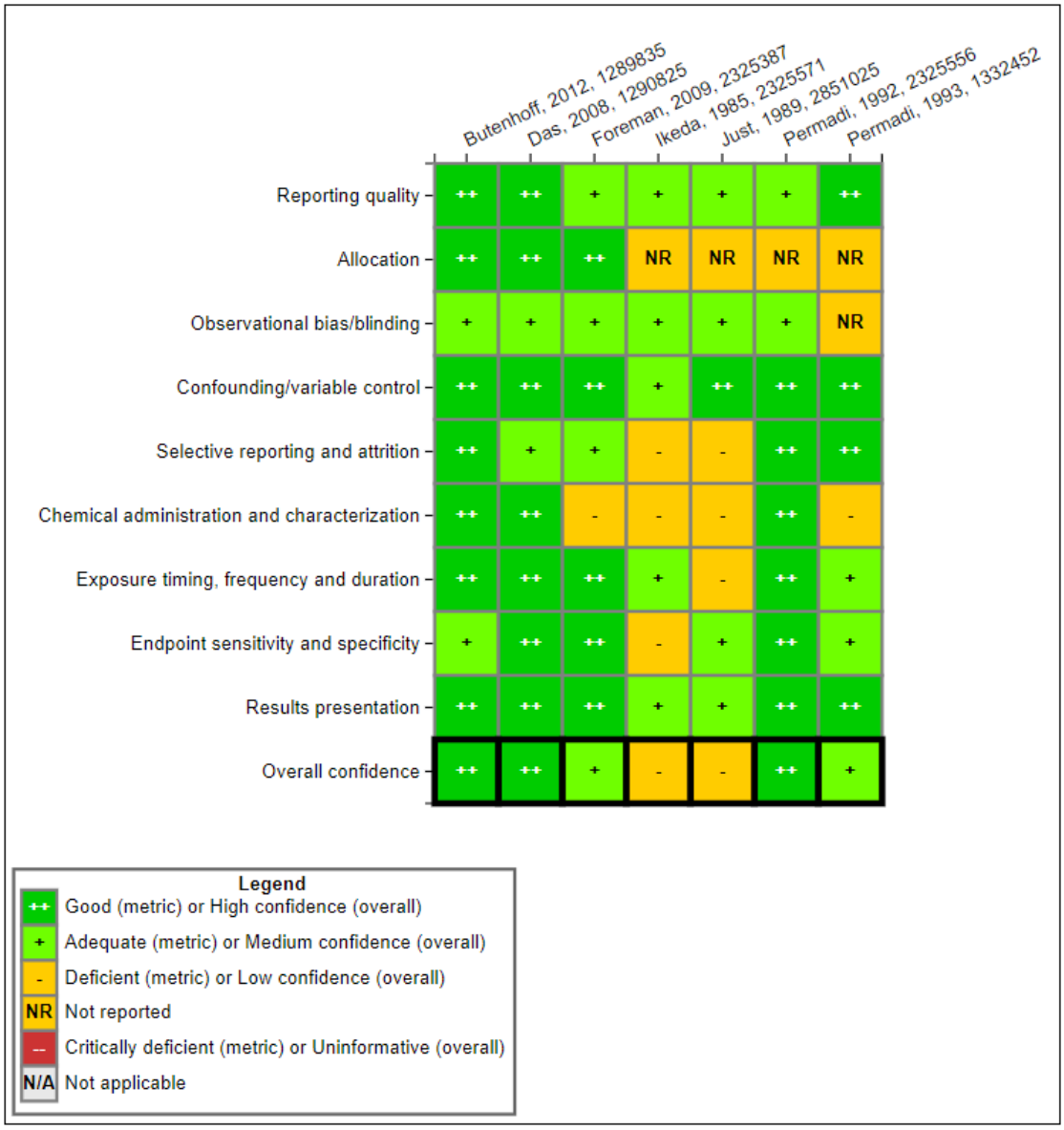


Figure 2-2. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA) exposure (see interactive data graphic for rating rationales).

The following health outcome categories were investigated by the studies listed in Figure 2-2: thyroid effects (Butenhoff et al., 2012), liver effects (Butenhoff et al., 2012; Foreman et al., 2009; Das et al., 2008; Permadi et al., 1993; Permadi et al., 1992; Just et al., 1989; Ikeda et al., 1985), developmental effects (Das et al., 2008), and reproductive effects (Butenhoff et al., 2012).

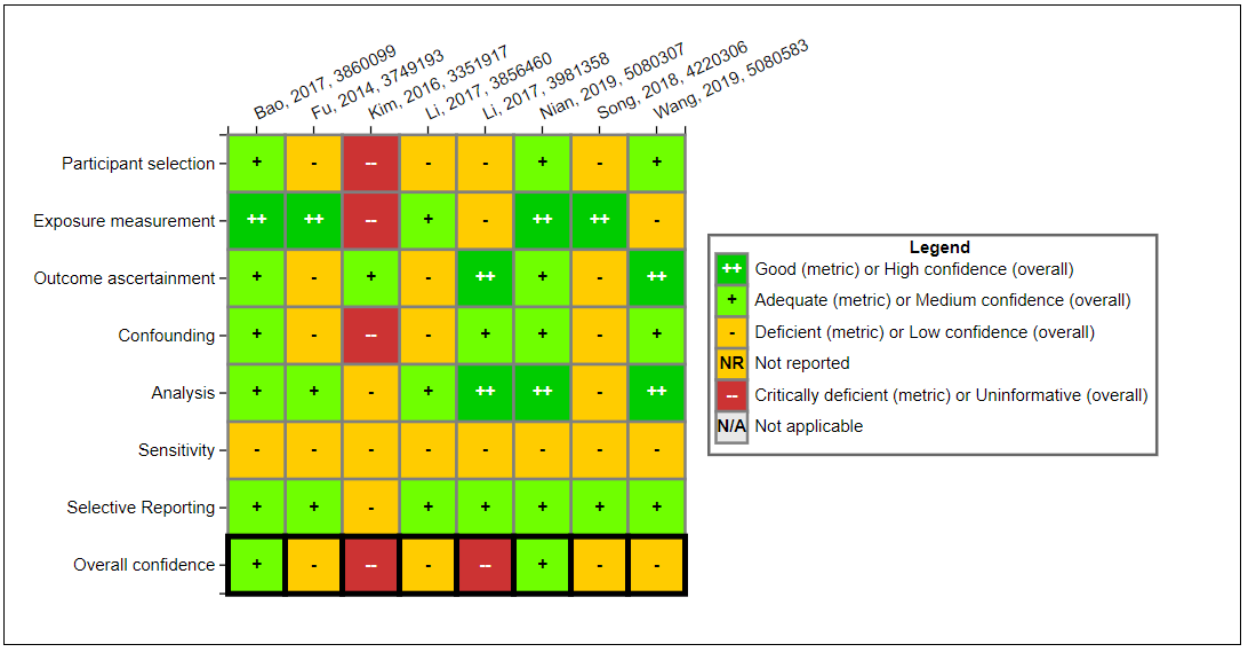


Figure 2-3. Evaluation results for epidemiological studies assessing effects of perfluorobutanoic acid (PFBA; interactive data graphic for rating rationales).

The following health outcome categories were investigated by the studies listed in Figure 2-3: thyroid effects (Li et al., 2017b; Kim et al., 2016), liver effects (Nian et al., 2019), developmental effects (Li et al., 2017a), reproductive effects (Song et al., 2018), blood lipids (Fu et al., 2014), hypertension/blood pressure (Bao et al., 2017), and renal function (Wang et al., 2019).

3. TOXICOKINETICS, EVIDENCE SYNTHESIS, AND EVIDENCE INTEGRATION

3.1. TOXICOKINETICS

Animal evidence has shown that perfluorobutanoic acid (PFBA), like other perfluorinated chemicals, is well absorbed following oral administration and distributes to all tissues of the body (Burkemper et al., 2017). However, a study evaluating the volume of distribution concluded that distribution is predominantly extracellular (Chang et al., 2008). Because of its chemical resistance to metabolic degradation, PFBA appears to be primarily eliminated unchanged in urine and feces.

Toxicokinetic studies of PFBA in rats, mice, and monkeys have been performed, providing information on the absorption, distribution, metabolism, and excretion (ADME) of PFBA (Burkemper et al., 2017; Chang et al., 2008). Also, Russell et al. (2015) evaluated the metabolism of 6:2 fluorotelomer alcohol (6:2 FTOH) in mouse, rat, and human hepatocytes, showing that PFBA is a metabolite of 6:2 FTOH, and evaluated PFBA toxicokinetics (TK) after inhalation and oral exposure of rats to 6:2 FTOH. The distribution of PFBA in human tissues has also been investigated (Pérez et al., 2013). Information on the absorption and distribution of PFBA to the serum and liver specifically has also been investigated in a number of toxicological studies (Gomis et al., 2018; Butenhoff et al., 2012; Foreman et al., 2009; Das et al., 2008).

3.1.1. Absorption

Chang et al. (2008) conducted a set of toxicokinetic experiments in which Sprague-Dawley (S-D) rats (3 male and 3 female) were given either a single intravenous (i.v.) or oral dose (30 mg/kg body weight) of ammonium perfluorobutanoic acid (NH_4^+PFBA). The serum area-under-the-concentration-curve (AUC) was 1,090 ± 78 and 239 ± 5 (μg-h/mL) in male and female rats, respectively, after i.v. dosing and 1,911 ± 114 and 443 ± 42 in males and females, respectively, after oral dosing. That the AUC after oral dosing was almost two times higher than after i.v. dosing is theoretically impossible but may be a statistical result from the small sample size (n = 3/group) or due to a problem in dosing. But the result indicates 100% oral absorption.

In other experiments, Chang et al. (2008) administered 3–300 mg/kg oral doses to male and female S-D rats. As expected, the concentration of PFBA in the serum increased with dose in a fairly linear fashion up to 100 mg/kg PFBA; however, the serum concentration of PFBA in rats dosed orally to 300 mg was approximately 60% the concentration at 100 mg/kg. Maximum concentration (C_{max}) values were similar in males and females following oral exposures to 30 mg/kg PFBA (131 ± 5 and 136 ± 12 μg/mL, respectively), but the time to peak concentration (T_{max}) differed

between sexes: 1.25 ± 0.12 hours for males and 0.63 ± 0.23 hours for females. Both values, however, indicate that absorption to the serum was fairly rapid in rats.

C_{max} values for male and female mice were also similar at lower doses (10 mg/kg; 50.50 ± 5.81 and 52.86 ± 2.08 µg/mL), but differed at 30 mg/kg (119.46 ± 13.86 and 151.20 ± 6.92 µg/mL) and 100 mg/kg (278.08 ± 20.38 and 187.97 ± 15.90 µg/mL). C_{max} and T_{max} values for rats and mice at 30 mg/kg appear to be similar; however, the T_{max} was higher in female mice than in male mice (the opposite relationship compared to rats).

3.1.2. Distribution

Burkemper et al. (2017) investigated the distribution of PFBA in male CD-1 mice (*n* = 4) given a single i.v. dose of radiolabeled [^{18}F]-PFBA (~0.074 MBq/µL). At 4 hours postinjection, the [^{18}F]-PFBA was detected in every tissue investigated, with the majority of the dose found in the stomach (~7.5% injected dose/g). Concentrations in the blood, lung, liver, kidney, intestines, and skin were all similar (~2-3%). Compared with perfluorooctanoic acid (PFOA) and perfluorohexanoic acid (PFHxA), the concentration of PFBA was much lower in the liver (~27 and ~20%, respectively). Chang et al. (2008) estimated volumes of distribution (V_d, mL/kg) for NH_4^+PFBA in male and female rats (209 ± 10 and 173 ± 21 at 30 mg/kg orally), mice (152 and 107 at 10 mg/kg orally; 296 and 134 at 30 mg/kg orally), and cynomolgus monkeys (526 ± 68 and 443 ± 59 at 10 mg/kg i.v.) (N = 3 animals/sex/dose group for all species); these values indicate that NH_4^+PFBA is primarily distributed in the extracellular space.

Distribution in rats and mice was also examined in multiple toxicological studies of PFBA (see Table 3-1). Although limited in scope (i.e., PFBA was only measured in the liver and blood serum), these studies demonstrated consistently that PFBA does distribute to the liver compartment in both species. Butenhoff et al. (2012) observed that liver concentrations of PFBA (µg/g) were higher in male and female S-D rats exposed to PFBA for 28 days versus rats exposed for 90 days. The ratio between liver concentrations (µg/g) and serum concentrations (µg/mL) ranged from 26%–47% in the 28-day rats and 16%–31% in the 90-day rats. In both exposure groups, the concentration of PFBA in the serum or liver was drastically reduced following a 3-week recovery period. Das et al. (2008) investigated the distribution of PFBA to the liver in both pregnant and nonpregnant rats as well as in Postnatal Day (PND) 1 and PND 10 pups. Serum levels of PFBA did not seem to differ drastically between pregnant and nonpregnant rats, but the low dose (35 or 175 mg/kg) liver concentrations in pregnant animals were approximately two to three times that of nonpregnant animals; this difference was attenuated in high-dose (350-mg/kg) animals. As would be expected, both the serum and liver concentrations in PND 1 pups were much greater than those in PND 10 pups. Das et al. (2008) corroborated the observations by Butenhoff et al. (2012) and Chang et al. (2008) that serum PFBA concentrations are higher than liver concentrations. The ratios of liver to serum PFBA concentration observed in Chang et al. (2008) were 22%–27% in male rats and 20%–23% and 15%–17% in male and female mice, respectively. These differences in liver/serum concentrations were also observed in various genetic strains of mice exposed to

35–350 mg/kg PFBA: 38%–73% in wild-type mice, 13%–35% in peroxisome proliferator-activated receptor alpha (PPARα) null mice, and 20%–33% in humanized PPARα mice (Foreman et al., 2009).

Table 3-1. Serum and Liver concentrations of perfluorobutanoic acid (PFBA) following subchronic or gestational exposure

Dose group (mg/kg-d)	Serum (µg/mL)	Liver (µg/g)	Serum (µg/mL)	Liver (µg/g)
	Pregnant dams (Das et al., 2008)		Nonpregnant female mice (Das et al., 2008)	
0	0.002 ± 0.001	0.003 ± 0.002	0.006 ± 0.003	0.038 ± 0.017
35	3.78 ± 1.01	1.41 ± 0.42	1.96 ± 1.0	0.51 ± 0.20
175	4.44 ± 0.65	1.60 ± 0.25	2.41 ± 1.65	0.86 ± 0.55
350	2.49 ± 0.60	0.96 ± 0.18	2.67 ± 1.2	0.89 ± 0.38
	PD1 male and female neonates (Das et al., 2008)		PD10 male and female neonates (Das et al., 2008)	
0	Not detected	0.004 ± 0.001	0.002 ± 0.002	0.003 ± 0.001
35	0.56 ± 0.15	0.22 ± 0.05	0.11 ± 0.03	0.04 ± 0.01
175	0.61 ± 0.39	0.29 ± 0.14	0.14 ± 0.07	0.04 ± 0.02
350	0.37 ± 0.14	0.24 ± 0.08	0.12 ± 0.05	0.04 ± 0.02
	28-d male rats (Butenhoff et al., 2012)		90-d male rats (Butenhoff et al., 2012)	
0	0.04 ± 0.05	<0.05	<0.01	<0.05
1.2	--	--	6.10 ± 5.22	1.34 ± 1.24
6	24.65 ± 17.63	7.49 ± 4.46	13.63 ± 9.12	3.07 ± 2.03
30	38.04 ± 23.15	17.42 ± 8.15	52.22 ± 24.89	16.09 ± 9.06
150	82.20 ± 31.83	37.44 ± 18.12	--	--
	28-d female rats (Butenhoff et al., 2012)		90-d female rats (Butenhoff et al., 2012)	
0	0.01 ± 0.01	0.05 ± 0.03	0.07 ± 0.06	<0.05
1.2	--	--	0.23 ± 0.14	0.05 ± 0.02
6	0.34 ± 0.13	0.16 ± 0.04	0.92 ± 0.52	0.15 ± 0.08
30	1.72 ± 0.88	0.434 ± 0.174	5.15 ± 3.29	0.91 ± 0.55
150	10.30 ± 4.50	2.70 ± 1.47	--	--

Pérez et al. (2013) investigated the distribution of PFBA in multiple tissues in cadavers in Tarragona County, Spain. PFBA was detected in liver, brain, lung, and kidney samples, but was below the level of detection in bone. Lung and kidney samples by far had higher PFBA concentrations (304 and 464 ng/g, respectively) than brain or liver samples (14 and 13 ng/g, respectively). For both the lungs and kidneys, PFBA was detected in greater quantities than any of the other 20 per- and polyfluoroalkyl substances (PFAS) compounds analyzed. The observation

that PFBA was observed in the greatest quantities in kidney samples may be related to kidney reabsorption. Chang et al. (2008) observed that rats given 300 mg/kg PFBA orally excreted significantly greater amounts of PFBA in the urine than did rats given 100 mg/kg (90.16% vs. 50.99%) and the authors suggested this as evidence of saturation of a renal tubular reabsorption process.

3.1.3. Metabolism

PFBA has been shown to be a product of the metabolism of 6:2 FTOH in mice, rats, and humans (Russell et al., 2015; Ruan et al., 2014). However, there is no evidence of biotransformation of PFBA. It is expected that PFBA, a short-chain (C4) of perfluoroalkyl acids (PFAAs), is metabolically inert because of chemical stability the same as longer chain PFAA chemicals, including perfluorohexanesulfonate (PFHxS, C6), perfluorooctane sulfonate (PFOS, C8), and PFOA, C8.

3.1.4. Excretion

In an overview of the toxicology of perfluorinated compounds, Lau (2015) briefly summarized the excretion half-lives of seven compounds, including PFBA. All supporting data for that review pertinent to PFBA are included in this analysis.

Chang et al. (2008) investigated the excretion of PFBA in S-D rats, CD-1 mice, cynomolgus monkeys, and workers occupationally exposed to PFBA or compounds metabolized to PFBA. In S-D rats exposed orally to 30 mg/kg PFBA, there was a marked difference in the serum PFBA excretion constants (λ) between males and females, 0.075/hour and 0.393/hour, respectively, for oral exposure and 0.109/hour and 0.673/hour, respectively, for intraveneous exposure (See Appendix C for a complete discussion on whether the calculated elimination constants in various species are mono- or biphasic in nature). The difference in oral λ resulted in half-lives ($t_{1/2}$) of 9.22 and 1.76 hours for males and females.

Russell et al. (2015) attempted to evaluate the excretion of PFBA, formed as a metabolite of 6:2 FTOH, after inhalation exposures in rats (strain not stated). In single-day studies, the animals were exposed by inhalation for 6 hours and their blood levels monitored until 24 hours. However, there was a negligible decline in PFBA blood concentration after 0.5 and 5 ppm 6:2 FTOH exposures in male rats and after 0.5 ppm exposure in female rats, precluding estimation of half-life. An excretion half-life of 19 hours was estimated from the 5-ppm single-day data for 5 ppm in female rats. After a 23-day inhalation exposure to male rats, use of a TK model resulted in estimation of a 27.7-hour half-life for that sex, which may explain the inability to estimate a half-life from the single-day exposures. However, both estimates depend on the estimated yield (percent of 6:2 FTOH metabolized to PFBA), which was 0.2% for male rats and 0.02% for female rats. Given the low yields, small errors in the estimate of that parameter could result in significant errors in the estimated half-life. Hence, the results of Chang et al. (2008) will be used to represent the excretion in rats.

In male CD-1 mice, the $t_{1/2}$ was higher in mice exposed to 30 mg/kg PFBA (16.25 ± 7.19 hours) than in mice exposed to 10 mg/kg (13.34 ± 4.55 hours); however, the $t_{1/2}$ in male mice exposed to 100 mg/kg was lower than both (5.22 ± 2.27 hours). This possibly indicates that the simple one-compartment model used to describe the kinetic data was not sufficient. There was no appearance of dose-dependence on $t_{1/2}$ in female mice, with values ranging from 2.87 ± 0.30 hours at 10 mg/kg to 2.79 ± 0.30 hours at 100 mg/kg.

Cynomolgus monkeys (N = 3/sex) displayed a clear biphasic excretion pattern, with a rapid decline in the initial (α) phase and a slower decline in the second (β) phase. Notably, the β phase began at around 24 hours and was observed because samples were also taken at 2, 4, 7, and 10 days, while in rodents, samples were only collected to 24 hours. Thus, it is possible that a β phase would have been observed in mice and rats had serum sampling been continued for a longer duration. Serum excretion half-lives for the α and β phases in male monkeys exposed to 10 mg/kg PFBA via i.v. injection were 1.61 ± 0.06 hours and 40.32 ± 2.36 hours, respectively; $t_{1/2}$ in female monkeys were 2.28 ± 0.14 hours and 41.04 ± 4.71 hours, respectively.

Excretion of PFBA from the serum in humans was also investigated by Chang et al. (2008). In the initial occupational study, male workers (n = 3) exposed to either PFBA or related fluorinated compounds had their baseline PFBA serum concentration determined. Following the voluntary removal from the workplace, workers had blood samples taken over a period of 8 days to estimate half-lives of excretion. Given the small sample size of the initial occupational study, a second study was conducted in which seven male and two female workers had blood samples taken immediately before a vacation and upon returning to the production facility (minimum elapsed time was 7 days). For the male workers in the initial study, $t_{1/2}$ of excretion from the serum ranged from 28.6 to 109.7 hours (1.2 to 4.6 days). For the 9 workers in the second study, the $t_{1/2}$ ranged from 44 to 152 hours (1.9 to 6.3 days), with an average value of 72 hours (95% confidence interval [CI]: 1.8–4.2 days). While quantitative data was not provided, the study authors report that serum excretion half-lives did not differ between male and female test subjects. Therefore, it appears there are strong sex differences in serum excretion in rodent species, but the data in cynomolgus monkeys and humans do not indicate such a difference.

Using the default method of $BW^{0.75}$ scaling and standard species BWs of 0.25 kg in rats and 70 kg in humans, the half-life in humans is predicted to be 4.1 times greater than rats. Given half-lives of 9.22 and 1.76 h in male and female rats, one would then predict half-lives of 37.8 h in men and 7.2 h in women. While the value for men based on the $BW^{0.75}$ scaling approach is within a factor of 2 of the value determined by Chang et al. (2008), BW0.75 scaling it is not understood to be based on data for this class of chemicals (i.e., with serum binding and clearance mechanisms known to occur for PFAS) and would lead to a lower prediction of human health risk at a given exposure. Further, while only two women participated in the Chang et al. (2008) study, that the observed elimination for them was 8 and 16 times slower than predicted by BW0.75 is an unlikely occurrence, even given the small sample size, and use of BW0.75 scaling (applied to the half-life in

female rats) could under-predict the risk of exposure by an order of magnitude. Therefore, use of BW0.75 as an alternative means of extrapolation is not considered further here.

Excretion in the urine appears to be the major route by which PFBA is excreted from the body. In both rats and mice, females are observed to have higher percentages of the dose excreted in urine at 24 hours (100.68%–112.37% and 65.44%–67.98%, respectively) compared with males (50.99%–90.16% and 34.58%–35.16%, respectively). This is consistent with evidence that organic anion transporters expressed in the kidneys of rodents reabsorb PFAS (Yang et al., 2009; Weaver et al., 2010) and are more highly expressed in male rodents (Kato et al., 2002; Cerrutti et al., 2002; Buist et al., 2002; Ljubojevic et al., 2007, 2004). However, both Yang et al. (2009) and Weaver et al. (2010) observe that PFBA is not an active substrate of OAT1, OAT2, or OATP1a1. Therefore, while the observed sex-difference in urinary excretion of PFBA is consistent with the literature for reabsorption of PFAS in general in the kidney in male rodents, the mechanism for this reabsorption for PFBA specifically is not currently know. Sex-differences in urinary excretion rates are not observed in primates, with both female and male cynomolgus monkeys having rates similar to those of male mice (36.2% and 41.69%, respectively) Chang et al. (2008). The excretion of PFBA in feces in rats and mice was very low compared with the excretion in urine, but higher in mice than in rats (4.10%–10.92% and 0.16%–2.99%, respectively).

3.1.5. Summary

Collectively, while the PFBA excretion half-lives for male and female rats appear to be shorter than for male and female mice, respectively, data suggest a strong sex-specific toxicokinetic difference for both species (i.e., females appearing to have a much faster excretion rate than males). Humans have a longer serum excretion half-life (~days) than rodents (~hours), while the half-lives in monkeys are comparable to both (α-phase: ~hours, β-phase: ~days). See Table 3-2 for a summary of PFBA toxicokinetics.

Table 3-2. Summary of toxicokinetics of serum perfluorobutanoic acid (PFBA) (mean ± standard error)

Species/ sex	Study design	Excretion half-life (h)	AUC (µg-h/mL)	Clearance (mL/h)	Volume of distribution (mL/kg)	Reference
Rats						
Male	30 mg/kg i.v. dose	6.38 ± 0.53	1,090 ± 78	7.98 ± 0.57	253 ± 6	Chang et al. (2008)
	30 mg/kg oral dose	9.22 ± 0.75	1,911 ± 114	4.63 ± 0.28	209 ± 10	
Female	30 mg/kg i.v. dose	1.03 ± 0.03	239 ± 5	27.65 ± 0.55	187 ± 3	
	30 mg/kg oral dose	1.76 ± 0.26	443 ± 42	14.32 ± 1.36	173 ± 21	
Mice						
Male	30 mg/kg oral dose	16.25 ± 7.19	2,869 ± 6,116	0.37 ± 0.80	296	Chang et al. (2008)
Female	30 mg/kg oral dose	3.08 ± 0.26	999 ± 42	0.87 ± 0.04	134	
Monkeys						
Male	10 mg/kg i.v. dose	1.61 ± 0.06 (α) 40.32 ± 2.36 (β)	112 ± 6	494 ± 61	526 ± 68	Chang et al. (2008)
Female	10 mg/kg i.v. dose	2.28 ± 0.14 (α) 41.04 ± 4.71 (β)	159 ± 8	224 ± 19	443 ± 59	
Humans						
Males and females	NV	Study 1: 28.6−109.71 Study 2: 72 (mean)	NV	NV	NV	Chang et al. (2008)

NV = not available.

3.2. NONCANCER EVIDENCE SYNTHESIS AND INTEGRATION

For each potential health effect discussed below, the synthesis describes the database of available studies, as well an array of the experimental animal study results (the primary evidence available for this PFAS) across studies (note: effect levels presented in these arrays are based on statistical significance[4] and/or biological significance; examples relevant to interpretations of biological significance include directionality of effect [e.g., statistically significantly decreased cholesterol/triglycerides is of unclear toxicological relevance], tissue-specific considerations for magnitude of effect [e.g., statistically nonsignificant increase of ≥10% in liver weight may be considered biologically significant]), significant finding at a single, lower dose level but not at multiple, higher dose levels may be interpreted as potentially spurious. For this section, evidence to inform organ/system-specific effects of PFBA in animals following developmental exposure are

[4]Throughout the assessment, the phrase "statistical significance" indicates a *p*-value < 0.05, unless otherwise noted.

discussed in the individual organ/system-specific sections (e.g., liver effects after developmental exposure are discussed in the liver effects effects). Evidence of other effects informing potential developmental effects (e.g., vaginal opening, eyes opening) is discussed in the "Developmental Effects" section.

3.2.1. Thyroid Effects

Human Studies

Two studies reported on the association between PFBA exposure and thyroid hormones or disease. One study on congenital hypothyroidism was considered uninformative[5] due to concerns with participant selection, confounding, and exposure measurement (Kim et al., 2016). In one *low* confidence study (Li et al., 2017b) examining thyroid hormones, among participants without thyroid disease, there were inverse associations reported with thyroxine (T4), free triiodothyronine (T3), and thyroid-stimulating hormone (TSH), with only the latter being statistically significant (Pearson correlation coefficient = −0.348, $p < 0.01$).

Animal Studies

Two *high* confidence studies reported in three publications from the same research group evaluated the effects of PFBA exposure on thyroid, specifically hormone levels, histopathology, and organ weight (Butenhoff et al., 2012; van Otterdijk, 2007a, b).[6] There were some outcome-specific considerations for study evaluations that were influential on the overall study rating for thyroid effects, but none of these individual domain-specific considerations were judged to be critical, and all studies considered further in this section were rated as *high* or *medium* confidence (see Figure 3-1).

[5]Clicking on the hyperlinked study evaluation determination will take users to the HAWC visualization for that study evaluation review. From there, users can click on individual domains to see the basis for that decision. In the subsequent hazard sections, hyperlinked endpoint names will take users to the HAWC visualization for that endpoint, from which users can click on the endpoint or studies to see the response data from which the visualization is derived.

[6]The Butenhoff et al. (2012) study reported the findings of two unpublished industry reports: a 28-day and 90-day gavage study fully reported in van Otterdijk (van Otterdijk, 2007a, b). While these industry reports were conducted at the same facility and largely by the same staff, they were conducted independently of one another and at different times: July 26, 2006 through September 15, 2006 for the 28-day study and April 5, 2007 through August 6, 2007 for the 90-day study. Throughout the document, both Butenhoff et al. (2012) and the relevant industry report are cited when discussing effects observed in these reports. Although one study evaluation was performed for this group of citations in HAWC, the overall confidence level of *high* applies to both the 28-day and 90-day reports.

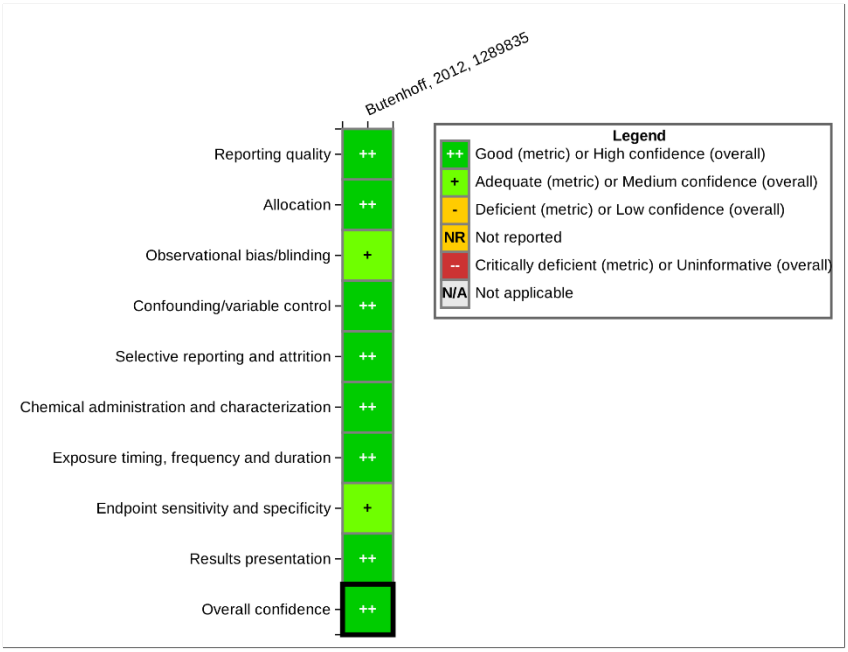


Figure 3-1. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA) exposure on the thyroid (see interactive data graphic for rating rationales).

Organ weight

Absolute and relative thyroid weights were increased (~twofold) at the end of treatment in male rats exposed to 6 or 30 mg/kg-day for 28 days compared with controls, but although elevated, they were not significantly increased at 150 mg/kg-day (Butenhoff et al., 2012; van Otterdijk, 2007a). Thyroid weights were not significantly increased in male rats following the recovery period or in female rats following the treatment or recovery period. Thyroid weight was not measured in rats exposed to NH_4^+PFBA for 90 days (Butenhoff et al., 2012; van Otterdijk, 2007b).

Thyroid hormones

Male rats exposed to NH_4^+PFBA for 28 days via gavage exhibited significant decreased total thyroxine (T4) and free T4 (fT4) levels compared with controls (see Table 3-3 and Figure 3-2). Total T4 was reduced 59%, 66%, and 79% and free T4 was reduced 46%, 50%, and 66% at 6, 30, and 150 mg/kg-day, respectively (Butenhoff et al., 2012; van Otterdijk, 2007a). Free T4 concentrations had returned to control levels at all doses 21 days after exposure ended, but total T4 levels remained decreased in the 150 mg/kg-day group (−23%) and was significantly increased (24%) in the 30-mg/kg-day group. TSH levels were not affected by exposure to NH_4^+PFBA at any exposure level. No treatment-related effects on any of the thyroid hormone measures were observed in female rats exposed for 28 days, although total T4 changes followed a similar pattern

(not significantly decreased) at the highest tested dose (Butenhoff et al., 2012; van Otterdijk, 2007a).

Table 3-3. Percent change in thyroid hormones due to perfluorobutanoic acid (PFBA) exposure in short-term and subchronic oral toxicity studies

Animal group	Dose (mg/kg-d)			
	1.2	6	30	150
Free T4				
28 d; male S-D rats (Butenhoff et al., 2012)		**−46**	**−50**	**−66**
28 d; female S-D rats (Butenhoff et al., 2012)		−0.5	+18	−25
90 d; male S-D rats (Butenhoff et al., 2012)	[a]	−9	−30	
90 d; female S-D rats (Butenhoff et al., 2012)	−6	+27	−15	
Total T4				
28 d; male S-D rats (Butenhoff et al., 2012)		**−59**	**−66**	**−79**
28 d; female S-D rats (Butenhoff et al., 2012)		−8	+27	−31
90 d; male S-D rats (Butenhoff et al., 2012)	13	−15	−39	
90 d; female S-D rats (Butenhoff et al., 2012)	+16	+14	−21	

Bolded cells indicate statistically significant changes compared to controls; shaded cells represent doses not investigated in the individual studies.
[a]No sample available due to insufficient sample volume for assay.

Decreased total T4 and free T4 levels were also observed in male rats exposed to NH_4^+PFBA via gavage for 90 days (Butenhoff et al., 2012; van Otterdijk, 2007b). Total T4 levels were not dose dependent. Total T4 increased 13% and decreased 15% following 1.2 and 6 mg/kg-day, respectively. In rats exposed to the highest dose tested (30 mg/kg-day NH_4^+PFBA), total T4 was significantly reduced 39%. Free T4 was also reduced in the 30-mg/kg-day dose group, but comparison to a control group was not possible due to insufficient sample volume in the control group. However, the decrease in free T4 appeared to be monotonic with increasing dose, and the decrease in the 30-mg/kg-day group (30%) was statistically significant compared with the free T4 concentration in the 1.2-mg/kg-day group. No statistically significant treatment-related effects were observed in female rats exposed to NH_4^+PFBA for 90 days, although total T4 was

nonsignificantly decreased at the highest dose [30 mg/kg-day; Butenhoff et al. (2012); van Otterdijk (2007b)].

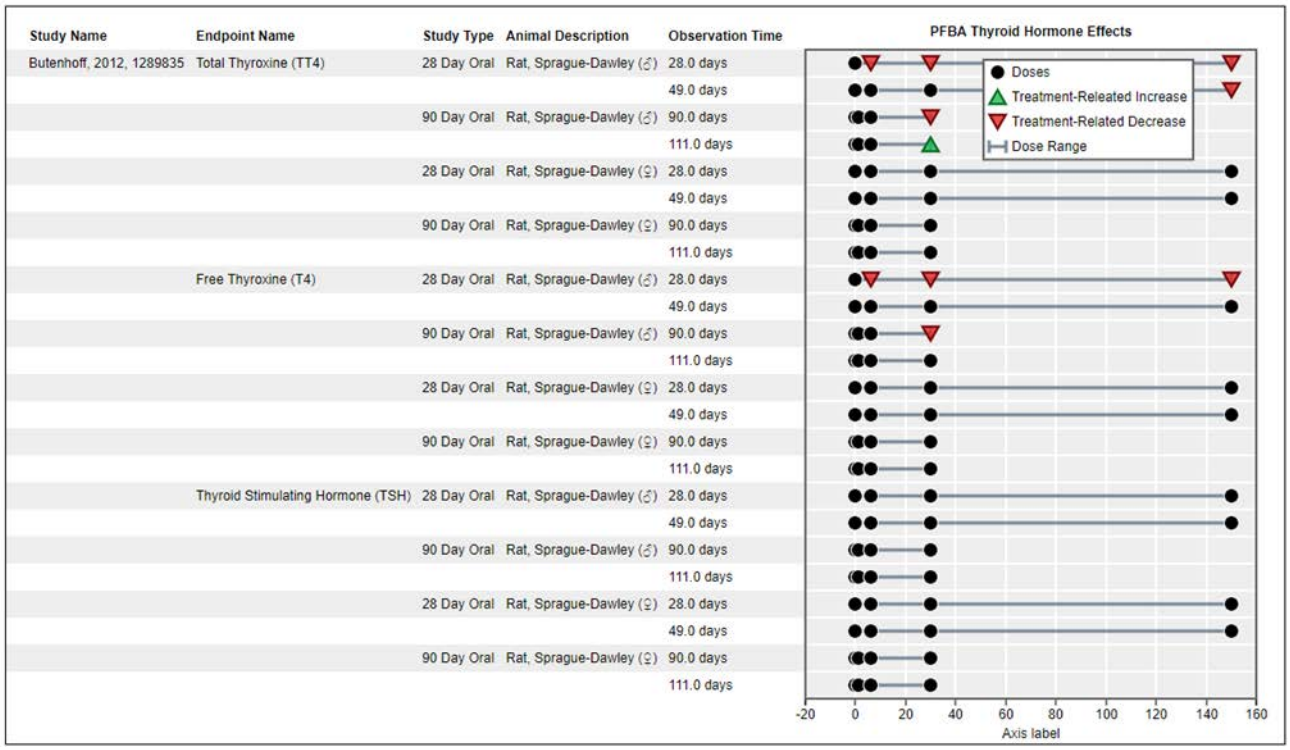


Figure 3-2. Thyroid hormone response to ammonium perfluorobutanoic acid (NH_4^+PFBA) exposure (see interactive data graphic and rational for study evaluations for thyroid hormone effects in Health Assessment Workspace Collaborative [HAWC]).

Histopathology

Butenhoff et al. (2012) and van Otterdijk (2007a, 2007b) also investigated thyroid histopathological and histomorphological effects in male and female rats resulting from NH_4^+PFBA exposure (see Table 3-4 and Figure 3-3). Incidence of follicular hypertrophy/hyperplasia increased in males exposed to 30 mg/kg-day (9/10) and 150 mg/kg-day (7/10) for 28 days compared with control (3/10), with all observed lesions graded by the study authors as of "minimal" severity (trend test p = 0.0498; Cochran Armitage test, performed by the U.S. Environmental Protection Agency [EPA]). Additionally, in the 150-mg/kg-day dose group, three out of the affected seven animals were observed to have lesions graded as "slight," a severity level greater than "minimal." Female rats treated for 28 days with 150 mg/kg-day NH_4^+PFBA had 40% incidence (4/10) of minimal lesions compared with 3/10 minimal lesions observed in the control group. Comparison of the 6-mg/kg-day group to control was not possible (the thyroid of only one animal was available for

testing). Thyroid histopathology was not examined in the 30-mg/kg-day females. No treatment-related effects were seen in the recovery groups. In contrast to the histopathological examination, the histomorphometric analysis reported that no effects on thyroid cell height or colloidal area in either the treatment or recovery groups. Follicular hypertrophy/hyperplasia was also observed to increase in male rats exposed to 30 mg/kg-day (9/10) for 90 days compared to controls when considering all lesions (9/10 vs. 4/10; Cochran Armitage trend $p = 0.0108$) and lesions graded "slight" (5/10 vs. 0/10; Cochran Armitage trend $p < 0.0001$).

Table 3-4. Incidence and severity of thyroid follicular hypertrophy/hyperplasia due to perfluorobutanoic acid (PFBA) exposure in short-term and subchronic oral toxicity studies

Animal group (n = 10 in all groups)	Dose (mg/kg-d)				
	0	**1.2**	**6**	**30**	**150**
28 d; male S-D rats (Butenhoff et al., 2012)	3 (min)		3 (min)	**9 (min)**	7 (4 min, 3 mild)
90 d; male S-D rats (Butenhoff et al., 2012)	4 (min)	6 (min)	4 (min)	9 (4 min, **5 mild**)	

Bolded cells indicate statistically significant changes compared with controls; shaded cells represent doses not investigated in the individual studies. Severity normalized to four point scaled as follows: min = minimal severity; mild = mild/slight severity; mod = moderate severity; sev = marked severity.

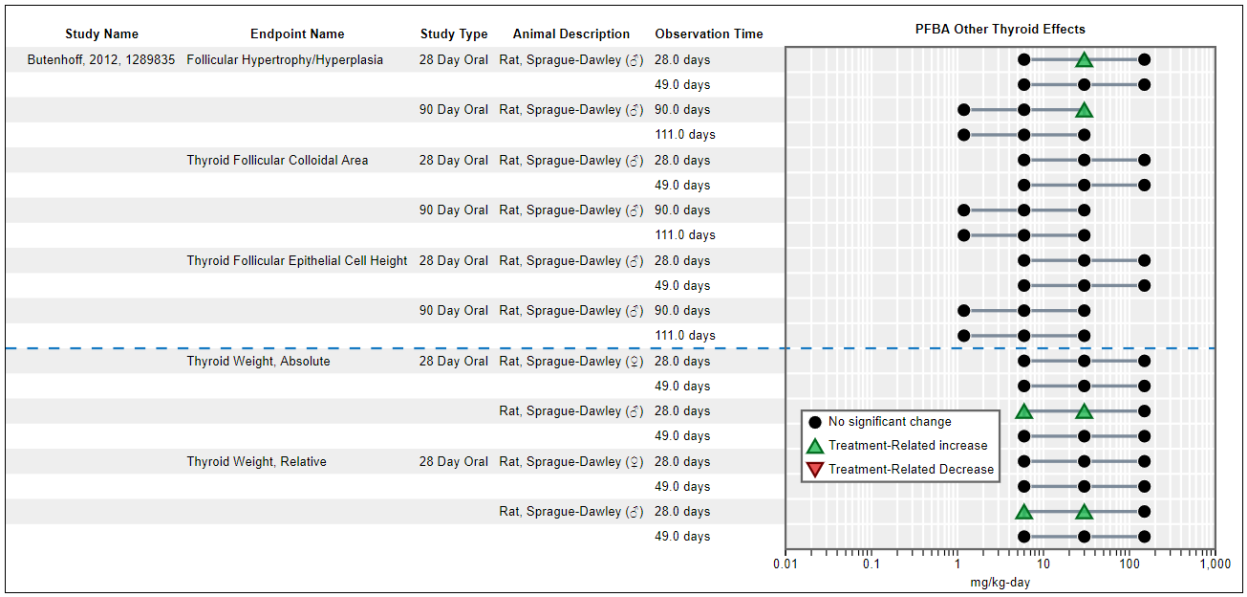


Figure 3-3. Thyroid histopathology and organ-weight responses to ammonium perfluorobutanoic acid (NH_4^+PFBA) exposure (see interactive data graphic and rational for study evaluations for other thyroid effects in Health Assessment Workspace Collaborative [HAWC]).

Evidence Integration Summary

Inverse associations were observed between PFBA exposure and thyroid hormone levels in the one available informative human study (Li et al., 2017b). However, given the *low* confidence in the study methods and the lack of biological coherence across the hormone changes, the available human evidence did not notably contribute to the evidence integration judgment on PFBA-induced thyroid effects.

The animal evidence comes from two *high* confidence experiments conducted by the same laboratory (Butenhoff et al., 2012; van Otterdijk, 2007a, b), which reported PFBA-induced perturbation of the thyroid in one species and sex (male S-D rats) across two different exposure durations. The reported PFBA exposure-induced effects across thyroid hormone measures (i.e., adult males, reductions in total and/or free T4; T3 was not measured) were consistent, dose dependent, and were associated with increasing absolute and relative thyroid weights and histopathology (follicular hypertrophy/hyperplasia). These decreases were of a concerning magnitude (≥50% in some PFBA exposure groups) and perturbations in total T4 were shown to persist at least 21 days after the termination of 90-day exposure to the highest dose (150 mg/kg-day), but not lower doses (in fact, total T4 was increased at 30 mg/kg-day). No effects (e.g., increases) on TSH in exposed rats were observed. The observed pattern of effects on the thyroid (i.e., decreased total and free T4 without a compensatory increase in TSH) after PFBA exposure is consistent with thyroid perturbations following exposure to other PFAS, including the structurally related compound perfluorobutane sulfonate [PFBS; U.S. EPA (2018b)]. Taken together, the consistent changes in total and free T4, thyroid weights, and histopathology across the two available oral PFBA exposure experiments are biologically coherent and plausible.

Several aspects of the animal evidence base decrease the strength or certainty of the evidence. Although there is coherence across different measures of thyroid toxicity in male rats, there is some inconsistency of effects across durations of exposure: some effects are seen in the 28-day study but not the 90-day study and the magnitude of change of some effects are larger in the short-term than in the subchronic study. However, the overall pattern of decreased thyroid hormones in the absence of a coordinated increase in TSH and commensurate alterations in thyroid tissue weight and histopathology, is consistent with hypothyroxinemia. Hypothyroxinemia has been defined as a low percentile value of serum free T4 (ranging from the 2.5th percentile to the 10th percentile of free T4), with a TSH level within the normal reference range.

While the organ-weight increases and histopathological effects (follicular hypertrophy) observed in (Butenhoff et al., 2012) are consistent with hypothyroxinemia, it is unclear by which mechanism these changes occurred. Rodents are more sensitive to these histopathological changes (follicular hypertrophy), which can then develop into follicular tumors (U.S. EPA, 1998). Increased thyroid follicular hypertrophy supports the finding that the thyroid hormone economy is perturbed. It is likely that the observed hypothyroxinemia was due to increased metabolism or competitive displacement of T4 (Butenhoff et al., 2012). Although there were no thyroid effects

(e.g., hormone or histopathological changes) observed in females at any dose or treatment duration, this may be related to the toxicokinetics of PFBA because clearance rates in rats are faster in females (see Section 3.1.4) and the direction of thyroid hormone and organ-weight responses in the tested dose groups were consistent with those observed in males.

There are many similarities in the production, regulation, and functioning of thyroid hormones between rodents and humans. While differences exist, including the timing of in utero thyroid development and hormone turnover rates, rodents are considered to be a good model for evaluating the potential for thyroid effects in humans (Zoeller et al., 2007). More specifically, the observed decreases in total and/or free T4 in the absence of increases in TSH are considered biologically relevant to humans (Crofton, 2004; Lau et al., 2003). While TSH is an indicator that the thyroid system has been perturbed, it does not always change when serum T4 is decreased. Adverse neurological outcomes have been demonstrated following hypothyroxinemia without any changes in T3 or TSH (Crofton, 2004; Hood and Klaassen, 2000). The typical compensatory feedback loop involves microsomal enzymes that induce uridine 5′-diphospho-glucuronosyltransferase (UDP-GT), affecting the thyroid gland by increasing T4 glucuronidation which in turn reduces serum T4. In this case the typical response to reduced serum free T4 is an increased production of TSH (Hood and Klaassen, 2000), which can lead to thyroid hyperplasia or rat follicular tumors. In that way, observation of thyroid histopathology can be an indication of perturbations in TSH levels over time even in situations where increased TSH is not observed at the time histopathology is measured (Hood et al., 1999). However, rodents have been shown to have a unique sensitivity to thyroid follicular hyperplasia (leading to development of follicular tumors) that is considered less relevant to humans (U.S. EPA, 1998). However, the coherent and consistent perturbations to thyroid hormone economy and the resultant increased thyroid histopathology indicates that PFBA is exerting some effect on the thyroids of exposed male rats. Even considering the increased sensitivity of rodents to thyroid follicular hyperplasia compared to humans, thyroid hormone perturbations are considered relevant to humans and may even be more sensitive to change in humans compared to rodents (U.S. EPA, 1998).

However, a notable data gap exists: studies evaluating the effect of PFBA on neurodevelopment or on thyroid measures after developmental exposure (see Section 3.2.3 "Developmental Effects") were not identified, thus leaving uncertainty as to the potential for more sensitive developmental effects of PFBA exposure on the thyroid and/or nervous systems. During developmental lifestages, such as gestational/fetal and postnatal/early newborn, thyroid hormones are critical in a myriad of physiological processes associated with somatic growth and maturation and survival mechanisms such as thermogenesis, pulmonary gas exchange, and cardiac development (Sferruzzi-Perri et al., 2013; Hillman et al., 2012). It is important that thyroid hormones are at sufficient levels during times that are critical to brain development and functioning as well as in the growth, development, and functioning of numerous organ system processes, including basal metabolism, reproductive, hepatic, sensory (auditory, visual) and

immune systems.(Forhead and Fowden, 2014; Gilbert and Zoeller, 2010; Hulbert, 2000). Perinatal and postnatal lifestages are more susceptible because their compensatory feedback responses are absent or not fully developed and they have low thyroid hormone reserves (Morreale de Escobar et al., 2004; Zoeller and Rovet, 2004). Further, thyroid hormones are critically important in early neurodevelopment as they directly influence neurogenesis, synaptogenesis, and myelination (Puig-Domingo and Vila, 2013; Stenzel and Huttner, 2013). Several human epidemiologic studies have demonstrated key relationships between decreased circulating levels of thyroid hormones such as T4 in a pregnant woman and in utero and early postnatal life neurodevelopmental status. For example, children born euthyroid but who were exposed to thyroid hormone insufficiency in utero (e.g., ≤10th percentile free T4), present with cognitive impairments (e.g., decreased intelligence quotient [IQ], increased risk of expressive language) and/or concomitant abnormalities in brain imaging (Korevaar et al., 2016; Henrichs et al., 2010; Lavado-Autric et al., 2003; Mirabella et al., 2000). Increasing the concern for potential developmental effects on the thyroid system, sensitive effects on THs have been consistently observed after developmental exposure to the structurally related PFAS (e.g., PFBS).

Taken together, the available studies provide ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause thyroid toxicity in humans (see Table 3-5). This judgment is based primarily on a short-term and subchronic study in male rats reporting a consistent and coherent pattern of thyroid effects, generally at PFBA exposure levels ≥30 mg/kg-day, although some notable effects were observed after exposure to 6 mg/kg-day.

Table 3-5. Evidence profile table for thyroid effects

Evidence profile table for PFBA thyroid effects					
Evidence integration summary judgment There is ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause adverse thyroid effects in humans based on the results of two *high* confidence studies in adult male rats showing a largely consistent and biologically coherent pattern of thyroid hormone effects following short-term (28 d) or subchronic (90 d) oral exposure, generally at PFBA exposure levels ≥30 mg/kg-d (although some notable effects were observed after 6 mg/kg-d).					
Summary of humans, animal, and mechanistic evidence (only oral exposure studies available)					
Studies, outcomes, and confidence	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Key findings and interpretation**	**Evidence stream summary**	**Inferences across evidence streams**
Evidence from studies of exposed humans					
THYROID HORMONES *Low* confidence Li et al. (2017b)	• No factors noted	• Evidence demonstrating lack of coherence of associations across hormones • *Low* confidence study	• One *low* confidence study reported inverse association with free T4, free T3, and TSH, with only the latter being statistically significant	• Single *low* confidence study with unexplained lack of biological coherence	*Human relevance:* Rats are a reliable model of thyroid effects in humans, and the observed pattern of changes in rodents are consistent with hypothyroxinemia in humans. Thus, the thyroid effects reported in animal bioassays after less than lifetime PFBA exposure are considered relevant to humans. Notably, humans exhibit reduced sensitivity to developing thyroid
Evidence from in vivo animal studies					
HORMONE LEVELS *High* confidence Butenhoff et al. (2012); van Otterdijk (2007a, 2007b)	• Consistency across studies in thyroid hormone changes (i.e., for total and free $T_{4)}$ in male rats across two *high confidence* studies of varied design (i.e., exposure duration) • Dose-response gradient	• Evidence demonstrating lack of coherence (no compensatory TSH increase in response to decreased free or total T4 and tissue changes)	• Decreases in free and total T4 in male rats exposed to >6 mg/kg-d in two *high* confidence studies following short-term or subchronic exposure	• Consistent and biologically coherent results for thyroid hormone levels, organ weights and histopathology from two *high*	

	• Coherence with other effects in the thyroid (histopathology) • Large magnitude of effect (as high as 79%)			confidence studies in rats exposed to >6 mg/kg-d (thyroid hormones) and 30 mg/kg-d (histopathology) • Thyroid effects observed only in males may be explained by differences in toxicokinetics • Uncertainties remain as to how organ weights and histopathology are affected in the absence of TSH increases	follicular hyperplasia as compared to rats. *Cross-stream coherence:* The human evidence was considered unreliable, so coherence could not be evaluated. *Susceptible populations and lifestages:* The developing fetus and children are susceptible to altered thyroid hormone status. However, a key data gap exists due to a lack of developmental studies on potential thyroid or nervous system effects. *Other inferences:* The observed thyroid effects are consistent with effects of other PFAS, including the related PFAS, PFBS. The MOA is unknown and unstudied. Most notably, the observed PFBA-induced changes in organ weight and histopathology without effects on TSH introduce uncertainty.
HISTOPATHOLOGY *High* confidence Butenhoff et al. (2012); van Otterdijk (2007a, 2007b)	• Consistency across studies in thyroid histopathological changes (i.e., follicular hypertrophy/hyperplasia) in male rats at 30 mg/kg-day across two high confidence studies of varied design (i.e., exposure duration) • Coherence with other effects in the thyroid (hormone levels)	• Unexplained lack of significant effects at highest tested dose, 150 mg/kg-d in one study Lack of coherence between histopathology and TSH levels	• Follicular hypertrophy/hyperplasia was observed in male rats exposed to 30 mg/kg-d in *high* confidence short-term and subchronic studies • No histopathological effects at 150 mg/kg-d after short-term exposure (untested with subchronic exposure)		
ORGAN WEIGHT *High* confidence Butenhoff et al. (2012); van Otterdijk (2007a, 2007b)	• Increases in absolute and relative thyroid weights at 6 and 30 mg/kg-d are coherent with the observed histopathology (i.e., increased hypertrophy/hyperplasia at 30 mg/kg-d) in this study	• Short term study • Unexplained inconsistency in lack of treatment-related effects at highest tested dose, 150 mg/kg-d	• Increase in thyroid weight (absolute and relative) at 6 and 30 mg/kg-d in a *high* confidence short-term study • No change in weights at 150 mg/kg-d		

3.2.2. Hepatic Effects

Human Studies

One epidemiology study reported on the relationship between PFBA exposure and serum biomarkers of liver injury. This study (Nian et al., 2019) was cross-sectional and was classified as *medium* confidence given minor concerns over participant selection, outcome ascertainment, and confounding. Sensitivity was considered *deficient* due to low exposure levels and narrow contrast for PFBA (detected in 52%, median [interquartile range (IQR)] = 0.03 ng/mL [0.01–1.6 ng/mL]), which likely reduced the study's ability to detect an effect. The study found no association between serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), total bilirubin, or cholinesterase with PFBA exposure, but given the sensitivity concerns, this is difficult to interpret.

In addition, one *low* confidence cross-sectional study (Fu et al., 2014) examined the association between PFBA exposure and blood lipids. No association was reported; however, the exposure levels in the study population were very low with narrow contrast (median = 0.1 ng/mL), so the study had poor sensitivity to detect an effect.

Animal Studies

Hepatic effects were evaluated in multiple *high* and *medium* confidence, short-term- and subchronic-duration studies in rats and mice (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a, b; Permadi et al., 1993; Permadi et al., 1992) and in one *high* confidence developmental toxicity study in mice (Das et al., 2008). Some outcome-specific considerations for study evaluations were influential on the overall study rating for liver effects, but none of these individual domain-specific limitations were judged as unlikely to be severe or have a notable impact on the study results, and all studies considered further in this section were rated as *high* or *medium* confidence (see Figure 3-4).

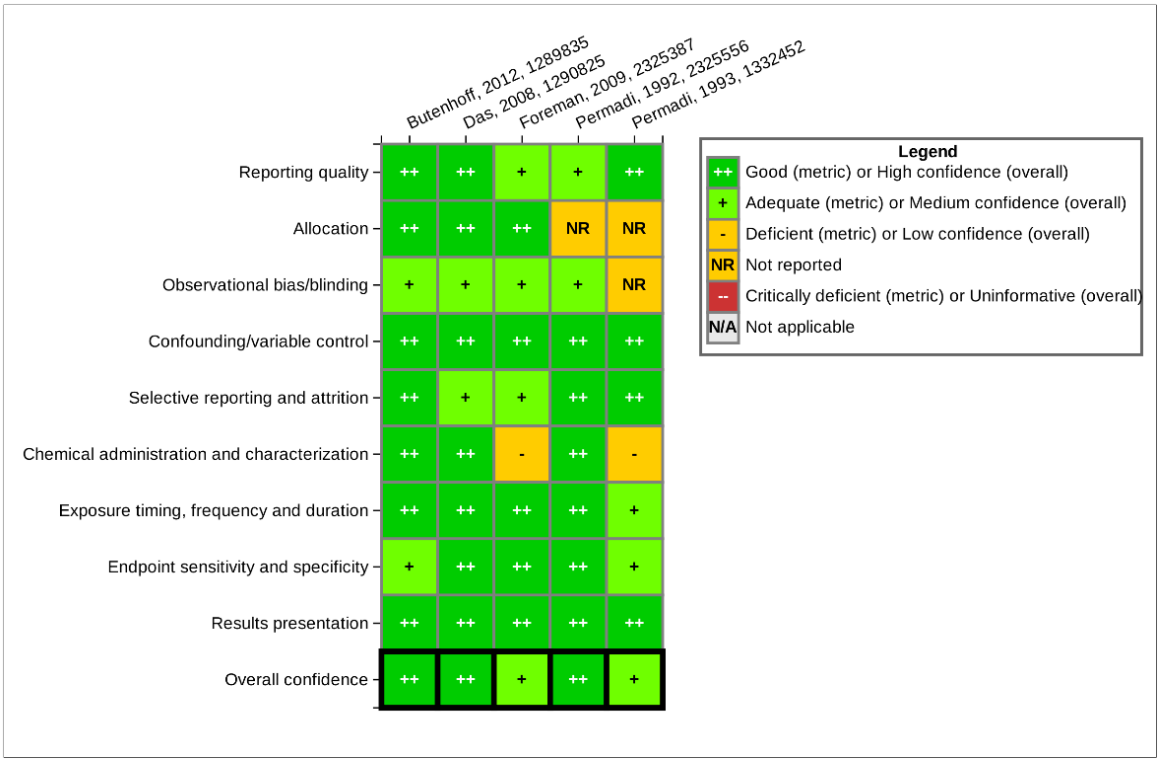


Figure 3-4. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA) exposure on the liver (see interactive data graphic for rating rationales).

One *low* confidence, short-term study also reported hepatic effects (Ikeda et al., 1985). This study was judged as *low* confidence given concerns over allocation of animals, reporting/attrition concerns, characterization of the test compound, and endpoint sensitivity.

Endpoints evaluated in the studies reporting liver effects include liver weights, histopathological changes, and serum biomarkers of effect.

Organ weight

Short-term and subchronic exposure studies consistently demonstrated increased liver weight in rodents exposed to PFBA (see Table 3-6 and Figure 3-5). Liver weight is commonly reported as either absolute weight or relative to body weight. In general, relative liver weight is the preferred metric as it accounts for individual variations in body weight, either due to the exposure being studied or to interindividual variability. Both absolute and relative liver weight are presented in this section for the sake of completeness; results based on absolute liver weight closely track those for relative liver weight.

Table 3-6. Percent increase in relative liver weight due to perfluorobutanoic acid (PFBA) exposure in short-term and subchronic oral toxicity studies

Animal group	Dose (mg/kg-d)						
	1.2	6	30	35	150	175	350
28 d; male S-D rats (Butenhoff et al., 2012; van Otterdijk, 2007a)		5	**24**		**48**		
28 d; female S-D rats (Butenhoff et al., 2012; van Otterdijk, 2007a)		−1	0		−3		
90 d; male S-D rats (Butenhoff et al., 2012; van Otterdijk, 2007b)	9	7	**33**				
90 d; female S-D rats (Butenhoff et al., 2012; van Otterdijk, 2007a)	0	−3	3				
28 d; PPARα wild-type male SV/129 mice (Foreman et al., 2009)				**61**		**101**	**112**
28 d; humanized PPARα male SV/129 mice (Foreman et al., 2009)				**38**		**63**	**81**
28 d; PPARα null male SV/129 mice (Foreman et al., 2009)				3		1	7
Pregnant P_0 female CD-1 mice on GD 18 (Das et al., 2008)				9		**28**	**32**
Nonpregnant P_0 female CD-1 mice on GD 18 (Das et al., 2008)				14		**32**	**29**
F_1 male and female CD-1 mice on PND 1 (Das et al., 2008)				9		**30**	**41**

Bolded cells indicate statistically significant changes compared with controls; shaded cells represent doses not investigated in the individual studies.

The only null study (Ikeda et al., 1985) reported that relative liver weight was not increased over controls in male S-D rats exposed to 0.02% PFBA in the diet for 2 weeks (approximately 20 mg/kg-day). However, this study was judged to be *low* confidence based on concerns over reporting, exposure characterization, and endpoint sensitivity/selectivity. Conversely, following 10 days of dietary exposure to 0.02% PFBA, relative liver weight was observed to be increased 38% in male C57Bl/6 mice in a *medium* confidence study (Permadi et al., 1993). Twenty-eight days of daily gavage exposure to ≥35 mg/kg-day PFBA significantly increased relative liver weights in adult male wild-type (+/+) or humanized PPARα (hPPARα) Sv/129 male mice (Foreman et al., 2009). The relative liver weight of wild-type male mice was increased by 61%, 101%, and 112% at 35, 175, and 350 mg/kg-day, respectively. Increased relative liver weight was also observed at these same dose groups in humanized PPARα (hPARα) male mice, although they were somewhat less than those observed in wild-type mice: 38%, 63%, and 81%. Relative liver weight was not changed in PPARα null (−/−) mice (Foreman et al., 2009). A similar profile of increased relative liver weight

was also observed in male S-D rats exposed to ≥30 mg/kg-day NH_4^+PFBA for 28 days (Butenhoff et al., 2012; van Otterdijk, 2007a): relative liver weights were increased 24 and 48% at 30 and 150 mg/kg-day. Relative liver weights in both dose groups were observed to return to control levels following a 21-day recovery period. Female rats exposed at the same dose levels did not experience increases in relative liver weights (1–3% decrease).

Similar to increases following 28-day exposures, relative liver weights were also observed to increase in male S-D rats exposed to NH_4^+PFBA for 90 days (Butenhoff et al., 2012; van Otterdijk, 2007b), with relative liver weights increased 33% at 30 mg/kg-day. As with the short-term exposure, relative liver weights returned to control values following a 21-day recovery period after the termination of the subchronic exposure. As observed in the short-term study, exposure to NH_4^+PFBA for 90 days did not increase liver weights in female rats (3% decreases to 3% increases). In a developmental toxicity study in CD-1 mice, exposure to NH_4^+PFBA increased relative (to body weight) liver weights in pregnant (measured on GD 18) and nonpregnant P_0 females (Das et al., 2008) at ≥175 mg/kg-day. Relative liver weights were increased by 28% and 32% at 175 and 350 mg/kg-day (respectively) in pregnant mice, whereas relative liver weights were increased 32 and 29% in nonpregnant mice at the same dose levels. No effect on liver weights was observed in the subset of dams followed until after weaning (PND 22). Similar magnitudes of relative liver weights increases were also observed in F_1 animals at PND 1: 30% and 41% at 175 and 350 mg/kg-day, respectively. However, in animals at PND 10, no change in relative liver weights was observed. The lack of an effect on PND 10 in F_1 or P_0 animals on PND 22 may be because these animals were not exposed during lactation and, therefore, had a 10- or 22-day recovery period compared with offspring or dams whose liver weights were measured on PND 1 and GD 17. This observation of a lack of an effect following a recovery period is consistent with the findings of the subchronic and short-term exposures in adult animals (Butenhoff et al., 2012; van Otterdijk, 2007a, b).

In conclusion, effects on relative liver weights in adult male rats and mice were observed at ≥30 or 35 mg/kg-day following subchronic or short-term exposures (respectively), whereas effects in adult pregnant and nonpregnant female mice (exposed during pregnancy) and their offspring were only observed at higher doses (≥175 mg/kg-day). Adult female rats were only exposed up to 150 mg/kg-day in the subchronic study (Butenhoff et al., 2012; van Otterdijk, 2007b), so it is currently unclear whether these animals would exhibit the same effects at the exposure levels used in the developmental toxicity study (Das et al., 2008). Regardless, the data for relative liver weight seem to indicate that male animals are more susceptible to this effect than female animals, possibly due to the observation that females have a much faster (5-6 times greater) excretion rate than males (see Section 3.1.4 for details).

Changes in absolute liver weight across all studies were generally consistent with those observed for relative liver weight. Following 10 days of dietary exposure to 0.02% (w/w) PFBA, absolute liver weights were observed to be increased 64% in male C57Bl/6 mice (Permadi et al., 1993; Permadi et al., 1992). Absolute liver weights were also increased 27% and 45% following

28 days of exposure to 30 or 150 mg/kg-day NH_4^+PFBA, respectively (Butenhoff et al., 2012; van Otterdijk, 2007a). No effects were observed in female rats following exposure or in male rats following a 21-day recovery. Similar to increases following 28-day exposures, liver weights were also observed to increase due to treatment in male S-D rats exposed to NH_4^+PFBA for 90 days (Butenhoff et al., 2012; van Otterdijk, 2007b), with absolute liver weights increased by 23%. Liver weights returned to control levels following a 21-day recovery period. As observed in the short-term study, exposure to NH_4^+PFBA for 90 days did not increase liver weights in female rats (~3%–8% increases). In a developmental toxicity study in CD-1 mice, exposure to NH_4^+PFBA increased absolute liver weights in pregnant and nonpregnant P_0 females (Das et al., 2008) at ≥175 mg/kg-day. Absolute liver weights were increased by 24% and 35% at 175 and 350 mg/kg-day, respectively, in pregnant mice, whereas absolute liver weights were increased 34% and 21% at those same doses in nonpregnant P_0 females. Similar magnitudes of absolute liver weights increases (27% and 32%) were also observed in F_1 animals at PND 1at 175 and 350 mg/kg-day (Das et al., 2008). As with relative liver weights, no effect was observed in offspring at PND 10 or pregnant P_0 animals at postweaning (PND 22).

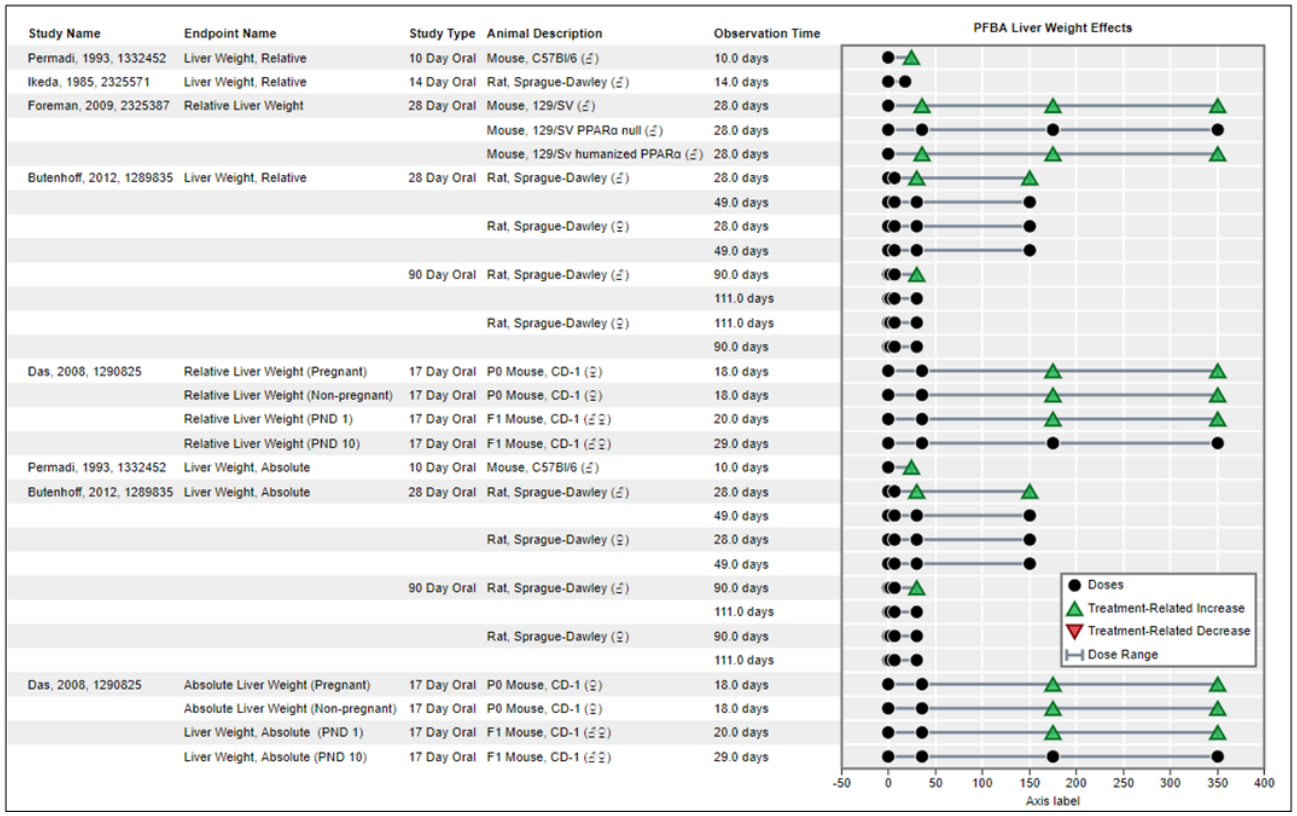


Figure 3-5. Liver-weight response to ammonium perfluorobutanoic acid (NH_4^+PFBA) or perfluorobutanoic acid (PFBA) exposure (see interactive data graphic and rational for study evaluations for liver-weight effects in Health Assessment Workspace Collaborative [HAWC])

Histopathology

Histopathological examination of the livers of mice and rats across three separate gavage studies of 28-day (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a) or 90-day (Butenhoff et al., 2012; van Otterdijk, 2007b) exposure duration revealed significant, dose-dependent alterations and lesions (see Table 3-7 and Figure 3-6).

Both wild-type and hPPARα mice exposed to PFBA for 28 days developed hepatocellular hypertrophy at doses ≥35 mg/kg-day, whereas PPARα null mice did not develop hypertrophic lesions at any dose following 28-day exposures (Foreman et al., 2009). Although the incidence and severity of the hypertrophic lesions was similar between wild-type and hPPARα mice at higher doses, hPPARα mice developed more severe lesions at 35 mg/kg-day than did the wild type mice (5/10 severe lesions vs. 0/10, respectively). Hepatocellular hypertrophy was also observed in 6/10 S-D rats exposed to 150 mg/kg-day PFBA for 28 days (Butenhoff et al., 2012; van Otterdijk, 2007a) 9/10 rats exposed to 30 mg/kg-day PFBA for 90 days (Butenhoff et al., 2012; van Otterdijk, 2007b). In both cases, no lesions were observed in animals following a 21-day recovery period.

hPPARα mice were much less susceptible to the development of hepatic focal necrosis following a 28-day exposure to PFBA compared to wild-type mice. Wild-type mice developed hepatic focal necrosis (with inflammatory cell infiltration) at 175 mg/kg-day (6/10) and 350 mg/kg-day (9/10), whereas focal necrosis was observed in only 1/10 and 2/10 hPPARα and PPARα null mice at 175 and 350 mg/kg-day, respectively (Foreman et al., 2009). For all strains, most of the necrotic lesions were judged to be mild in severity. By comparison, in rats exposed to PFBA for 28 days, there was no observed increase in hepatocellular coagulative necrosis (Butenhoff et al., 2012; van Otterdijk, 2007a). Effects on hepatocellular necrosis in rats were also not observed following 90-day exposures to PFBA (Butenhoff et al., 2012; van Otterdijk, 2007b).

Following exposure to 350 mg/kg-day for 28 days, centrilobular and periportal vacuolation was observed in PPARα null and humanized mice, respectively, while no vacuolation was reported for wild-type mice (Foreman et al., 2009). No quantitative data was reported for these effects, so examining the dose-response or magnitude of effect across doses was not possible. The lack of vacuolation in wild-type animals is consistent with the lack of vacuolation in rats exposed to PFBA for 90 days in (Butenhoff et al., 2012; van Otterdijk, 2007b), where 4/10 control animals were reported to exhibit vacuolation, but incidence dropped to 1/10 in the low dose group and no vacuolation was observed at higher doses. Although the number of studies was small, mice did seem to be more sensitive to the development of hepatocellular lesions compared to rats, possibly owing to the observed differences in toxicokinetics between the two species: mice are observed to have serum excretion half-lives approximately 2-times longer than rats at similar exposure levels (see Section 3.14 and Table 3-2 for details).

Table 3-7. Incidence and severity of liver histopathological lesions due to perfluorobutanoic acid (PFBA) exposure in short-term and subchronic oral toxicity studies

Animal group (*n* = 10 in all groups)	Dose (mg/kg-d)							
	0	**1.2**	**6**	**30**	**35**	**150**	**175**	**350**
Hypertrophy								
28 d; male rats (Butenhoff et al., 2012; van Otterdijk, 2007a)	0		0	0		**6 (min)**		
90 d; male rats (Butenhoff et al., 2012; van Otterdijk, 2007b)	0	0	0	**9 (5 min, 4 mild)**				
28 d; PPARα wild-type male mice (Foreman et al., 2009)	0				**10 (4 mild, 6 mod)**		**10 (1 mild, 1 mod, 8 sev)**	**10 (sev)**
28 d; hPPARα male mice (Foreman et al., 2009)	0				**10 (1 mild, 4 mod, 5 sev)**		**10 (2 mod, 8 sev)**	**10 (sev)**
28 d; PPARα null male mice (Foreman et al., 2009)	0				0		0	0
Coagulative necrosis								
90 d; male rats (Butenhoff et al., 2012; van Otterdijk, 2007b)	0		0	0		0		
Focal necrosis[a]								
28 d; PPARα wild-type male mice (Foreman et al., 2009)	0				1 (mild)		**6 (2 min, 4 mild)**	**9 (8 mild, 1 mod)**
28 d; hPPARα male mice (Foreman et al., 2009)	0				1 (min)		1 (min)	2 (min)
28 d; PPARα null male mice (Foreman et al., 2009)	0				0		1 (min)	2 (min)
Vacuolation								
28 d; PPARα wild-type male mice (Foreman et al., 2009)	None reported							
28 d; hPPARα male mice (Foreman et al., 2009)	Periportal vacuolation reported to increase at 350 mg/kg-d, compared to controls							
28 d; PPARα null male mice (Foreman et al., 2009)	Centrilobular vacuolation reported to increase at 350 mg/kg-d, compared to controls							

Bolded cells indicate statistically significant changes compared to controls; shaded cells represent doses not investigated in the individual studies. Severity normalized to four point scaled as follows: min = minimal severity; mild = mild/slight severity; mod = moderate severity; sev = marked severity.
[a]Incidence of focal necrosis for the positive control of Wy-14,643 (a known PPARα activator) was 3 total (1 minimal, 2 mild) at 50 mg/kg-day exposure.

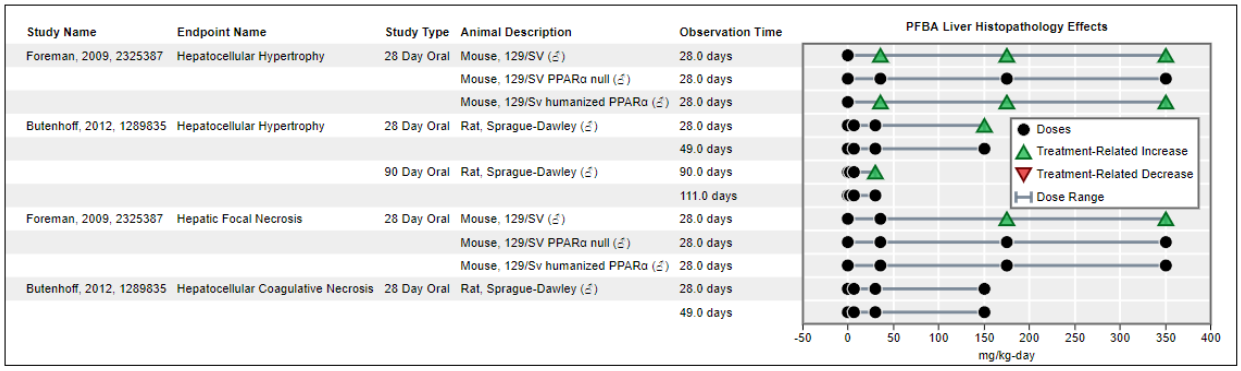


Figure 3-6. Liver histopathology response to ammonium perfluorobutanoic acid (NH_4^+PFBA) or perfluorobutanoic acid (PFBA) exposure (see interactive data graphic and rationale for study evaluation for liver histopathology effects in Health Assessment Workspace Collaborative [HAWC]).

Serum biomarkers

Serum biomarkers associated with altered liver function or injury including ALT, AST, ALP, total protein, albumin, and total bilirubin were not significantly changed in male or female S-D rats exposed to up to 150 mg/kg-day PFBA for 28 days (Butenhoff et al., 2012; van Otterdijk, 2007a). However, prothrombin time (a measure of clotting time induced by the liver-produced prothrombin protein) was decreased at 150 mg/kg-day and ≥6 mg/kg-day in males and females, respectively, although decreases were rather small (~5–9% relative to control) and were reported to be within the concurrent reference range for S-D rats. Some alterations in serum biomarkers were also observed in rats exposed to PFBA for 90 days: ALP was increased 32% in male rats exposed to 30 mg/kg-day and bilirubin was decreased 21% and 13% in male and female rats (respectively) exposed to 30 mg/kg-day (Butenhoff et al., 2012; van Otterdijk, 2007a). ALT was not affected by PFBA exposure in wild-type, PPARα null, or hPPARα mice (Foreman et al., 2009). Cholesterol levels were decreased 20% and 27% in male rats exposed to 30 and 150 mg/kg-day PFBA for 28 days. Cholesterol levels returned to control values following recovery and no effects on cholesterol were seen in male rats exposed to PFBA for 90 days. No clear explanation exists to describe why cholesterol levels might be changed after 28, but not 90, days of PFBA exposure.

Mechanistic Evidence and Supplemental Information

The liver effects observed in the PFBA database consist of increased liver weight, increased incidence of hepatocellular hypertrophy, and (to a lesser degree) hepatocellular necrosis. Increased liver weight and hepatocellular hypertrophy can be associated with changes that are adaptive in nature (Hall et al., 2012), and not necessarily indicative of adverse effects unless observed in concordance with other clinical, pathological markers of overt liver toxicity (see PFBA

Protocol; Appendix A). The IRIS PFAS Assessment Protocol (which addresses PFBA) describes that the panel recommendations from Hall et al. (2012) can be used to judge whether observed hepatic effects are adverse or adaptive in nature. However, given that Hall et al. (2012) was focused on framing non-cancer liver effects in the context of progression to liver tumors, the protocol further indicates that "…consultation of additional relevant information will be considered to interpret the adversity of noncancer liver effects over a lifetime exposure, taking into account that effects perceived as adaptive can progress into more severe responses and lead to cell injury." In the case of PFBA, the "additional relevant information" consists of multiple *in vitro* mechanistic studies, an *in vivo* study investigating PFBA-induced liver effects in wildtype humanized PPARα mice, and, PPARα-null mice (Foreman), as well as evidence from other PFAS that help elucidate possible modes of action of PFBA liver toxicity.

Many of the hepatic effects caused by exposure to perfluorinated compounds such as PFBA have been attributed to activation of the peroxisome proliferator-activated receptor alpha (PPARα[7]) (Rosenmai et al., 2018; Bjork and Wallace, 2009; Foreman et al., 2009; Wolf et al., 2008). Due to reported cross-species differences in PPAR signaling potency and dynamics the potential human relevance of some hepatic effects has been questioned, particularly is it relates to PPARα activation. The goal of the qualitative analysis described in this section is to evaluate the available mechanistic evidence for PFBA-induced liver effects and to assess the biological relevance of effects observed in animal models to possible effects in humans.

While the database is smaller for PFBA than for some other PFAS, in vitro studies demonstrate that PFBA activates PPARα in both rodent and human cell lines. Studies using rodent cell lines or COS-1 cells transfected to express rodent PPARα generally report that exposure to PFBA consistently results in activation of PPARα and increased expression of PPARα-responsive genes (Rosen et al., 2013; Wolf et al., 2012; Bjork and Wallace, 2009; Wolf et al., 2008). However, while PFAS generally have been shown to activate PPARα, shorter chain PFAS such as PFBA appear to be weak activators. For example, Bjork and Wallace (2009) showed that PFBA is a weaker activator of PPARα in primary rat and human hepatocytes than is either the six-carbon PFHxA or eight-carbon PFOA. PFBA is also one of the weakest mouse and human PPARα activators compared with other longer chain PFAS [i.e., C5–C12; Rosen et al. (2013); Wolf et al. (2012); Wolf et al. (2008)]. These studies also observed diminished effects and transcription levels in human cell lines (primary hepatocytes) or COS-1 cells transfected with human PPARα compared to mice (primary hepatocytes or transfected COS-1 cells). One study using the human hepatoma cell line HepG2 also reported activation of PPARα after exposure to PFBA for 24 hours, further demonstrating that the human PPARα can be activated by PFBA (Rosenmai et al., 2018). Interestingly, when modeling the slope of PPARα activation in human hepatoma cells for various PFAS, Rosenmai et al. (2018)

[7]PPARα is a member of the nuclear receptor superfamily that can be activated endogenously by free fatty acid derivatives. PPARα plays a role in lipid homeostasis, but it is also associated with cell proliferation, oxidative stress and inflammation (NJDWQI, 2017; Angrish et al., 2016; Mellor et al., 2016; Hall et al., 2012).

observed that PFBA (slope = 7.4×10^{-3}) was a stronger activator than PFOA (slope = 4.9×10^{-3}). Foreman et al. (2009) investigated PPARα activation in the liver of mice following in vivo exposure to PFBA. The PPARα-responsive gene CYP4A10 was activated to a greater degree in wild-type mice than in humanized mice, but acyl-CoA oxidase (ACO, active in β-oxidation and lipid metabolism) appeared to be activated to a similar magnitude in both wild-type and humanized mice. The known PPAR activator Wy-14,643 activated CYP4A10 and ACO to a similar magnitude in humanized PPARα mice compared to PFBA but to a lesser degree in wild-type mice. Neither gene was activated following exposure to PFBA or Wy-14,643 in PPARα null mice.

One in vivo study (Foreman et al., 2009) provided evidence that oral PFBA exposure elicits apical, toxicological effects in humanized PPARα mice. This study showed that increased liver weight and hepatocellular hypertrophy were induced following exposure to ≥35 mg/kg-day PFBA in wild-type and hPPARα mice. While magnitude of liver-weight increases was larger for wild-type mice, the effect on hypertrophy was the same for wild-type and hPPARα mice at higher exposures. Conversely, hPPARα mice had more severe lesions at lower doses compared with wild-type mice. Increased liver weight and hypertrophy were also seen in positive controls treated with Wy-14,643.

Enlargement of the liver represents one of the most common observations associated with chemical exposures via the oral route in laboratory animals and humans. In addition to measured increases in the mass of liver tissue, histological evaluation typically reveals isolated or multifocal areas of hepatocellular hypertrophy. The swelling of hepatocytes may include accumulation of lipid material (e.g., micro- or macro-vesicular steatosis), organellar growth and proliferation (e.g., peroxisomes, endoplasmic reticulum), increased intracellular protein levels (e.g., Phase I and II enzymes), and altered regulation of gene expression (e.g., stress response, nuclear receptors) (for review see: Batt and Ferrari, 1995). Importantly, hepatocellular hypertrophy alone is morphologically indistinguishable between an adaptive or toxic response sans further indicators of cell status (Williams and Iatropoulos, 2002), such as reduced glutathione levels, mitochondrial integrity, receptor-dependent or independent signal transduction pathway activity (e.g., pro-survival versus pro-cell death balance), or redox state, for example. Although hepatocellular hypertrophy is commonly attributed to receptor-dependent organellar growth and proliferation (e.g., peroxisome proliferator-activated receptor [PPAR] mediated), the milieu of pathways involved in modulating hepatocyte structural and functional response to chemicals are diverse (Williams and Iatropoulos, 2002). For example, hepatocyte swelling has also been associated with cell death processes, in particular oncosis or oncotic necrosis (Weerasinghe and Buja, 2012). Several liver diseases or conditions such as ischemia-reperfusion injury, drug-induced liver toxicity, and partial hepatectomy, have noted oncosis (oncotic necrosis) upon cellular/tissue examination (for review see: Kass 2006; Jaeschke and Lemasters 2003), and are not dependent upon peroxisome proliferation or PPAR signaling. Rather, cellular alterations such as a transition in mitochondrial membrane permeability and caspase activation (especially Caspase-8) have been identified as key mediators or tipping points for a shift from a hypertrophic (oncotic) hepatocellular

phenotype to apoptotic and/or primary necrotic cell death (Lemasters et al., 2002; Van Cruchten and Van Den Broeck, 2002). As such, an assumption that chemical-induced hepatocellular hypertrophy is by default a distinctly proliferative/growth response associated exclusively with PPAR signaling may not be accurate.

Although no PFBA-specific studies investigated activation of other isoforms of PPAR (e.g., PPARγ) or additional pathways (e.g., constitutive androstane receptor [CAR] or pregnane X receptor [PXR]), evidence from human cell culture experiments involving PFOS and PFOA, two of the most heavily studied PFAS, suggest the possibility of other non-PPARα MOAs operational in liver toxicity. For example, PFOA and/or PFOS exposure is associated with PPARγ activation (Beggs et al., 2016; Buhrke et al., 2015), and increased mRNA levels of CAR and PXR responsive genes (Abe et al., 2017; Zhang et al., 2017). Activation of these hepatic nuclear receptors play an important role in regulating responses to xenobiotics, as well as in energy and nutrient homeostasis (di Masi et al., 2009). Animal studies of other PFAS also provide some evidence suggesting that nuclear receptor pathways other than PPARα may be involved in PFAS-induced liver effects. For example, two separate in vivo studies using PPARα null animal models report increases in absolute and relative liver weight (Das et al., 2017; Rosen et al., 2017) as well as hepatocellular hypertrophy and lipid accumulation (Das et al., 2017) following PFHxS or PFNA exposure. Multiple in vivo studies have also evaluated activation of CAR and PXR in rodents exposed to PFDA: PFDA exposure in wild-type C57BL6/6J mice led to increased nuclear translocation of CAR and mRNA levels of CAR/PXR responsive genes [*CYP2B10* and *CYP3A11*; Abe et al. (2017)]; these effects were not observed in CAR or PXR null mice. PFDA has also been observed to activate PXR in human HepG2 cells (Zhang et al., 2017) and increase mRNA levels of CAR/PXR-regulated genes (*CYP2B6* and *CYP3A4*) in primary human hepatocytes (Rosen et al., 2013).

In addition to hypertrophy, Foreman (2009) also observed additional histopathological effects. Hepatic focal necrosis was statistically significantly increased following exposure of wild-type mice to ≥175 mg/kg-day PFBA. While no statistically significant increases in focal necrosis were seen at any dose in PPARα null or humanized mice, necrosis was seen to increase slightly in the highest dose compared to controls (2/10 vs. 0/10) in hPPARα; it is possible that exposure to higher doses of PFBA would elicit increased necrotic effects in hPPARα mice. Interestingly, no statistically significant increase in focal necrosis was observed in any mouse strain treated with Wy-14,643 in this study. That PFBA exposure resulted in liver necrosis in wild-type mice, but not PPARα null mice, suggests that PPARα is required for the development of this lesion. However, the observation that the positive control for PPARα activation, Wy-14,643, did not also result in this lesion (in this study), as well as suggestive evidence of increased necrosis in hPPARα mice, provides evidence that a PPARα-independent, complementary and/or multifaceted mode of action may be active in the observed liver toxicity. Supporting this conclusion is the observation that centrilobular and periportal vacuolation (i.e., lipid accumulation) was increased compared with controls in PPARα null and humanized mice after exposure to 350 mg/kg-day PFBA, with

greater vacuolation in PPARα null mice than in humanized mice. Vacuolation was not reported in wild-type mice, and results for the vacuolation endpoints were only provided for the control and low-dose groups for the PPARα null and hPPARα mice. This result is consistent with Das et al. (2017) who reported that PFAS increased both accumulation and oxidation of lipids in the liver of exposed mice, with accumulation occurring faster than oxidation. Thus, while vacuolation occurs in humanized PPARα mice, oxidation is also induced (as evidenced by the upregulation of ACO), limiting lipid accumulation to a degree. However, in PPARα null mice, accumulation of lipids in the livers of exposed animals must be occurring through a PPARα-independent mechanism. Thus, PFBA appears to result in increased lipid accumulation in the liver via a PPARα-independent mechanism, and while humanized mice do exhibit an increase in β-oxidation via ACO upregulation, this increase in lipid catabolism is not sufficient to overcome the increased lipid deposition in the liver.

The observation of increased liver weight, increased incidence of hepatocellular hypertrophy, vacuolation, and necrosis in wildtype and humanized PPARα mice is important when considered in the context of the recommendations of the Hall et al. (2012) paper. In interpreting "histological changes caused by an increase in liver weight", exactly the situation observed in PFBA-exposed hPPARα mice in Foreman et al. (2009), Hall et al. (2012) suggests that coincident histological evidence of liver injury/damage can be used to support the conclusion that the liver weight increases/histological changes (i.e., hypertrophy) are adverse. Among the histological changes that Hall et al. (2012) identifies as sufficient supporting evidence is necrosis and steatotic vacuolar degeneration, with the study authors further differentiating between macro-vesicular vacuolation (considered non-adverse) and micro-vesicular vacuolation. Micro-vesicular vacuolation is described by the presence of hepatocytes partially or completely filled with multiple small vacuoles without displacement of the nucleus (Kleiner and Makhlouf, 2015). This pattern of vacuolation is precisely what is observed by Foreman et al. (2009) in hPPARα mice exposed to PFBA. Additionally, focal necrosis is observed in wildtype mice in Foreman et al. (2009). Hence, according to the Hall recommendations, observation of liver weight increases, hypertrophy, micro-vesicular vacuolation, and necrosis across wildtype and hPPARα mice is consistent with a determination that these interconnected PFBA-induced liver effects meet the criteria for adversity.

Accumulation of lipids in the liver is an apical key event (decreased fatty acid efflux resulting in lipid accumulation) leading to hepatic steatosis (Angrish et al., 2016; Kaiser et al., 2012) and has been observed in animal toxicological studies following exposure to numerous environmental agents that ultimately cause steatosis (Joshi-Barve et al., 2015; Wahlang et al., 2013). Sustained steatosis can progress to steatohepatitis and other adverse liver diseases such as fibrosis and cirrhosis (Angrish et al., 2016). Therefore, that vacuolation occuring in null PPARα mice indicates a PPARα-independent mechanism for the accumulation of lipids in the liver possibly as a precursor to more severe forms of liver injury. The occurrence of vacuolation in humanized mice further supports the human relevance of the observed hepatic toxicity.

Overall, evidence specific to PFBA and from other potentially relevant PFAS provide support for both PPARα dependent and independent pathway contributions to hepatic toxicity, and further, that activation of humanized PPARα by PFBA can likewise result in hepatic effects of concern. Additionally, application of the recommendations from Hall et al. (2012) clearly support the conclusion that the multiple and interconnected effects observed in the livers of exposed animals meet the criteria for adversity.

Evidence Integration Summary

No association between PFBA and circulating levels of multiple serum biomarkers of hepatic injury were observed in the only available, *medium* confidence epidemiologic study with reduced sensitivity (Nian et al., 2019). These null findings from a single study with low sensitivity did not influence the evidence integration judgments.

Hepatic effects associated with oral exposures to PFBA have been consistently observed in *high* or *medium* confidence short-term and subchronic studies in adult mice or rats of both sexes (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a, b; Permadi et al., 1993; Permadi et al., 1992), and in a developmental toxicity study in mice (Das et al., 2008). Overall, changes in liver weights and histopathology (hepatocellular hypertrophy) were consistently observed across two species, with effects occurring in male adult rats and mice, female pregnant or nonpregnant adult mice, and in male and female neonatal mice. In particular, increases in liver weight and hepatocellular hypertrophy incidence occurred at similar dose levels across species, occurred at multiple doses, and appeared to be dose-related (i.e., increasing magnitude of effect with increasing dose).

Increased liver weights were consistently observed in male, but not female, adult rats following 28- or 90-day exposures (Butenhoff et al., 2012; van Otterdijk, 2007a, b), as well as in male wild-type and hPPARα mice, pregnant and nonpregnant female mice, and neonatal male and female mice on PND 1 (Foreman et al., 2009; Das et al., 2008; Permadi et al., 1993; Permadi et al., 1992). For male rodents, the doses at which effects occurred appeared to differ appreciably across species, but wild-type PPARα mice seemed to exhibit greater magnitudes of effect versus humanized PPARα mice or rats. As noted above, female pregnant and nonpregnant mice, along with their offspring, exhibited effects only at higher doses compared with adult male rats and mice, possibly relating to the observation that female rodents eliminate PFBA much more rapidly than males (see Section 3.1.4).

Liver histopathology was also consistently observed across PFBA studies (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a, b), although differences in the type and/or severity of lesions differed somewhat across species and durations of exposure. Wild-type and hPPARα mice were both observed to develop hepatocellular hypertrophy following 28 days of oral exposure to PFBA, whereas only wild-type mice developed hepatic focal necrosis (Foreman et al., 2009). PPARα null mice developed neither of these lesions in response to exposure. Adult male rats were also observed to develop hepatocellular hypertrophy, but not coagulative necrosis,

following 28 or 90 days of exposure (Butenhoff et al., 2012; van Otterdijk, 2007a, b). Again, differences in toxicokinetics may explain somewhat the differences in lesion incidence across species, with rats eliminating PFBA much more rapidly than mice. Interestingly, PPARα null and hPPARα mice were observed to develop centrilobular and periportal vacuolation, whereas wild-type mice did not. This possibly indicates the accumulation of lipids within the liver. Increased liver weights were concurrently observed at all doses with hepatocellular hypertrophy in wild-type and hPPARα mice following short-term exposure (Foreman et al., 2009). However, in wild-type mice, liver weight increases occurred at lower doses than did focal necrosis in the same study (Foreman et al., 2009), while focal necrosis was not observed in hPPARα mice in the presence of liver weight changes at any dose. In male rats, changes in liver weight were seen at lower doses than hepatocellular hypertrophy following 28-day exposures, whereas both effects were observed at the same dose following 90-day exposures (Butenhoff et al., 2012; van Otterdijk, 2007a, b).

Changes in serum biomarkers of liver function and/or injury were not consistently observed across exposure durations or concurrently with hepatocellular lesions. In the 28-day study in rats, prothrombin time alterations were observed only at 150 mg/kg-day; no changes in ALT, AST, or ALP were observed. While increased ALP and increased hepatocellular hypertrophy were both observed in male rats exposed to 30 mg/kg-day for 90 days in the subchronic study, no concurrent increase in ALT and AST was observed at this exposure level. Further, while decreased bilirubin was observed, this is inconsistent with what would be expected as a marker of liver injury (i.e., an increase in bilirubin); therefore, this observation is of unclear toxicological significance. Lastly, cholesterol levels were decreased in a dose-dependent manner following the 28-day, but not the 90-day, exposure. As a whole, the various clinical chemistry endpoints, as measurements of liver toxicity, are inconsistent across endpoints and durations of exposure, and thus did not influence the evidence integration judgements.

One characteristic of the evidence base for PFBA is the sparsity of chemical-specific mechanistic data to inform the human relevance of the observed increases in liver weight and hypertrophic lesions in rats and mice. In the one study that does provide chemical-specific information, PFBA exposure to wild-type and hPPARα mice increased both liver weights and hepatocellular hypertrophy. Only wild-type mice were observed to develop focal necrosis, possibly indicating that activation of PPARα was a necessary step in the mode of action for developing this lesion. However, hepatic focal necrosis was not observed in any group (wild-type, hPPARα, or PPARα null mice) exposed to the positive control (the PPARα activator Wy-14,643) in wild-type mice. Further, increased vacuolation was only reported in PPARα-null and hPPARα mice, an observation consistent with in vivo evidence for longer chain PFAS (Das et al., 2017). This observation (increased vacuolation) in PPARα-null and humanized mice indicates that lipid accumulation in the liver is occurring, at least in part, through a PPARα-independent mechanism, and that either the lack of, or attenuated activity of, PPARα-induced lipid catabolism is not sufficient to overcome the increased accumulation. This strongly suggests a complementary or multifaceted

mode of action for development of PFBA-induced hepatic effects. Indeed, based on evidence from other PFAS chemicals, it appears that there are non-PPARα mechanisms relevant to hepatic effects. In vivo and in vitro studies of PFOA, PFOS, PFDA, and PFNA demonstrate that PFAS exposure can activate PPARγ, CAR, and PXR (Abe et al., 2017; Das et al., 2017; Zhang et al., 2017; Beggs et al., 2016; Buhrke et al., 2015; Rosen et al., 2013) and that activation of these receptors results in the hepatic effects observed in PPARα null mice.

Thus, multiple lines of evidence, taken as a whole, indicate that the liver toxicity observed in rodents due to PFBA exposure is likely adverse, relevant to humans, and dependent on multiple biological pathways (i.e., both PPARα-dependent and -independent pathways). Even considering a PPARα-only mode of action, human PPARα is observed to be activated by PFBA exposure in vitro and evidence in humanized PPARα mice (increased liver weight and increased hepatocellular hypertrophy, which is observed to be more severe than that in wild-type mice) indicates that the PPARα-mediated components of the undefined MOA(s) appear relevant to human toxicity, given that the effects are observed in animals with human PPARα receptors. Further, the existing evidence base also supports the operation of PPARα-independent pathways for other hepatotoxic effects given the direct observation of increased vacuolation in PPARα null mice in response to PFBA exposure, an observation also seen in humanized PPARα mice. Even in the absence of PPARα activity, hepatic toxicity occurs that is possibly the precursor to more clearly adverse liver disease (e.g., steatohepatitis, fibrosis, and cirrhosis). Thus, while there is uncertainty in relating the sensitivity of hepatic changes observed in rodents to humans given the generally decreased sensitivity of human responses to PPARα agonism, evidence from PFBA studies and studies on other PFAS, indicate that PPARα alone cannot be identified as the exclusive mode of action for PFBA-induced liver effects. Lastly, independent of conclusions regarding PPARα as the mode of action, consideration of the recommendations from Hall et al. (2012) also support a determination that the observed hepatic effects in rodents are relevant to humans. Hall et al. (2012) recommends that coincident histological evidence of liver injury/damage can be used to support the conclusion that liver weight/hypertrophic effects are adverse. It is clear from the in vivo evidence in rodents that PFBA induces a constellation of effects in the liver, including increased liver weight, hypertrophy, vacuolation, and necrosis. Therefore, according to Hall et al. (2012), these coincident effects is consistent with the conclusion that PFBA-induced liver effects in rodents meet the criteria for adversity.

The available animal evidence for effects on the liver includes multiple *high* and *medium* confidence studies with consistent effects across multiple species, sexes, exposure durations, and study designs (e.g., exposures during pregnancy); it exhibits coherence between the effects on liver weights and histopathology and a clear biological gradient (increasing effect with increasing dose); and the evidence is interpreted to be relevant to humans. Taken together, the available studies provide ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause hepatic toxicity in humans (see Table 3-8), given relevant exposure circumstances. This judgment is based

primarily on a series of short-term, subchronic, and developmental studies in rats and mice, generally exhibiting effects at PFBA exposure levels ≥30 mg/kg-day.

Table 3-8. Evidence profile table for hepatic effects

Evidence profile table for PFBA hepatic effects					
Evidence integration summary judgment There is ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause adverse hepatic effects based on the results of six *high* and *medium* confidence studies (in rats and mice) showing biologically consistent, coherent, and plausible effects (across durations and study types) on liver weights and hepatocellular histopathology after oral exposure to PFBA at ≥30 mg/kg-d following short-term (28 d), subchronic (90 d), or gestational (17 d) exposures. These findings are interpreted as relevant to humans.					
Summary of human and animal, and mechanistic evidence (only oral exposure studies available for human and animal evidence)					
Studies, outcomes, and confidence	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Key findings and interpretation**	**Evidence stream summary**	**Inferences across evidence streams**
Evidence from studies of exposed humans					*Human relevance:* • There is uncertainty in relating the sensitivity of hepatic changes observed in rodents to humans given the generally decreased sensitivity of human responses to PPARα agonism, as compared to rodent responses • However, evidence from one *medium* confidence in vivo study indicates that activation of PPARα alone cannot explain the liver effects observed after PFBA exposure, and that the PPARα-mediated
SERUM BIOMARKERS *Medium* confidence Nian et al. (2019) (liver biomarkers) *Low* confidence Fu et al. (2014) (lipids)	• No factors noted	• Null results in studies with poor sensitivity (i.e., biased towards the null).	• No association observed between PFBA serum concentrations and liver biomarkers or blood lipids in studies with poor sensitivity due to narrow exposure contrast	Null findings in studies with limited sensitivity	
Evidence from in vivo animal studies					
ORGAN WEIGHTS *High* confidence Butenhoff et al. (2012); van Otterdijk (2007a, 2007b) Das et al. (2008) Permadi et al. (1992) Permadi et al. (1993)	• Consistent increases in liver weight in male adult rats and mice, pregnant and nonpregnant female mice, and male and female neonatal mice across four *high*	• No factors noted	• Increased liver weight in male adult rats exposed to ≥30 mg/kg-d in *high* confidence short-term and subchronic	Consistent, dose-dependent, and biologically coherent effects on liver weights and histopathology from *high* and *medium*confidence studies in adult male	

Ikeda et al. (1985) *Medium* confidence Foreman et al. (2009)	confidence studies and one *medium*-confidence study of varied design (i.e., exposure duration, lifestage) • Evidence of dose-response with increased magnitude of effect with increased dose • Coherence with histopathology results in male rats and mice, especially at high doses • Magnitudes of effect as high as 112%		experiments. Reduced effects in females may be attributable to toxicokinetic differences • Increased liver weight in pregnant or nonpregnant female mice and in PND 1 neonates exposed to ≥175 mg/kg-d • Increased liver weight in male wild-type PPARα and hPPARα mice exposed to ≥35 mg/kg-d in a *medium*-confidence short-term study. No effects were seen in PPARα null mice	rats across short-term and subchronic exposure durations and in male and female mice exposed as adults or developmentally PPARα-dependence observed for some effects (focal necrosis) but other effects (vacuolation) occur in animals lacking PPARα activity (null mice) or in animals with ostensibly attenuated PPARα activity (humanized mice) Clear sex dependence seen in the *high* confidence subchronic and short-term studies that is possibly related to toxicokinetic differences (i.e., longer half-lives in male rats vs. female rats) (see Section 2.1). Some liver effects (increased liver weight) observed in female mice in the *high* confidence mouse developmental toxicity study, but at doses higher than those tested in the *high* confidence	contribution to liver hypertrophy occurs in exposed animals with human PPARα. Additionally, there is indirect evidence from in vivo and in vitro studies of other PFAS (PFOA, PFOS, PFDA, PFHxA, PFHxS) indicating the involvement of multiple nuclear receptors other than PPARα in hepatic effects, including PPARγ, CAR, and PXR • Observed effects in PPARα null and humanized mice may be precursors to clearly adverse health outcomes such as steatosis • Consideration of the Hall et al. (2012) recommendations supports a conclusion that the PFBA-induced liver effects in rodents are adverse and relevant to humans given the observation of multiple coincident

				subchronic and short-term study	effects (liver weight increases, hypertrophy, vacuolation, and necrosis). Given the limited PFBA-specific information on the potential MOA for noncancer liver effects and the apparent involvement of both PPARα-dependent and independent mechanisms in the available studies, the liver effects reported in rodent bioassays are interpreted as relevant to humans (see additional discussion in Section 3.2.2) *Cross-stream coherence:* The human evidence was not interpretable, so could not be evaluated *Susceptible populations and lifestages:* None identified, although those with pre-existing liver disease could
HISTOPATHOLOGY *High* confidence • Butenhoff et al. (2012); van Otterdijk (2007a, 2007b) *Medium* confidence • Foreman et al. (2009)	• Consistent findings of hepatic histopathological changes (e.g., hepatocellular hypertrophy, focal necrosis) in male rats and mice (wild type and hPPARα) across two studies (one *high* confidence and one *medium* confidence) of varied design (i.e., exposure duration) • Coherence with liver-weight effects, especially at higher doses		• Hepatocellular hypertrophy observed in male rats exposed to 30 mg/kg-d in a *high* confidence subchronic study • Hepatocellular hypertrophy observed in male wild-type PPARα and hPPARα mice exposed to ≥35 mg/kg-d in a *medium* confidence short-term study • Hepatic focal necrosis observed in male wild-type PPARα mice exposed to ≥175 mg/kg-d in a *medium* confidence short-term study • Hepatic vacuolation observed in male PPARα-null mice and hPPARα mice at 350 mg/kg-d • Reduced effects in females may be attributable to		

			toxicokinetic differences (see Section 2.1)		potentially be at greater risk *Other inferences:* The MOA for liver effects is not fully established, although available evidence indicates that multiple pathways are likely involved
SERUM BIOMARKERS *High* confidence • Butenhoff et al. (2012); van Otterdijk (2007a, 2007b)		• Unclear biological relevance and/or toxicological significances of the incoherent observations of alterations in clinical chemistry measures (i.e., increased ALP but not ALT or AST, and decreased bilirubin when increased bilirubin expected)	• Increased ALP and decreased bilirubin in male or male and female (respectively) rats exposed to 30 mg/kg-d in a *high* confidence study		
Mechanistic evidence and supplemental information					
Biological events or pathways	**Species or model systems**	**Key findings, limitations, and interpretation**		**Evidence stream summary**	
MOLECULAR EVENTS—PPARα	In vitro and/or in vivo (animal and human): multiple in vitro studies in rat and/or human liver cell lines or cells transfected with rat and/or human PPARα evaluating PPARα activation; one in vivo study in mice evaluating PPARα activation	*Key finding:* PFBA-induced increased expression of PPARα-responsive genes in primary rat and human hepatocytes, cells transfected with rat or human PPARα and wild-type and hPPARα mice *Limitations:* small database investigating PPARα activation due to PFBA exposure; some inconsistencies regarding the strength of activation and/or interspecies differences		Overall, studies in rodent and human in vitro and in vivo models suggest that PFBA induces hepatic effects, at least in part, through PPARα. The evidence also suggests a role for PPARα-independent pathways in the MOA for noncancer liver effects of PFBA.	
MOLECULAR EVENTS—OTHER PATHWAYS	Evidence from multiple in vivo and in vitro studies of other PFAS (PFOA, PFOS, PFDA, PFHxA, PFHxS) that demonstration the	*Key finding:* indirect evidence of alternative pathways following observation of effects in humanized PPARα mice exposed to PFBA, direct evidence from other PFAS that multiple non-PPARα pathways activated following exposure			

	activation of multiple nuclear receptors, including PPARγ, CAR, and PXR Indirect evidence from in vivo study in mice that non-PPARα pathways likely active in PFBA-induced toxicity	*Limitations:* no PFBA specific in vitro data; only one in vivo study providing indirect evidence		
ORGAN-LEVEL EFFECTS	In vivo (animal): one short-term, *medium* confidence study in mice	*Key finding:* observation of increased liver weight and increased hepatocellular hypertrophy/vacuolation in humanized PPARα mice; concurrent observation that a known PPAR activator (Wy-14,643) did not elicit the same effects (focal necrosis) as PFBA exposure in wild-type mice; evidence suggests the activity of non-PPARα pathways in liver toxicity *Limitations:* only one in vivo study		

3.2.3. Developmental Effects

This section describes studies of PFBA exposure and potential early life effects or developmental delays, as well as effects attributable to developmental exposure. The latter includes all studies where exposure is limited to gestation and/or early life. As such, this section has some overlap with evidence synthesis and integration summaries for other health systems where studies evaluated the effects of developmental exposure (see Sections 3.2.2 and 3.2.4 on potential "Hepatic" and "Reproductive Effects," respectively). Synthesis descriptions of studies across sections may vary in detail, depending on the impact the data has on summarizing the evidence relevant to that hazard; typically earlier hazard sections will include a more detailed discussion that is then cited in later sections.

Human Studies

The one epidemiologic study that investigated developmental effects (birth weight, gestational age) due to PFBA exposure (Li et al., 2017a) was deemed *uninformative*.

Animal Studies

A standardized suite of potential developmental effects was evaluated in one *high* confidence developmental toxicity study in mice (Das et al., 2008). There were some outcome-specific considerations for study evaluations that were influential on the overall study rating for developmental effects, but none of these individual domain-specific considerations were judged to be deficient, and the Das et al. (2008) study considered further in this section was rated as *high* confidence (see Figure 3-7). Endpoints evaluated in the study included time to eye opening, full litter resorption, postnatal survival, vaginal opening, preputial separation, body weights, and morphological evaluations (see Table 3-9 and Figure 3-8).

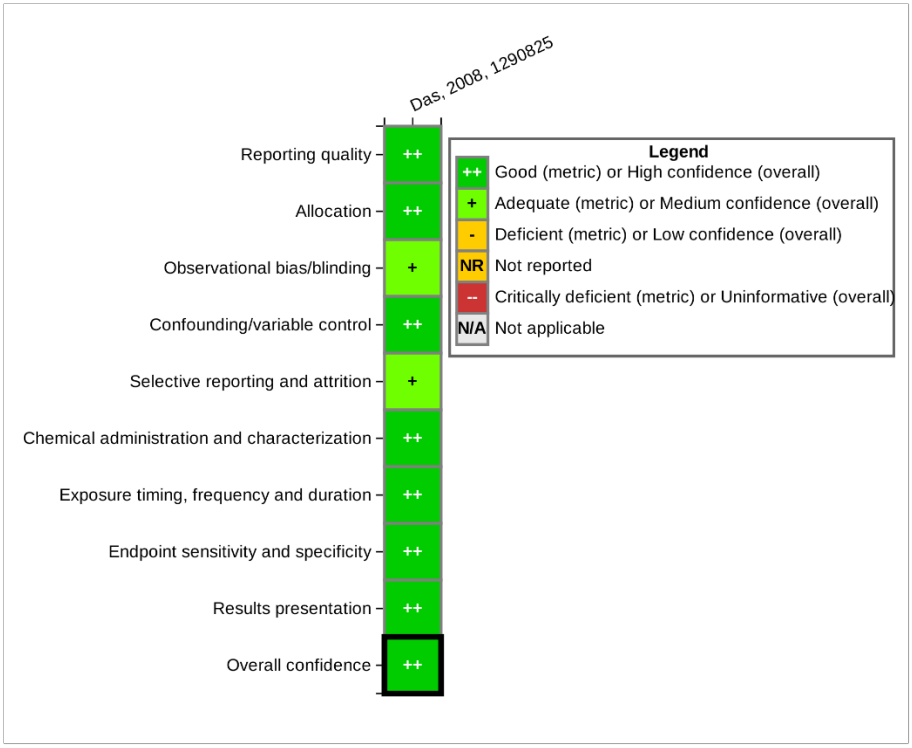


Figure 3-7. Evaluation results for animal studies assessing developmental effects of perfluorobutanoic acid (PFBA) exposure (see interactive data graphic for rating rationales).

Oral exposure from Gestation Day (GD) 1 to 17 of CD-1 mice (male and female offspring were evaluated) to NH_4^+PFBA resulted in delayed eye opening by 1.1, 1.4, and 1.5 days compared to controls at 30, 175, and 350 mg/kg-day, respectively (Das et al., 2008). There were also significantly increased full litter resorptions at 350 mg/kg-day (33 vs. 7% in controls), although there were no effects on the number of implants or live fetuses. Additionally, although not statistically significant, postnatal survival was consistently reduced at PNDs 7, 14, and 21 by approximately 5%. The male and female pubertal landmarks (preputial separation and vaginal opening, respectively) were delayed. Preputial Separation was delayed by 2.3 days at 350 mg/kg-day while vaginal opening was delayed 3.3 and 3.6 days (175 and 350 mg/kg-day, respectively). No changes were observed in neonatal or postweaning body weight. Anatomical changes were observed (renal dilation, fetal hydronephrosis, and absent testis) but were randomly distributed among the treatment groups, including controls, and thus were not attributable to PFBA exposure.

Table 3-9. Developmental effects observed following perfluorobutanoic acid (PFBA) exposure in a developmental toxicity study

Animal group	Dose (mg/kg-d)		
	35	175	350
Full-litter resorptions;[a] Pregnant P_0 female CD-1 mice on GD 18 (Das et al., 2008)	1/29	4/28	8/29
Survival to PND 1; F_1 male and female CD-1 mice on PND 1 (Das et al., 2008)	−2%	1%	−3%
Survival to PND 7; F_1 male and female CD-1 mice on PND 7 (Das et al., 2008)	−1%	−1%	−5%
Survival to PND 14; F_1 male and female CD-1 mice on PND 14 (Das et al., 2008)	−1%	−1%	−6%
Survival to PND 21; F_1 male and female CD-1 mice on PND 21 (Das et al., 2008)	−2%	−1%	−6%
Delayed eye opening; F_1 male and female CD-1 mice (Das et al., 2008)	−6%	−8%	−8%
Delayed vaginal opening; F_1 female CD-1 mice (Das et al., 2008)	−6%	−7%	−9%
Delayed preputial separation; F_1 male CD-1 mice (Das et al., 2008)	−1%	−3%	−7%

[a]Control group incidence: 2/29.

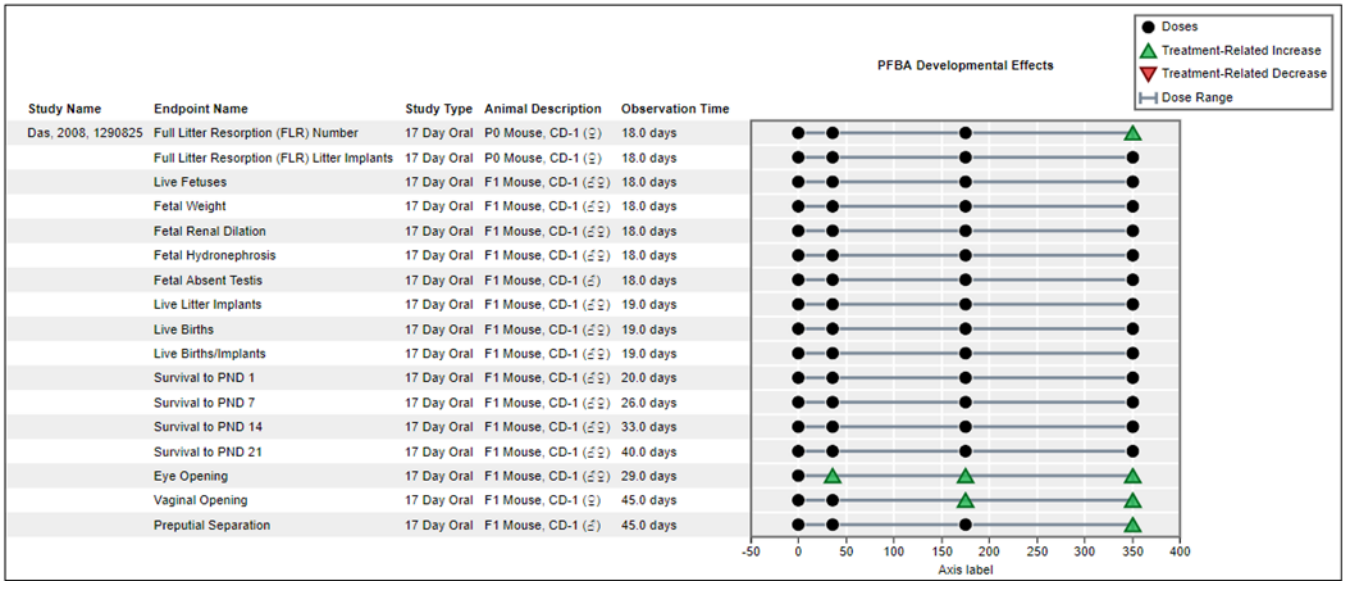


Figure 3-8. Pre- and postnatal developmental effects to gestational ammonium perfluorobutanoic acid (NH_4^+PFBA) exposure (see interactive data graphic and rational for study evaluations for developmental effects in Health Assessment Workspace Collaborative [HAWC])

Evidence Integration Summary

No informative human studies were available that investigated the potential developmental toxicity of PFBA.

Coherent effects on developmental maturation were observed in one *high* confidence study in mice (Das et al., 2008) following in utero exposure to PFBA. The developmental effects of PFBA exposure in this study included delayed eye opening, full-litter resorption, decreased survival, fetal absent testis, and delays in vaginal opening and preputial separation, although pup growth and body weight were unaffected. These effects indicate that PFBA appears to disrupt the normal gestational and postnatal development of exposed fetuses. One factor increasing the strength of evidence is that effects on the developing fetus (e.g., delayed eye opening; delays in the development of the male and female reproductive systems) are seen following exposure to other PFAS, most notably the structurally related compound perfluorobutane sulfonate [PFBS; U.S. EPA (2018b)], but other, longer chain PFAS as well. Following exposure to ≥200 mg/kg-day PFBS (U.S. EPA, 2018b) or 5 mg/kg-day perfluorooctanoic acid [PFOA; Lau et al. (2006)] or perfluorooctane sulfonate [PFOS; Lau et al. (2004)], similar delays in eye opening (~1.5 days) were observed in mice. Similarly, following exposure to ≥200 mg/kg-day PFBS, time to vaginal opening was increased by >3 days (Feng et al., 2017), and time to vaginal patency was increased ~3 days in mice exposed to 20 mg/kg-day PFOA (Lau et al., 2006) and ~2 days in rats exposed to 30 mg/kg-day PFOA (Butenhoff et al., 2004). Time to pubertal milestones was also delayed in male rodents exposed to PFOA: preputial separation was delayed ~1.5 days in mice exposed to 20 mg/kg-day (Lau et al., 2006) and ~2 days in rats exposed to 30 mg/kg-day PFOA (Butenhoff et al., 2004). Thus, qualitatively, a consistent pattern of delayed pubertal milestones is observed following exposure to related PFAS, increasing certainty in the evidence available for PFBA.

Data gaps in the developmental toxicity database include a lack of information on the thyroid and nervous system following gestational exposure. Given that other PFAS (i.e., PFBS) observed perturbations in thyroid hormone levels following gestational exposure and that PFBA induces changes in thyroid hormone levels in exposed adult animals, it is possible that PFBA would also alter normal thyroid function in the developing fetus. As both PFBA and PFBS evidence bases lack studies on developmental neurotoxicity, a potential consequence of altered thyroid function during development, this represents an important unknown.

Thus, considering the coherent suite of developmental effects, primarily developmental delays, observed following PFBA exposure, as well as similar effects observed following exposure to multiple other PFAS (including the structurally similar PFBS), there is ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause adverse developmental effects in humans (see Table 3-10), given relevant exposure circumstances. The basis for this judgment is a single *high* confidence gestational exposure study in mice, with multiple adverse effects occurring at PFBA exposure levels ≥175 mg/kg-day (with delays in eye opening occurring at ≥35 mg/kg-day). Notably, even in the absence of evidence informing potential similarities of effects between PFBA

and other PFAS regarding gestational thyroid function, the available PFBA-specific developmental effects alone support this judgment.

Table 3-10. Evidence profile table for developmental effects

Evidence profile table for PFBA developmental effects					
Evidence integration summary judgment There is ***sufficient evidence*** to indicate that PFBA exposure has the potential to cause adverse developmental effects in humans. This judgment is based on the results of one *high* confidence study in mice showing a biologically coherent pattern of developmental effects, generally at PFBA exposure levels ≥175 mg/kg-d (with delays in eye opening occurring at ≥35 mg/kg-d) following gestational (GD 1–17) oral exposure. In the absence of evidence to the contrary, these findings are as assumed to be relevant to humans.					
Summary of human, animal, and mechanistic evidence					**Inferences across evidence streams**
Studies, outcomes, and interpretation	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Key findings and interpretation**	**Evidence stream summary**	
Evidence from studies of exposed humans					*Human relevance:* In the absence of evidence to the contrary, the developmental effects observed in mice are considered relevant to humans *Cross-stream coherence:* NA (no informative studies in humans) *Susceptible populations and lifestages:* Pregnancy and early life *Other inferences:* No MOA information available
No informative studies were identified					
Evidence from in vivo animal studies (oral exposure)					
DEVELOPMENTAL MILESTONES *High* confidence Das et al. (2008)	• Low risk of bias in one *high* confidence study in male and female mice • Evidence of dose-response with increased magnitude of effect with increased exposure concentration • Observed developmental delays were Internally coherent (within-study) and consistent across related PFAS	• No factors noted	• Dose-dependent delays in eye opening in male and female mice at ≥35 mg/kg-d • Delayed preputial separation in males at 350 mg/kg-d • Dose-dependent delays in vaginal opening in females at 175 and 350 mg/kg-d • Increased full litter resorption at 350 mg/kg-d • No effects on pup weight or survival	Coherent delays in developmental milestones, with multiple alterations observed at ≥175 mg/kg-d; this effect is consistent with effects seen for other PFAS	

	compounds, including PFBS and PFOS				

3.2.4. Reproductive Effects

Human Studies

One *low* confidence cross-sectional study (Song et al., 2018) examined the association between PFBA exposure and semen parameters. There was no evidence of an association between PFBA exposure and decreased semen quality (correlation coefficients were −0.03 for semen concentration and 0.2 for progressive motility), although issues were noted during study evaluation regarding the ability of this study to detect an effect. The study also did not report important methodological details.

Animal Studies

Two *high* confidence studies reported in three publications from the same research group (Butenhoff et al., 2012; van Otterdijk, 2007a, b) evaluated the effects of PFBA exposure on reproductive organ weights in rats (see Figure 3-9). In addition, one *high* confidence developmental toxicity study (Das et al., 2008) reported several delays in reproductive system development (e.g., vaginal opening; preputial separation) after gestational exposure. These latter results are synthesized and integrated with other studies examining developmental outcomes (see Section 3.2.3) given the apparent coherence of findings of developmental delays after PFBA exposure and the general lack of other studies or effects on reproduction, including an absence of studies on functional measures (see discussion below).

Organ weight

Short-term exposure (28 days) to PFBA in male S-D rats increased absolute epididymis weight (note: absolute organ weights are typically preferred for these reproductive organ measures) 10% compared to controls, but only at the lowest dose [6 mg/kg-day; Butenhoff et al. (2012); van Otterdijk (2007a)]. In a separate cohort, this effect was not seen following a 3-week recovery period (at 49 days) from exposure at any dose (6, 30, or 150 mg/kg-day). Changes in absolute or relative testis weight were not observed in rats following either 28 days of exposure or during the recovery period. Similarly, no changes in absolute or relative ovary weight were observed in rats following short-term (28 days) PFBA exposure and did not arise during the recovery period (Butenhoff et al., 2012; van Otterdijk, 2007a).

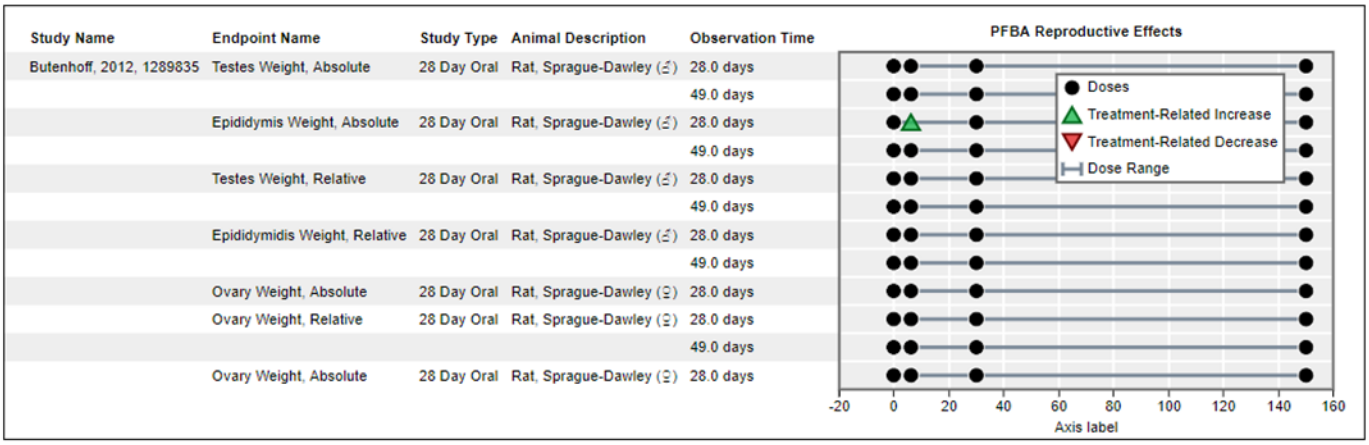


Figure 3-9. Reproductive effects to ammonium perfluorobutanoic acid (NH_4^+PFBA) exposure (see interactive data graphic and rational for study evaluations for reproductive effects in Health Assessment Workspace Collaborative [HAWC])

Evidence Integration Summary

The database of studies examining the potential for PFBA exposure to elicit effects on reproductive parameters is limited to one human and one animal study reported in three publications from the same research group (Butenhoff et al., 2012; van Otterdijk, 2007a, b) (*low* and *high* confidence studies, respectively). There is one *high* confidence animal study that observed delayed development of the reproductive system (i.e., delayed vaginal opening and preputial separation) following gestational PFBA exposure (Das et al., 2008). These latter results are synthesized and integrated in the developmental effects section (see Section 3.2.3) and not discussed further in this section.

In the only available human study (a *low* confidence study), no association was observed between semen quality and PFBA exposure. Null findings in a single study with low sensitivity (biased towards the null) are not interpreted to influence the evidence integration judgments.

The available animal evidence is sparse, limited to evaluations of reproductive organ-weight measurements in a *high* confidence short-term experiment evaluating a cohort of rats immediately after exposures ended and another cohort evaluated 21 days postexposure (Butenhoff et al., 2012), both of which were largely null.

Given the sparsity of evidence on potential reproductive effects, the relative insensitivity of the outcome measures (organ weights) in animals, and the largely null findings, there is ***insufficient evidence*** to determine whether PFBA exposure has the potential to cause reproductive effects in humans (other than the developmental delays discussed in Section 3.2.3; see Table 3-11).

Table 3-11. Evidence profile table for reproductive effects

Evidence profile table for PFBA reproductive effects					
Evidence integration summary judgment There is ***insufficient evidence*** to determine whether PFBA exposure has the potential to cause reproductive effects in humans (other than the developmental delays discussed in Section 3.2.3).					
Summary of human, animal, and mechanistic evidence judgments					
Studies, outcomes, and confidence	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Key findings and interpretation**	**Evidence stream summary**	**Inferences across evidence streams**
Evidence from studies of exposed humans					*Human relevance:* Organ-weight changes in rats are considered relevant to humans in the absence of evidence to the contrary *Cross-stream coherence:* No reliable evidence to compare *Susceptible populations and lifestages:* None identified *Other inferences:* No MOA information available
SEMEN PARAMETERS *Low* confidence Li et al. (2017b)	• No factors noted	• *Low* confidence study • High risk of bias	• No association between PFBA exposure and semen quality	No association with semen parameters in single *low* confidence study with poor sensitivity	
Evidence from in vivo animal studies (oral or inhalation exposure)					
ORGAN WEIGHTS *High* confidence Butenhoff et al. (2012)	• No factors noted	• Lack of dose dependence • Lack of coherence with other measures (i.e., testis weight) • Lack of toxicological relevance of increased epididymal weight	• Increased epididymal weight in rats exposed to 6 mg/kg-day, but not higher doses in a *high* confidence short-term study • No change in testis or ovary weights	Largely null findings in the only available study that examined organ weights, but not any more sensitive measures of potential reproductive effects	

3.2.5. Other Noncancer Health Effects

In addition to the potential health effects outlined above, some epidemiological studies have examined the potential for associations between PFBA exposure and blood pressure and renal function, while a number of experiments in rats and mice have examined potential effects of PFBA exposure on body weight (note: these data were used to inform interpretation of the health effects discussed in prior sections), hematological effects, and ocular effects. Given the paucity of studies available and/or the lack of consistent or coherent effects of PFBA exposure, there is ***insufficient evidence*** to determine whether any of these evaluated outcomes might represent potential human health hazards of PFBA exposure. Additional studies on these health effects could modify these interpretations.

Human Studies

One *medium* confidence cross-sectional study (Bao et al., 2017) examined the association between PFBA exposure and blood pressure and reported statistically significant increased odds of hypertension (OR = 1.10 [95% CI: 1.04–1.17 per ln-PFBA, ng/mL]) and increased systolic blood pressure (β = 0.80 mm HG [95% CI: 0.25–1.34 per ln-PFBA, ng/mL]). This is despite narrow exposure contrast (median 0.16 ng/mL, IQR 0.01–0.54). While this was a *medium* confidence study, there is remaining potential for bias; this includes outcome misclassification resulting from the volatility of blood pressure and its measurement at a single time point as well as the cross-sectional design. In the absence of additional confirmatory epidemiology studies, or other supportive findings (e.g., from animal studies), the results of this observational study alone are interpreted as "insufficient evidence".

One *low* confidence cross-sectional study (Wang et al., 2019) examined the association between PFBA exposure and renal function. They reported statistically significant lower estimate glomerular filtration rate (β: −0.5, 95% CI −0.8, −0.1 [change in GFR (mL/min/1.73 m^2) per 1 ln-serum PFAS (ng/mL)]) and higher, though not significant, odds of chronic kidney disease (OR: 1.1, 95% CI 1.0,1.2) despite low exposure levels. However, there is potential for reverse causation in this association. In essence, as described in Watkins et al. (2013), decreased renal function (as measured by decreased GFR or other measures) could plausibly lead to higher levels of PFAS, including PFBA, in the blood. This hypothesis is supported by data presented by Watkins et al. (2013), although there is some uncertainty in the conclusions because of the use of modeled exposure data as a negative control and the potential for the causal effect to occur in both directions. Consequently, there is considerable uncertainty in interpreting the results of studies of this outcome.

Animal Studies

Body-weight changes were evaluated in multiple *high* and *medium* confidence short-term and subchronic-duration studies in rats and mice (Butenhoff et al., 2012; Foreman et al., 2009; Das

et al., 2008; van Otterdijk, 2007a, b). In general, no PFBA-related effects on body weight were seen in any study. Foreman et al. (2009) reported that body weighs were not affected in any exposure group of Sv/129 mice. Initial and final body weights were statistically significantly lower in humanized PPARα (hPPARα) Sv/129 mice exposed to 350 mg/kg-day PFBA compared to all other groups, but this was explained by random assignment of animals; body weights in this group actually increased slightly across the duration of study, indicating the lower measured body weights were not treatment related. However, the change in body weight across the duration of the study was not changed at any dose in any group of animals, indicating that PFBA exposure had no deleterious effect on adult body weight in mice. Maternal, preweaning, and postweaning body weights were not altered by PFBA exposure in CD-1 mice (Das et al., 2008). Adult body weights were not altered in S-D rats exposed to PFBA for either 28 or 90 days (Butenhoff et al., 2012; van Otterdijk, 2007a, b). PFBA appears to not affect body weight across multiple species, exposure durations, or lifestages.

Some evidence of effects on the hematological system were observed in S-D rats exposed to PFBA. Following 28 days of exposure, no effects other than prothrombin time (PT; a measure of clotting potential) were observed (van Otterdijk, 2007a, b). In males, PT was statistically significantly decreased 6% following exposure to 150 mg/kg-day PFBA, whereas in females, statistically significant decreases of 4 and 5% were observed in the 6- and 30-mg/kg-day dose groups, respectively. PT was decreased 4% in the 150-mg/kg-day dose group in females, but the decrease was not statistically significant. Following the recovery period, no statistically significant decreases in PT were seen in male rats, but consistent 7–8% decreases in PT were observed in all exposed female dose groups. Hematological effects were more pronounced following 90-day exposures. In males, red blood cell counts, hemoglobin, and hematocrit were decreased 4, 6, and 5%, respectively, and red blood cell distribution width was increased 5% following exposure to 30 mg/kg-day PFBA. While the number of RBCs and RBC distribution width were observed to return to control values following recovery, hemoglobin and hematocrit remained decreased 5% relative to control. Mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were decreased 2–3% in female rats exposed to 30 mg/kg-day PFBA. However, these effects were observed to return to control levels following recovery. Taken as a whole, although some hematological effects were observed in exposed rats, the effect sizes were quite small, they generally were observed to return to control levels following a recovery period, and there was no consistency of effects across exposure durations or sexes.

Ocular effects were also observed in rats exposed to PFBA for 28 or 90 days (van Otterdijk, 2007a, b). In male rats exposed for 28 days, a delayed bilateral pupillary reflex was observed at 150 mg/kg-day. While examination of neuronal tissue (including the optic nerve) revealed no histopathological effects, ocular histological effects were observed. Outer retinal degeneration, characterized as a loss of 25–30% of photoreceptors, was observed along with a decrease (20–35%) in retinal thickness. Ocular effects were also observed in the 90-day subchronic study:

delays in pupillary dilation were observed at weeks 8 and 12 in rats exposed to 30 mg/kg-day. These delays were reported to be unilateral in nature, not consistent across the treatment period, and were low in incidence. No ocular histopathological results were reported for the 90-day subchronic study. Thus, while some ocular effects were observed following PFBA exposure, there were inconsistencies in effects across durations, with greater effects following short-term exposures than in subchronic exposures. This limited the interpretability of the observed effects.

3.3. CARCINOGENICITY

No human or animal studies were available to inform the potential for PFBA exposure to cause genotoxicity or cancer.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

The currently available studies provide ***sufficient evidence*** for hazard with respect to the potential for thyroid, liver, and developmental effects in humans given relevant PFBA exposure conditions. These judgments are based on data from short-term (28-day exposure), subchronic (90-day exposure), and developmental (17-day gestational exposure) oral-exposure studies in rodents. Further characterizations of the exposure conditions potentially relevant to the indicated hazards are provided in Section 5. A summary of the justifications for the evidence integration judgments for each of the main hazard sections is provided below and organized by health effect and further summarized in Table 4-1.

The judgment of ***sufficient evidence*** to indicate the potential for PFBA exposure to cause thyroid toxicity in humans is based primarily on a short-term and subchronic study in male rats reporting a consistent and coherent pattern of hormonal, organ weight, and histopathological changes, generally at PFBA exposure levels ≥30 mg/kg-day, although some notable effects were observed at 6 mg/kg-day. For effects to the thyroid in exposed animals, PFBA-induced perturbations were observed in one species and sex (male rats) across two different exposure durations (short-term and subchronic). Consistent, dose-dependent decreases in total and free T4 were observed independent of any effect on TSH, which is a pattern of hormone perturbation that is consistent with hypothyroxinemia. Additionally, increased thyroid weights and increases in thyroid follicular hypertrophy were observed. However, while the observed thyroid histopathological changes support the potential for PFBA to disrupt the thyroid hormone economy, rodents are uniquely sensitive to the development of thyroid follicular hypertrophy and tumor development (U.S. EPA, 1998) compared with humans. The similarities in the production and regulation of thyroid hormone homeostasis between rodents and humans, and the consistency of the observed pattern of effects with changes observed in humans, the effects in rodents were considered relevant to humans. A detailed discussion of thyroid effects is included in Section 3.2.1.

The judgment of ***sufficient evidence*** to indicate the potential for PFBA exposure to cause hepatic toxicity in humans, given relevant exposure circumstances, is based primarily on a series of short-term, subchronic, and developmental studies in rats and mice, generally exhibiting effects at PFBA exposure levels ≥30 mg/kg-day. The PFBA-induced effects were observed in two species and one sex (male rats and mice) across multiple exposure durations (short-term, subchronic, and gestational). Consistent, coherent, dose-dependent, and biologically plausible effects were observed for increased liver weights and increased incidences of hepatic histopathological lesions.

Supporting the biological plausibility and human relevance of these effects is mechanistic information that suggests non-PPARα modes of action may explain some of the observed effects in exposed rodents, and that observed effects may be precursors to clearly adverse health outcomes such as steatosis. Supporting this conclusion is evidence from other PFAS that have consistently shown that longer chain PFAS can activate non-PPARα nuclear receptors, including PPARγ, CAR, and PXR, although there is uncertainty in inferring a similar relationship for the short-chain, PFBA.

The judgment of ***sufficient evidence*** to indicate the potential for PFBA exposure to cause developmental effects in humans (given relevant exposure circumstances), including increased prenatal effects (full-litter resorptions) and delays in developmental milestones (days to eye opening, vaginal opening, and preputial separation) without effects on fetal (pup) survival or growth is based on a single study in mice exposed gestationally to PFBA. Although the observed developmental effects due to PFBA exposure were investigated in only one *high* confidence study, they demonstrate a constellation of effects affecting the developing organism that is internally coherent (within-study) and consistent across related PFAS compounds, including PFBS and PFOS.

There was ***insufficient evidence*** to determine whether PFBA exposure has the potential to cause reproductive toxicity (in adults), effects on hematological or clinical chemistry markers, ocular effects, changes in blood pressure, or effects on renal function in humans. Other potential health outcomes have not been evaluated in the context of PFBA exposure. Most notably, studies focusing on the potential for PFBA exposure to affect the immune system, the thyroid or nervous system in developing organisms, or mammary glands represent important data gaps given the associations observed for other PFAS, such as PFBS, PFOA and PFOS (ATSDR, 2018b, U.S. EPA).

Table 4-1. Evidence integration summary for health effects with *sufficient evidence* for hazard

Evidence stream scenarios	Evidence in studies of humans[a]	Evidence in animal studies[a]	Inferences across evidence streams
No Studies, or Low Confidence or Conflicting Evidence	**Developmental** **Hepatic** **Thyroid**		
Strong Mechanistic Evidence Alone			
One High or Medium Confidence Apical Study without Supporting or Conflicting Evidence		**Developmental**	**Developmental:** presumed human relevance; unknown MOA
Multiple High or Medium Confidence Apical Studies with Some Inconsistency or Important Uncertainties		**Thyroid** **Hepatic**	**Thyroid:** presumed human relevance; unknown MOA **Hepatic:** Mechanistic evidence supports human relevance; MOA involves PPARα-dependent and independent pathways
Multiple High or Medium Confidence Apical Studies with Strong Support (e.g., MOA understanding supporting biological			

Stronger Evidence Stream Scenarios

[a]May include consideration of studies informing biological plausibility: for studies in humans, this includes studies of human tissues or cells, and other relevant simulations; for animal studies, this includes ex vivo and in vivo experiments, and other relevant simulations.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

No human or animal studies were available to inform the potential for PFBA exposure to cause genotoxicity or cancer.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFESTAGES

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

In adult animals exposed subchronically, PFBA exposure was consistently observed to elicit stronger responses in male rats compared with female rats. The reason for this sex dependence is most likely due to differences in toxicokinetics between males and females. The serum half-life of PFBA following a single oral dose of 30 mg/kg-day is approximately 9 hours, compared to 2 hours for females (see Table 3-1). Urinary excretion rates are much faster in female rodents compared to male rodents (approximately 50-90% faster), possibly due to renal reabsorption of PFBA in male rats by organic anion transporters. Further, specifically relevant to hepatic effects, the liver concentrations of PFBA following subchronic exposure to 30 mg/kg-day is approximately 16-fold higher in males than in females [16.09 vs. 0.91 mg/kg-day; Butenhoff et al. (2012); van Otterdijk (2007a, 2007b)]. No difference in serum half-lives was observed in monkeys exposed to a single i.v. dose of 10 mg/kg: 1.61 hours for males versus 2.28 hours in females (Chang et al., 2008). Also, while quantitative data were not provided, serum excretion half-lives were reported to not differ between males and females in the one occupational study available (Chang et al., 2008). Additionally, effects on liver weight were observed in pregnant and nonpregnant mice (Das et al., 2008). Developmental effects were also observed in female fetuses/neonates (full litter resorption, delayed eye opening, delayed vaginal opening) and male fetuses/neonates [full litter resorption, delayed eye opening, delayed preputial separation; Das et al. (2008)], with no clear difference in sensitivity. Therefore, while there does appear to be a clear sex-dependence for some PFBA-induced health effects in adult rodents, the observed lack of sex-specific sensitivity for other effects in adult and immature rodents and the apparent lack of toxicokinetic differences between sexes in primates (and a single human occupational study) preclude the identification of males as a broadly sensitive subpopulation for PFBA-induced health effects in humans.

Lastly, given the effects seen in pregnant mice (increased liver weights, full-litter resorptions) and the developing organism (fetal/postnatal death and delays in time to eye opening, vaginal opening, and preputial separation), it is possible that pregnancy and early life represent two sensitive lifestages to PFBA exposure.

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

The PFBA oral toxicity database provides ***sufficient evidence*** that oral exposure to PFBA has the potential to cause adverse thyroid, hepatic, and developmental effects in humans based on multiple *high* and *medium* confidence animal toxicity studies (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a, b; Permadi et al., 1993; Permadi et al., 1992).

There are no available human or animal toxicity studies to inform the potential for PFBA to cause adverse effects via inhalation. Likewise, there are no human or animal studies available to inform the potential for oral or inhalation exposure to cause genotoxicity or cancer.

5.2. NONCANCER TOXICITY VALUES

The noncancer oral toxicity values (i.e., reference doses) derived in this section are estimates of an exposure for a given duration to the human population (including susceptible subgroups and/or lifestages) that is likely to be without an appreciable risk of adverse health effects over a lifetime. The RfD derived in Section 5.2.2 corresponds to chronic, lifetime exposure and is the primary focus of this document. In addition, RfDs specific to each organ or system are provided (organ/system-specific RfDs), as these toxicity values may be useful in some contexts (e.g., when assessing the potential cumulative effects of multiple chemical exposures occuring simultaneously). Less-than-lifetime, subchronic toxicity values (including the subchronic RfD and organ/system-specific subchronic RfDs), which are derived in Section 5.2.2, correspond to exposure durations of between 30 days and 10% of the life span in humans. These subchronic toxicity values are presented because they may be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures).

5.2.1. Oral Noncancer Toxicity Values

5.2.2. Oral Reference Dose (RfD) Derivation

Study Selection

Given the identified hazards relating to thyroid, liver, and developmental effects, two *high* confidence studies reporting these effects were selected for the purpose of deriving an oral reference dose (RfD). The subchronic (Butenhoff et al., 2012) and developmental (Das et al., 2008) studies were selected to support RfD derivation given the ability of these study designs to estimate potential effects of lifetime exposure, as compared to short-term or acute studies. Both studies

used rats or mice as the laboratory animal species and used vehicle-exposed controls. Animals were exposed to reagent-grade NH_4^+PFBA (reported as >98% pure or as a 28.9% solution in distilled water; impurities not reported) via a relevant route (oral administration via gavage) and for a relevant duration (90 days or GD 1−17) of exposure.

Also available in the PFBA database are two short-term (i.e., 28 day) studies that provide information on the hepatic and thyroid effects of PFBA (Butenhoff et al., 2012; Foreman et al., 2009; van Otterdijk, 2007a). While these studies were used for qualitative hazard identification purposes (they supported the final evidence integration judgments for these endpoints and thus were critical for identification of these endpoints for dose-response analysis), they were ultimately not considered for use as the basis of the quantitative dose-response analyses. When developing a lifetime reference value, chronic or subchronic studies (and studies of developmental exposure) are generally preferred over short-term or acute studies. Likewise, subchronic and developmental studies are preferred when developing a subchronic RfD. However, while short-term studies were not used for the identification of points of departure (PODs), they were deemed relevant to decisions regarding the application of uncertainty factors for the purpose of derivation of toxicity values (see "Derivation of Candidate Toxicity Values" below).

In the liver, a pattern of adverse effects has been observed in mice and rats, with PFBA exposure resulting in increased liver weights (absolute and relative) in adult exposed animals (Butenhoff et al., 2012; Das et al., 2008; van Otterdijk, 2007b) in conjunction with histopathological lesions [i.e., hepatocellular hypertrophy; Butenhoff et al. (2012); van Otterdijk (2007b)]. As discussed in Section 3.2.2, the observed effects in the livers of exposed experimental animals are judged to be relevant to human health as evidenced by the observation of increased liver weights and increased hepatocellular hypertrophy in mice expressing human PPARα and increased vacuolation in humanized-PPARα and PPARα null mice. This strongly suggests a multi-faceted mode of action for liver effects consisting, in part, of non-PPARα mechanisms operant in humans (noting that activation of human PPARα by PFBA also results in hepatic changes). Further, the observation of vacuolation specifically indicates that the observed effects are possible precursors to clearly adverse downstream effects such as steatohepatitis, fibrosis, and cirrhosis. Thus, the observed pattern of liver effects in PFBA-exposed animals are judged to be adverse, relevant to human health, and appropriate to consider for reference value derivation. For the purposes of dose-response modeling, relative liver weights were chosen over absolute liver weights. Although body weights were not affected on average in any PFBA study, relative liver weights are still preferred because this measure of effect accounts for any changes in body weights that occur in individual animals (changes in body and liver weights are associated). For liver hypertrophy, severity information was available in addition to raw incidence. Therefore, both the total incidence of lesions and the incidence of "slight" severity lesions were considered for dose-response analysis.

A pattern of adverse effects in the thyroid is also observed in exposed rats that consists of decreased free and total T4 levels and increased incidence of thyroid follicular hypertrophy and

hyperplasia (Butenhoff et al., 2012; van Otterdijk, 2007b). Decreased thyroid hormone levels are judged to be relevant to human health given the many similarities in the production, regulation, and functioning of thyroid hormones between rodents and humans. For effects on T4, total T4 was chosen for dose-response modeling over free T4, based on the lack of data in the control group for free T4 (given insufficient volume for the assay). In addition, rodents are more sensitive to increases in thyroid follicular hypertrophy and hyperplasia, and thus changes in thyroid hormone levels are considered more relevant for deriving human health toxicity values. For this reason, the increases in thyroid hypertrophy/hyperplasia were not considered further for RfD derivation. Note, however, that decreased total T4 was seen at 6 mg/kg-day in rats exposed to PFBA for 28 days, but not in rats exposed for 90 days (where it was only observed at 30 mg/kg-day). However, this discrepancy may be explained by the difference in serum concentrations following 28- and 90-day exposures. Serum free T4 concentrations were higher in the 6 mg/kg-day dose group following 28-day exposures (24.7 µg/mL) versus 90-day exposures (6.1 µg/mL). This difference was reversed in the 30 mg/kg-day dose group for the 28-day and 90-day animals, being 38.0 µg/mL versus 52.2 µg/mL, respectively. Because it is likely that serum concentrations following chronic exposures will resemble those following subchronic exposures (more so than serum concentrations following short-term exposures), the effects on total T4 following subchronic exposure are deemed most appropriate for deriving lifetime and subchronic toxicity values.

Effects on the developing reproductive system included delays in vaginal opening and preputial separation (Das et al., 2008). EPA's Reproductive Toxicity Guidelines (U.S. EPA, 1996) state that "[s]ignificant effects on … age at puberty, either early or delayed, should be considered adverse…" and thus supports considering these endpoints for reference value derivation. Delayed eye opening, also seen following PFOA exposure, is identified as a "simple, but reliable" indicator of impaired postnatal development by Das et al. (2008). Further, a delay of eye opening is a form of visual deprivation that prevents ocular visual signals from reaching the brain during a critical period of development (Wiesel, 1982). There is a time-sensitive critical period in the development of visual system during which the architecture of the visual cortex is established (Espinosa and Stryker, 2012), and accordingly, any alterations of the visual system during that time is considered adverse. Evidence in humans further supports the adversity of this endpoint given that infants born with congenital cataracts that interfere with the processing of visual signals have permanent visual defects if the cataracts are removed after the critical window for visual development (Wiesel, 1982). Therefore, any delay in the development of sight or development of the visual neurological system results in permanent functional decrements and is relevant to human health.

Full-litter resorption is a clear indicator of pregnancy loss and/or prenatal fetal mortality (note: number of implants and live fetuses were unaffected). Additionally, although the effect was not statistically significant as a result of pair-wise statistical tests, decreased survival to postnatal Day 21 is also a clear marker of fetal and neonatal mortality, and the magnitude of decreased survival observed (6%) is in excess of the response level typically used in benchmark dose

modeling of this type of developmental endpoint (i.e., 5%). Alongside the potential coherence of decreased postnatal survival with other effects on developmental maturation, this supports consideration of this endpoint for deriving PODs. As such, all observed gestational and/or developmental effects were considered adverse and suitable for reference value derivation.

Therefore, all outcomes relevant to the identified hazards (see Table 5-1) from the selected studies advanced for dose-response (i.e., excluding the short-term studies) were evaluated for toxicity value derivation as described below and in Appendix D.

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

Endpoint	Reference[a]	Exposure duration	Species/sex	POD derivation[b]
Liver				
Increased relative liver weight	Butenhoff et al. (2012)	Subchronic	S-D rat, male	Yes
		Gestational	CD-1 mouse, female	Yes
Increased absolute liver weight		Subchronic	S-D rat, male	No
		Gestational	CD-1 mouse, female	No
Increased liver hypertrophy		Subchronic	S-D rat, male	Yes
Thyroid				
Decreased total T4	Butenhoff et al. (2012)	Subchronic	S-D rat, male	Yes
Decreased free T4		Subchronic	S-D rat, male	No
Increased thyroid follicular hypertrophy		Subchronic	S-D rat, male	No
Developmental				
Increased full-litter resorptions	Das et al. (2008)	Gestational	CD-1 mouse, female	Yes
Decreased fetal/postnatal survival		Gestational	CD-1 mouse, male and female	Yes
Delayed eye opening		Gestational	CD-1 mouse, male and female	Yes
Delayed vaginal opening		Gestational	CD-1 mouse, female	Yes
Delayed preputial separation		Gestational	CD-1 mouse, male	Yes

[a]Both the Butenhoff et al. (2012) and Das et al. (2008) studies were rated as *high* confidence.
[b]See text for rationale for inclusion/exclusion from POD derivation.

Estimation or Selection of Points of Departure (PODs)

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR to represent a minimal, biologically significant level of change. The BMD technical guidance (U.S. EPA, 2012a) sets

up a hierarchy by which BMRs are selected, with the first and preferred approach using a biological or toxicological basis to define what minimal level of response or change is biologically significant. If that biological or toxicological information is lacking, then the BMD technical guidance recommends BMRs that can be used instead, specifically a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data. The BMRs selected for dose-response modeling of PFBA-induced health effects are listed in Table 5-2 along with the rationale for their selection.

Table 5-2. Benchmark response levels selected for benchmark dose (BMD) modeling of perfluorobutanoic acid (PFBA) health outcomes

Endpoint	BMR	Rationale
Liver		
Increased relative liver weight	10% relative deviation	A 10% increase in liver weight has generally been considered a minimally biologically significant response.
Increased liver hypertrophy	10% extra risk	Recommended as the standard BMR for dichotomous endpoints (U.S. EPA, 2012a) in the absence of information on a more appropriate, biologically based BMR; the endpoint is not considered a "frank" effect and does not support using a lower BMR.
Thyroid		
Decreased total T4	1 standard deviation	Toxicological evidence that would support identification of a minimally biologically significant response is lacking in adult animals. Further, evidence for the level of response in thyroid hormones associated with neurodevelopmental effects is inconsistent, with decreases of 10−25% being identified in human and rodent studies (Gilbert et al., 2016; Gilbert, 2011; Haddow et al., 1999). The BMD technical guidance (U.S. EPA, 2012a) recommends a BMR equal to 1 standard deviation for continuous endpoints when biological information is not sufficient to identify the BMR. In this case, the BMR based on 1 SD from the Butenhoff et al. (2012) study corresponds to a ~13% decrease, consistent with the levels of decreased T4 associated with neurodevelopmental decrements, thus strengthening the rationale for using a BMR = 1 SD for this endpoint.
Developmental		
Increased full-liter resorptions	5% extra risk	A 5% extra risk is recognized as appropriate for endpoints (i.e., pregnancy loss and/or fetal mortality) that are considered to be "frank" effects (U.S. EPA, 2012a). Further, both endpoints are observed in a nested developmental toxicity study which are generally recognized as supporting BMRs of 5% extra risk.
Decreased postnatal survival		

Endpoint	BMR	Rationale
Delayed eye opening	5% relative deviations	Biological evidence supports identification of a minimally significant decrease of visual input (1 d delayed eye opening) due to hypothyroxinemia during a critical period of retinal development (Espinosa and Stryker, 2012). Delays of 1 d in eye opening reduces the time available for visual cortex development related to orientation selectivity by approximately 20% (Espinosa and Stryker, 2012) and corresponds to ~6% change in Das et al. (2008). Further, delays in vaginal opening greater than or equal to 2 d have been used to define biologically relevant responses previously (U.S. EPA, 2013) and this magnitude in delay in Das et al. (2008) is also ~6%. Both levels of response are consistent with a 5% relative deviation. Lastly, a 5% change in other markers of growth/development in gestational studies (e.g., fetal weight) has generally been considered a minimally biologically significant response level.
Delayed vaginal opening		
Delayed preputial separation		

Where modeling was feasible, the estimated BMDLs were used as points of departure (PODs, see Table 5-4). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix D. Where dose-response modeling was not feasible, or adequate modeling results were not obtained, NOAEL or LOAEL values were identified based on biological rationales when possible and used as the POD. For example, for liver weight, a NOAEL would be chosen as the dose below that which causes at least a 10% change, consistent with the rationale for the selection of the BMR for that endpoint. If no biological rationale for selecting the NOAEL/LOAEL is available, statistical significance was used as the basis for selection. The PODs (based on BMD modeling or NOAEL/LOAEL selection) for the endpoints advanced for dose-response analysis are presented in Table 5-4.

Approach for Animal-Human Extrapolation of Perfluorobutanoic Acid (PFBA) Dosimetry

The PFAS protocol (see "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 scientifically sound and/or sufficiently validated 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 deriving oral reference doses (U.S. EPA, 2011). This hierarchy preferentially prioritizes adjustments that result in reduced uncertainty in the dosimetric adjustments (i.e., preferring chemical-specific values to underpin adjustments vs. use of default approaches).

No PBPK model is available for PFBA. But, as toxicokinetic data for PFBA exist in relevant animals (rats, mice, and monkeys) and humans, a data-informed extrapolation approach for estimating the dosimetric adjustment factor (DAF) can be used. Briefly, the ratio of the clearance (CL) in humans to animals, CL_H:CL_A, can be used to convert an oral dose-rate in animals

(mg/kg-day) to a human equivalent dose rate. Assuming the exposure being evaluated is low enough to be in the linear (or first order) range of clearance, the average blood concentration (C_{AVG}) that results from a given dose is calculated as:

$$C_{\text{AVG}}\ (\text{mg/mL}) = \frac{f_{\text{abs}} \times dose\ (\text{mg/kg/h})}{CL\ (\text{mL/kg/h})} \qquad (5\text{-}1)$$

where f_{abs} is the fraction absorbed and *dose* is average dose rate expressed at an hourly rate. Assuming equal toxicity given equal C_{AVG} in humans as mice or rats, and that f_{abs} is the same in humans as animals, the equitoxic dose (i.e., the human dose that should yield the same blood concentration [C_{AVG}] as the animal dose from which it is being extrapolated) is then calculated as follows:

$$HED = \frac{POD}{CL_{\text{A}}/CL_{\text{H}}} = POD \times \frac{CL_{\text{H}}}{CL_{\text{A}}} \qquad (5\text{-}2)$$

Thus, the DAF is simply CL_H:CL_A, the ratio of clearance in humans to clearance in the animal from which the POD is obtained.

However, clearance values are not reported for humans in the one toxicokinetic study available for PFBA (Chang et al., 2008). As clearance is a measure of average excretion, in order to calculate clearance, one also needs to evaluate a companion variable, the volume of distribution (V_d), which in turn requires a measure of total exposure or dose. Chang et al. (2008) did not report the V_d for humans. However, Chang et al. (2008) did report V_d for cynomolgus monkeys. Assuming that V_d for monkeys is a reasonable surrogate for V_d in humans, clearance, normalized to body weight, can be calculated as follows:

$$CL\ (\text{mL/kg-h}) = \ln(2) \times \frac{1}{t_{1/2}(\text{h})} \times V_{\text{d}}\ (\text{mL/kg}) \qquad (5\text{-}3)$$

As $t_{1/2}$ is required in the calculation of *CL,* these values must be determined from the data presented for humans in Chang et al. (2008). Chang et al. (2008) reported values for human subjects from two 3M facilities: Cottage Grove, MN and Cordova, IL. Cottage Grove had three subjects, which were not identified by gender. Cordova, IL had nine subjects, two of which were identified as female. The half-lives for those two women fell among the values of the other subjects (Cottage Grove and men from Cordova). Considering the minimal difference in $t_{1/2}$ observed between male and female monkeys, it was assumed that the available data were not sufficient to distinguish male and female humans. The analytic method used replaced concentration measurements below the limit of quantitation (LLOQ) with LLOQ/$\sqrt{2}$. For individuals where only two measurements were made, the resulting half-life estimate was then highly sensitive to this assumption. The two known female subjects (Cordova, IL), one male subject from Cordova, IL, and one subject from Cottage Grove, MN fell into this category; half-lives for these four subjects were

not used. Additionally, the last time point for Subject 2 from Cottage Grove, MN fell below the LLOQ and was also excluded from $t_{1/2}$ estimation. The mean and median $t_{1/2}$ values estimated from these data (8 total subjects, 20 observations) were 81.8 and 67.5 hours, respectively. Mixed effects modeling confirmed this half-life, estimating an approximate half-life of 67.9 hours when accounting for clustering (see Appendix C).

As discussed in Section 3.1.4, using the default method of $BW^{0.75}$ scaling and standard species BWs of 0.25 kg in rats and 70 kg in humans, the half-life in humans would be predicted to be 4.1 times greater than rats. Given half-lives of 9.22 and 1.76 h in male and female rats, one would then predict half-lives of 37.8 h in men and 7.2 h in women, much less than that estimated using the human data available from Chang et al. (2008). Scaling according to $BW^{0.75}$ is not understood to be based on data for this class of chemicals (i.e., with serum binding and clearance mechanisms known to occur for PFAS) and would lead to a lower prediction of human health risk at a given exposure (possibly up to an order of magnitude under-prediction). Therefore, use of $BW^{0.75}$ as an alternative means of extrapolation is not considered further here.

Using a value of 484.5 mL/kg for V_d for humans [average of male and female V_d values in monkeys, 526 and 443 mL/kg, respectively, Table 4, Chang et al. (2008)] and 67.9 hours for $t_{1/2}$ in male humans, CL in humans is estimated to be 4.95 mL/kg-h. See Table 5-3 for the DAFs for converting rat and mice PODs to human equivalent doses (HEDs).

Table 5-3. Rat, mouse, and human clearance values and data-informed dosimetric adjustment factors

Sex	Species	Animal *CL* (mL/kg-h)	Human *CL* (mL/kg-h)	DAF (CL_H:CL_A)
Male	Rat	23.63[a]	4.95[c]	0.209
	Mouse	13.33[b]		0.371
Female	Rat	102.89[a]		0.048
	Mouse	35.65[b]		0.139

Data from Tables 2, 3, 5, and 6 of Chang et al. (2008).
[a]Average CL values reported for oral and i.p. exposures reported in Table 2 of Chang et al. (2008).
[b]Average CL values reported for the 10- and 30-mg/kg dose groups reported in Table 3 of Chang et al. (2008); the CL value for the 100-mg/kg dose group was excluded as it was ~threefold and ~twofold higher for males and females, respectively, than the values reported at 10 or 30 mg/kg. This is perhaps due to saturation of renal absorption or serum binding.
[c]CL value for humans (male and female) as described above.

Therefore, human equivalent dose (HED) for considered health effects was calculated as follows, using relative liver weight observed in male rats in the subchronic Butenhoff et al. (2012) study as an example:

$$HED = POD\ (\text{mg/kg-d}) \times \frac{CL\ human\ (mL/kg\text{-}h)}{CL\ animal\ (mL/kg\text{-}h)} \qquad (5\text{-}4)$$

$$HED = 9.6\ (\text{mg/kg-d}) \times \frac{4.95\ (\text{mL/kg-h})}{23.63\ (\text{mL/kg-h})} = 2.01\ (\text{mg/kg-d})$$

There is uncertainty in applying this dosimetric approach given that the volume of distribution (V_d) was not measured in humans and in order to estimate clearance in humans, the human V_d was assumed to be equal to that in monkeys. One alternative approach to using the ratio of clearance values for animal:human dosimetric adjustments is to use the measured serum concentrations from toxicological studies as BMD modeling inputs and then to use the estimated human clearance values to calculate the HED. However, it is interpreted that there is a greater degree of uncertainty in this approach compared to the ratio of clearance values approach outlined above. First, the measured serum concentrations were reported to be taken 24 hours after the last exposure in the developmental toxicity study (Das et al., 2008) and was likely done so in the subchronic toxicity study (Butenhoff et al., 2012; van Otterdijk, 2007b) as well. Given the relatively short half-life of PFBA measured in mice and rats, it is likely that this end-of-exposure measurement of serum concentrations is not reflective of the average serum concentrations experienced by exposed animals. For example, the reported serum levels (see Section 2.1.1) in female mice of Das et al. (2008) did not correlate at all with exposure levels. Also, in order to estimate the HED in the absence of a validated PBPK model, the resulting POD (in units of serum concentrations) would need to be multiplied by the estimated human clearance value. Thus, in addition to the uncertainty in using end-of-exposure serum concentrations not reflective of average exposures, this approach would suffer from the same uncertainty surrounding the assumption that human and monkey volumes of distribution are equal in addition to the uncertainty in the human half-life. Therefore, the ratio of clearance values is considered to have less uncertainty than either serum concentration-based BMD modeling or use of default allometric dosimetric adjustments and this is the approach used.

Table 5-4 presents the PODs and estimated POD_{HED} values for the thyroid, liver, and developmental toxicity endpoints.

Table 5-4. Points of departure (PODs) considered for use in deriving candidate reference values for perfluorobutanoic acid (PFBA)

Endpoint/reference	Species/strain/sex	POD type/model	POD (mg/kg-d)	POD_{HED}[a] (mg/kg-d)
Increased relative liver weight (Butenhoff et al., 2012)	S-D rat, male	$BMDL_{10RD}$ Exp3 (LN-CV)	9.6	2.0
Increased relative liver weight (Das et al., 2008)	CD-1 mouse, P_0 female	$BMDL_{10RD}$ Exp4 (CV)	15	2.1
Increased liver hypertrophy[b] (Butenhoff et al., 2012)	S-D rat, male	$BMDL_{10ER}$ Weibull	5.4	1.1
Decreased total T4 (Butenhoff et al., 2012)	S-D rat, male	NOAEL[c] (15% decrease)	6	1.3
Increased full-litter resorption (Das et al., 2008)[d]	CD-1 mouse, P_0 female	$BMDL_{5ER}$ Log-Probit	21.2	3.0
Decreased postnatal survival (Das et al., 2008)[e]	CD-1 mouse, F_1 male/female	$BMDL_{5ER}$[d] Nested-Logistic	185.0	25.7
Delayed eyes opening[e] (Das et al., 2008)	CD-1 mouse, F_1 male/female	$BMDL_{5RD}$ Hill (CV)	4.9	0.7
Delayed vaginal opening[e] (Das et al., 2008)	CD-1 mouse, F_1 female	$BMDL_{5RD}$ Hill (CV)	3.8	0.5
Delayed preputial separation[e] (Das et al., 2008)	CD-1 mouse, F_1 male	$BMDL_{5RD}$ Exp3 (CV)	179.1	24.9

CV = constant variance.

[a]See discussion in "Approach for Animal-Human Extrapolation of PFBA Dosimetry" section for details on HED.

[b]Modeling results for all lesions are used here given greater model uncertainty when modeling only "slight" lesions (see Appendix D).

[c]No models provided adequate fit to the mean when using constant or nonconstant variance or the log-normal distribution.

[d]The Nested-Logistic model using no litter-specific covariate but accounting for intralitter correlation was the selected model; while this model estimated a BMD above the highest dose group in this data set (423 mg/kg-day vs. 350 mg/kg-day). The BMDL estimated was within the dose range (185 mg/kg-day). Given that the extrapolation above the dose range was modest (~20%) and the BMDL was within the dose range, the modeling results were deemed appropriate for use in POD derivation.

[e]All HED calculations used the DAF for female mice given exposures were to the pregnant animals.

Derivation of Candidate Toxicity Values for the Oral Reference Dose (RfD)

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002)and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* U.S. EPA (1994), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFBA. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as designated UF to be applied to the candidate POD_{HED} values are listed in Table 5-5. As discussed below, the short-term studies of thyroid and hepatic effects after PFBA exposure were considered for use in UF selection.

Table 5-5. Uncertainty factors for the development of the candidate values for perfluorobutanoic acid (PFBA)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$ = 3.16 ~3) is applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between mice or rats and humans following oral NH_4^+PFBA/PFBA exposure. Some aspects of the cross-species extrapolation of toxicokinetic processes have been accounted for by calculation of an HED through application of a DAF based on animal and human half-lives; however, some residual toxicokinetic uncertainty and uncertainty regarding toxicodynamics remains. Available chemical-specific data further support the selection of a UF of 3 for PFBA; see text below for further discussion.
UF_H	10	A UF_H of 10 is applied for interindividual variability in the absence of quantitative information on the toxicokinetics and toxicodynamics of NH_4^+PFBA/PFBA in humans.
UF_S	10 1	A UF_S of 10 is applied to endpoints observed in the subchronic study (Butenhoff et al., 2012; van Otterdijk, 2007b) for the purposes of deriving chronic toxicity values. See additional discussion on this decision below. A UF_S of 1 is applied to endpoints observed in the developmental toxicity study (Das et al., 2008); the developmental period is recognized as a susceptible lifestage where exposure during certain time windows (e.g., pregnancy and gestation) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991).
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, although the PFBA database is relatively small, *high* confidence subchronic and developmental toxicity studies are available in mice and rats. No multigenerational reproductive toxicity study is available for PFBA; however, while some developmental delays were observed in exposed animals, they were seen at HEDs comparable to other endpoints. Therefore, the lack of a multigenerational reproductive toxicity study is not expected to result in a large underestimation of toxicity (i.e., a much lower POD is not considered likely). Additionally, given the observation of effects on the thyroid in adult animals and developmental delays in gestationally exposed offspring, the database is limited by the lack of measures of thyroid toxicity in gestationally exposed offspring and the lack of a developmental neurotoxicity study. However, the difference between thyroid hormone effects in adults and neonates was less than threefold for the structurally related compound PFBS. This suggests that the recommended UF_D of 3 for PFBA is sufficient to account for the lack of measures of thyroid toxicity (and potential downstream neurodevelopmental effects) in gestationally exposed animals. Lastly, as potential immunotoxicity and mammary gland effects are of increasing concern across several members of the larger PFAS family, the lack of studies evaluating these outcomes following PFBA exposure are also limitations in the database.
UF_C	Table 5-7	Composite uncertainty factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$.

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002), the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans, and accounts for uncertainty regarding the toxicokinetic and toxicodynamic differences across species. As is usual in the application of this uncertainty factor, the toxicokinetic uncertainty is mostly accounted for through the application of dosimetric approaches for

estimation human equivalent doses (see Section 4.2.2). This leaves some residual uncertainty around the toxicokinetics and the uncertainty surrounding toxicodynamics. Typically, a 3-fold UF is applied for this uncertainty in the absence of chemical-specific information. This is the case for the thyroid and developmental endpoints. For the liver endpoints, there is chemical-specific information that should be further considered in determining the most appropriate value for the UF_A to account for the uncertainty.

Foreman et al. (2009) investigated the response to PFBA exposure in PPARα wild-type, PPARα null, and hPPARα mice for hepatic effects and observed either that effects were generally equivalent in wild-type versus humanized mice (liver weight, liver hypertrophy, see Table 3-6 and Table 3-7), that wild-type mice exhibited effects that humanized mice did not (focal hepatic necrosis), and that PPARα null mice generally did not exhibit hepatic effects. Additionally, in vitro studies suggest that human cells or cells transfected with human PPARα were less sensitivity to PPAR activation than rodent cells or rodent PPARα (Rosen et al., 2013; Wolf et al., 2012; Bjork and Wallace, 2009; Wolf et al., 2008). If PPARα were the only operant mode of action for noncancer effects in the liver, this observation might support reducing the remaining portion of the UF_A to 1, as it could be argued that humans are not as sensitive as wild-type rats to the hepatic effects of PFBA exposure (note: without evidence to the contrary, as noted in the previous paragraph, the toxicodynamic portion of this UF is typically assigned a value of 3 on the assumption that responses manifest in humans could be more sensitive than those observed in animals). However, there is additional evidence presented in Foreman et al. (2009) and other studies (see Section 2.2.5) that indicates that non-PPARα modes of action appear to be active in the livers of exposed rats. Specifically from Foreman et al. (2009), vacuolation is reported in the livers of PPARα null and humanized mice, but not wild-type mice, although the degree to which null or humanized mice are more susceptible to this effect is hard to characterize given the results are presented qualitatively. Vacuolation (i.e., the accumulation of lipids) is an important precursor event in the development of steatosis, which itself is a precursor to other adverse conditions such as steatohepatitis, fibrosis, and cirrhosis. As discussed in Section 2.2.5, this observation of PFBA-induced effects independent of PPARα activation is supported by in vitro and in vivo data which shows that other PFAS can activate other forms of PPAR (i.e., PPARγ) and additional pathways (i.e., constitutive androstane receptor [CAR] or pregnane X receptor [PXR]). Given the observation of apical liver effects in humanized PPARα mice and the observation that other modes of action appear to contribute to potential liver toxicity, the observation that humanized PPARα mice exhibit diminished responses for some hepatic effects attributable to PPARα activation cannot alone determine the appropriate value of the toxicodynamic portion of the UF_A. Therefore, given the remaining uncertainty in additional modes of action (MOAs) that appear to be active in PFBA-induced liver effects, and the relative contribution of these MOAs to toxicity in humans as compared with rodents, the value of the UF_A was set to 3 for the purposes of deriving toxicity values for hepatic effects. No mode of action information is available for thyroid or developmental effects; in the absence of information

suggesting otherwise, as noted above a UF_A (3) is also applied to these endpoints to account for any residual toxicokinetic and toxicodynamic uncertainty.

The short-term studies of Butenhoff et al. (2012), van Otterdijk (2007a), and Foreman et al. (2009) were also considered for potential use in informing the selection of the UF_S. More specifically, for several of the outcomes from which PODs were derived, comparisons between short-term exposure and subchronic exposure appeared possible (i.e., because of the inherent similarities in study design and experimental conduct). When comparing short-term to subchronic PFBA exposure for liver weight and thyroid hormone measures, there was no apparent increased sensitivity with longer exposure duration in terms of the magnitude of the observed effects at the same tested doses or the lowest doses at which effects were observed. In addition, given the toxicokinetics of PFBA, steady state levels in potential target tissues may not substantially increase with increasing exposure duration (Butenhoff et al., 2012; van Otterdijk, 2007a, b). In these studies, the latter conclusion seemed to be dose dependent, as PFBA levels actually decreased with longer exposures when comparisons are made at 6 mg/kg-day (~25 to 14 µg/mL in serum and ~7.5 to 3.1 µg/g in liver comparing 28 to 90 days of exposure), whereas levels were either substantially increased or similar when comparisons are made at 30 mg/kg-day (~38 to 52 µg/mL in serum and ~17.4 to 16.1 µg/mL in liver comparing 28 to 90 days of exposure). This indicates perhaps that steady-state conditions have been reached in the livers of exposed rats after only 28 days of exposure. Initially, this indicates that increased durations of exposure may not elicit increased effects in the target tissue as the LOAEL for liver weights is 30 mg/kg-day for male rats exposed to either 28 or 90 days. When also considering results from Foreman et al. (2009), and basing comparisons on human equivalent external concentrations (see Table 5-6 below for modeling results and application of dosimetric adjustments), it appears that liver weight is affected at equivalent doses across mice and rats and durations of exposure in the available studies.

Table 5-6. Comparison of liver-weight effects across species and durations of exposure

Reference	Species/strain/sex	Duration	POD type/model	POD (mg/kg-d)	POD_{HED} (mg/kg-d)
Relative liver weight (Butenhoff et al., 2012)	S-D rat, male	90 d	NOAEL	6	1.25
Relative liver weight (Butenhoff et al., 2012)	S-D rat, male	28 d	$BMDL_{10}$, Exp4 (NCV)	6.34	1.33
Foreman et al. (2009)	Sv/129 WT mouse, male	28 d	LOAEL	35	1.29[a]
Foreman et al. (2009)	Sv/129 hPPARα mouse, male	28 d	$BMDL_{10}$, Hill (NCV)	4.41	1.66

[a]As this data set only supported identification of a LOAEL; the LOAEL-to-NOAEL uncertainty factor was applied to facilitate comparison to the other HEDs for liver-weight effects.

However, this is not the case for all liver effects. Histopathological evaluations of the liver in male rats exposed to PFBA for 90 days shows that hepatocellular hypertrophy occurs at 30 mg/kg-day, whereas hypertrophy only occurs at 150 mg/kg-day in male rats exposed for 28 days (Butenhoff et al., 2012; van Otterdijk, 2007a, b). Thus, it is readily apparent that, while liver concentrations are equivalent following 28- or 90-day exposures, prolonged exposure (i.e., 90 days vs. 28 days) elicits adverse effects in the liver. Thus, taking into account the increased potential for some effects in the liver with increasing durations of exposure, and the large uncertainty associated with the lack of data on whether the effects observed in the subchronic study worsen after chronic exposure, the UF_S was set to 10 for the purposes of the liver endpoints. With regard to the thyroid effects, although no increased sensitivity was observed between short-term and subchronic exposure durations, it is still possible that chronic exposures could elicit stronger responses; therefore, the default UF_S was retained for the thyroid endpoints.

The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor. For example, for relative liver weight in adult rats from Butenhoff et al. (2012), the candidate value is calculated as:

$$\text{Candidate value for PFBA (ammonium salt)} = BMDL_{10} \div UF_c \quad (5\text{-}5)$$

$$\text{Candidate value} = 2.0 \left({}^{mg}/_{kg\text{-}d}\right) \div 1{,}000$$

$$\text{Candidate value} = 0.002 \left({}^{mg}/_{kg\text{-}d}\right)$$

$$\text{Candidate value} = 2.0 \times 10^{-3} \left({}^{mg}/_{kg\text{-}d}\right)$$

The candidate value for the free acid of PFBA is calculated using the ratio of molecular weights, as follows:

$$\text{Candidate value for PFBA (free acid)} = \text{Candidate value (ammonium salt)} \times \left(\frac{\text{MW free acid}}{\text{MW ammonium salt}}\right) \quad (5\text{-}6)$$

$$\text{Candidate value} = 0.002\left({}^{\text{mg}}/_{\text{kg-d}}\right) \times \left(\frac{214}{231}\right)$$

$$\text{Candidate value} = 0.002\left({}^{\text{mg}}/_{\text{kg-d}}\right) \times 0.926$$

$$\text{Candidate value} = 0.0019\left({}^{\text{mg}}/_{\text{kg-d}}\right)$$

$$\text{Candidate value} = 2 \times 10^{-3}\left({}^{\text{mg}}/_{\text{kg-d}}\right)$$

Table 5-7. Candidate values for perfluorobutanoic acid (PFBA).

Endpoint	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Increased relative liver weight (Butenhoff et al., 2012)	2.0	3	10	10	1	3	1,000	2.0×10^{-3}
Increased relative liver weight (Das et al., 2008)	2.1	3	10	10	1	3	1,000	2.1×10^{-3}
Increased liver hypertrophy (Butenhoff et al., 2012)	1.1	3	10	10	1	3	1,000	1.1×10^{-3}
Decreased total T4 (Butenhoff et al., 2012)	1.3	3	10	10	1	3	1,000	1.3×10^{-3}
Increased full-liter resorption (Das et al., 2008)	3.0	3	10	1	1	3	100	3.0×10^{-2}
Decreased postnatal survival (Das et al., 2008)	25.7	3	10	1	1	3	100	2.6×10^{-1}
Delayed eyes opening (Das et al., 2008)	0.7	3	10	1	1	3	100	7.0×10^{-3}
Delayed vaginal opening (Das et al., 2008)	0.5	3	10	1	1	3	100	5.0×10^{-3}
Delayed preputial separation (Das et al., 2008)	24.9	3	10	1	1	3	100	2.5×10^{-1}

[a] All values presented in this table are for the ammonium salt of PFBA; to calculate RfDs for the free acid of PFBA, consult Equation 5-6 above

Selection of Lifetime Toxicity Value(s)

Selection of organ/system-specific oral reference doses (osRfDs)

From among the candidate values presented in Table 5-7, organ/system-specific RfDs (osRfDs) are selected for the individual organ systems identified as hazards in Section 3. The osRfD values selected were associated with increased liver hypertrophy for liver effects, decreased total T4 for thyroid effects, and developmental delays (based on the candidate value for delayed time to vaginal opening) for developmental effects. The confidence decisions about the study, evidence base, quantification of the POD, and overall RfD for these organ/system-specific values are fully described in Table 5-8, along with the rationales for selecting those confidence levels. In deciding overall confidence, confidence in the evidence base is prioritized over the other confidence decisions. The overall confidence in the osRfD for liver effects is *medium*, whereas the confidence in the osRfDs for thyroid effects and developmental effects is *medium-low*. Selection of the overall RfD is described in the following section.

Table 5-8. Confidence in the organ/system-specific oral reference doses (osRfDs) for perfluorobutanoic acid (PFBA)

Confidence categories	Designation	Discussion
Liver RfD = 1 × 10^{-3} mg/kg-d		
Confidence in study[a] used to derive osRfD	High	Confidence in the study (Butenhoff et al., 2012; van Otterdijk, 2007b) is *high* given the study evaluation results (i.e., rating of *good* or *adequate* in all evaluation categories) and characteristics that make it suitable for deriving toxicity values, including the 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 supporting this hazard	Medium	Confidence in the evidence base for liver effects is *medium* because there are consistent, dose-dependent, and biologically coherent effects on organ weight and histopathology observed in multiple *high* and *medium* confidence studies. While the available mechanistic evidence also supports the human relevance of observed effects, there is a sparsity of chemical-specific information. One *in vivo* PFBA study is available that indicates non-PPARα modes-of-action are active in the development of liver effects, but no PFBA-specific studies investigated activation of other PPAR isoforms or additional pathways. Another limitation of the database for PFBA-induced liver effects is the lack of a chronic duration study.
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 was based on PFBA-specific toxicokinetic information, the latter of which introduces some uncertainty. Another source of potential uncertainty is that hypertrophy was only observed in the high dose group; however, modeling lesions of "slight" severity only increased model uncertainty, and thus data for all lesions served as the basis for BMD modeling.

Overall confidence in osRfD	Medium	The overall confidence in the osRfD is *medium* and is primarily driven by *medium* confidence in both the evidence base supporting this hazard and the quantitation of the POD using BMD modeling of data from a *high* confidence study.
Thyroid RfD = 1×10^{-3} mg/kg-d		
Confidence in study[a] used to derive osRfD	High	Confidence in the study (Butenhoff et al., 2012; van Otterdijk, 2007b) is *high* given the study evaluation results (i.e., rating of *good* or *adequate* in all evaluation categories) and characteristics that make it suitable for deriving toxicity values, including the 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 supporting this hazard	Medium	Confidence in the evidence base for thyroid effects is *medium* because there were consistent and coherent effects on hormone levels, organ weights, and histopathology in a single *high* confidence study. Confidence is decreased by the lack of coherence between histopathology and TSH, as well as the increased sensitivity of rodents for developing thyroid hypertrophy compared to humans. Another limitation of evidence base for thyroid effects is the lack of chronic-duration or developmental study.
Confidence in quantification of the POD_{HED}	Medium-low	Confidence in the quantification of the POD and osRfD is medium-low given the POD was based on a NOAEL (BMD modeling was unable to provide an adequate fit to the data) and dosimetric adjustment was based on PFBA-specific toxicokinetic information, the latter of which introduces some uncertainty. It may be noted however that a 15% decrease in total T4 levels, upon which the NOAEL was based, is consistent with a 13% decrease in total T4 that would correspond to a response level based on 1SD. Therefore, this NOAEL may not be substantially more uncertain than a BMD-based POD. This supports a determination that the confidence in the quantification of the POD is medium-low.
Overall confidence in osRfD	Medium-low	The overall confidence in the osRfD is *medium-low* and is primarily driven *medium* confidence in the evidence base; however, the *medium-to-low* confidence in the quantitation of the POD does warrant decreasing the overall confidence in the osRfD
Developmental RfD = 5×10^{-3} mg/kg-d		
Confidence in study[a] used to derive osRfD	High	Confidence in the study (Das et al., 2008) is *high* given the study evaluation results (i.e., rating of *good* or *adequate* in all evaluation categories) and characteristics that make it suitable for deriving toxicity values, including the 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 supporting this hazard	Medium	Confidence in the evidence base for developmental effects is *medium*. Although data are only available in gestationally exposed animals in a single *high* confidence developmental toxicity study, there were coherent delays in multiple developmental milestones (general development, puberty).
Confidence in quantification of the POD_{HED}	Medium-low	Confidence in the quantification of the POD and osRfD is *medium*-to-*low* given the POD was based on BMD modeling and dosimetric adjustment was based on PFBA-specific toxicokinetic information, the latter of which introduces some uncertainty. Other sources of uncertainty are the use of dosimetric adjustments based on the ratio of adult toxicokinetic parameters, and that the derived BMDL is approximately nine-fold below the observed range of the data.
Overall confidence in osRfD	Medium-low	The overall confidence in the osRfD is *medium-low* and is primarily driven by the *medium*-to-*low* confidence in the quantitation of the POD given the extrapolation below the range of the observed data. Modeling data from a *high* confidence study in

		a *medium*-confidence evidence base does not fully mitigate the *medium*-to-*low* confidence in the actual modeling results in this case.

[a]All study evaluation details can be found on HAWC.

Selection of Overall oral reference dose (RfD) and confidence statement

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

Table 5-9. Organ/system-specific oral reference dose (osRfD) values for perfluorobutanoic acid (PFBA)

System	Basis	POD	UF_C	osRfD (mg/kg-d)	Confidence
Hepatic	Increased hepatocellular hypertrophy in adult male S-D rats	$BMDL_{HED}$ from Butenhoff et al. (2012)	1,000	1×10^{-3}	*Medium*
Thyroid	Decreased total T4 in adult male S-D rats	$NOAEL_{HED}$ from Butenhoff et al. (2012)	1,000	1×10^{-3}	*Medium-low*
Developmental	Developmental delays after gestational exposure in CD1 mice[a]	$BMDL_{HED}$ from Das et al. (2008)	100	5×10^{-3}	*Medium-low*

[a]POD based on delayed vaginal opening used to represent three developmental delays observed in the study.

From the identified human health hazards of PFBA exposure and the derived osRfDs for effects in the liver, thyroid, and developing organism, an overall ***RfD of 1 × 10^{-3} mg/kg-day based on increased liver hypertrophy and decreased total T4*** is selected. These osRfDs are selected as the overall RfD as they represent effects in two different organ systems with the same osRfD value, including the osRfD with the highest confidence of all osRfDs derived (i.e., the hepatic osRfD, with *medium* confidence). The other available osRfD was interpreted with *medium-low* confidence and had a higher osRfD value; thus, this was not selected. While the overall confidence in the individual liver and thyroid osRfDs do differ slightly (*medium* for increased liver hypertrophy and *medium-low* for decreased total T4), an overall confidence of *medium* is selected for the final RfD. This confidence level of *medium* is supported given the two osRfDs come from the same *high* confidence study and that the evidence bases for both organ systems were rated as *medium*. The difference in the overall confidence for the two osRfDs was driven primarily by the confidence in the quantification of the osRfDs: *medium* for increased liver hypertrophy and *medium-low* for decreased total T4. However, as noted in Table 5-8, the use of the NOAEL approach for decreased

total T4 is not substantially more uncertain than using the BMD approach given the relatively similar values in PODs that would be derived using either approach. Hence, although the NOAEL approach is conceptually associated with more uncertainty than the BMD approach, the confidence in the quantification of the total T4 POD was only downgraded to *medium-low*, rather than *low* in this specific case. This supports the determination of *medium* confidence for the overall RfD based on liver and thyroid effects.

Another consideration in selecting the overall RfD is the difference in composite uncertainty factors across the three candidate osRfDs. The composite UF for the liver and thyroid osRfDs was greater than that for developmental effects (1,000 vs 100), stemming from not applying a UF_S for the developmental effects. Application of the larger composite UF for liver and thyroid effects results in osRfDs that are five-fold lower than the developmental osRfD and thus protective of PFBA-induced effects to the developing organism. If the osRfD for developmental effects were chosen as the overall RfD based on the application of a smaller composite UF, this would raise concerns that it would not be protective against potential liver and thyroid effects. Lastly, the selection of the overall RfD based on liver and thyroid effects is further supported by the fact that the confidence in that RfD is *medium*, compared with *medium-low* for developmental effects.

Increased liver hypertrophy and decreased total T4 was only observed in male rats exposed to PFBA, thus possibly identifying males as a susceptible population. However, as discussed in Section 3.3, this observation in rats may be driven primarily by the observed sex-dependent differences in toxicokinetics in rats. No compelling information is available that supports a similarly strong sex dependence in toxicokinetics in humans. Therefore, this RfD is presumed to be equally applicable to both male and female humans.

Subchronic Toxicity Values for Oral Exposure (subchronic oral reference dose [RfD]) Derivation

In addition to providing RfDs for lifetime exposures in multiple systems, this document also provides an RfD for less-than-lifetime, subchronic-duration exposures. In the case of PFBA, all studies used to calculate the RfDs were subchronic or gestational in duration. Therefore, the method to calculate the subchronic RfDs is identical to that used for calculating the RfDs, minus the application of a 10-fold UF_S for the subchronic studies (see Table 5-6). The individual organs and systems for which specific candidate subchronic RfD values were derived were the liver, thyroid, and the developing organism (see Table 5-10).

Table 5-10. Candidate subchronic oral reference dose (RfD) values for perfluorobutanoic acid (PFBA)

Endpoint	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	RfD (mg/kg-d)
Increased relative liver weight (Butenhoff et al., 2012)	1.8	3	10	1	1	3	100	1.8×10^{-2}
Increased relative liver weight (Das et al., 2008)	2.1	3	10	1	1	3	100	2.1×10^{-2}
Increased liver hypertrophy (Butenhoff et al., 2012)	1.1	3	10	1	1	3	100	1.1×10^{-2}
Decreased total T4 (Butenhoff et al., 2012)	1.3	3	10	1	1	3	100	1.3×10^{-2}
Increased full-liter resorption (Das et al., 2008)	3.0	3	10	1	1	3	100	3.0×10^{-2}
Increased fetal/neonatal death (Das et al., 2008)	25.7	3	10	1	1	3	100	2.6×10^{-1}
Delayed eyes opening (Das et al., 2008)	0.7	3	10	1	1	3	100	7.0×10^{-3}
Delayed vaginal opening (Das et al., 2008)	0.5	3	10	1	1	3	100	5.0×10^{-3}
Delayed preputial separation (Das et al., 2008)	24.9	3	10	1	1	3	100	2.5×10^{-1}

From the identified human health hazards of PFBA exposure and the derived candidate RfDs, osRfDs of 1×10^{-2} mg/kg-day are selected for liver effects (increased liver hypertrophy) and thyroid effects (decreased total T4) and an osRfD of 5×10^{-3} mg/kg-day is selected for developmental effects (developmental delays based on the candidate value for delayed vaginal opening). The selection of these candidate values over other candidates, as well as the confidence in these subchronic osRfDs is identical to the confidence in the osRfDs discussed in the prior section and presented in Table 5-8.

From these subchronic osRfDs, an ***overall subchronic RfD of 5×10^{-3} mg/kg-day based on developmental delays*** is selected. This osRfD is selected as the overall subchronic RfD as it is the lowest osRfD among the derived subchronic osRfDs, even though it is not the osRfD interpreted with the highest confidence. In the case of the subchronic RfD, selection does not need to consider differences in the composite UF as a value of 100 is applied to all PODs. This is due to all the studies considered for the subchronic RfD being subchronic or gestational duration studies. This results in the osRfD for developmental delays being 50% lower than the osRfD for liver or thyroid effects. Although the overall confidence in the osRfD for developmental delays (*medium-low*) is lower than for liver effects (*medium* confidence, see derivation of RfD section), selection of the developmental osRfD as the overall subchronic RfD is presumed to be protective of possible effects in other organ

systems. Selection of the liver osRfD, although having a stronger overall confidence determination, as the overall subchronic RfD would be considered inadequate to protect against potential developmental effects.

5.2.3. Inhalation Reference Concentration (RfC)

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

5.3. CANCER

5.3.1. Cancer Weight-of-Evidence Descriptor and Derivation of Cancer Risk Values

No studies were identified that evaluated the carcinogenicity of PFBA in humans or animals. In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the EPA concluded that there is *inadequate information to assess carcinogenic potential* for PFBA for any route of exposure. Therefore, the lack of data on the carcinogenicity of PFBA precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

REFERENCES

Abe, T; Takahashi, M; Kano, M; Amaike, Y; Ishii, C; Maeda, K; Kudoh, Y; Morishita, T; Hosaka, T; Sasaki, T; Kodama, S; Matsuzawa, A; Kojima, H; Yoshinari, K. (2017). Activation of nuclear receptor CAR by an environmental pollutant perfluorooctanoic acid. Arch Toxicol 91: 2365-2374. http://dx.doi.org/10.1007/s00204-016-1888-3

Angrish, MM; Kaiser, JP; Mcqueen, CA; Chorley, BN. (2016). Tipping the balance: Hepatotoxicity and the 4 apical key events of hepatic steatosis [Review]. Toxicol Sci 150: 261-268. http://dx.doi.org/10.1093/toxsci/kfw018

ATSDR (Agency for Toxic Substances and Disease Registry). (2018a). An overview of perfluoroalkyl and polyfluoroalkyl substances and interim guidance for clinicians responding to patient exposure concerns. Interim guidance revised 5/7/2018. https://stacks.cdc.gov/view/cdc/77114

ATSDR (Agency for Toxic Substances and Disease Registry). (2018b). Toxicological profile for perfluoroalkyls. Draft for public comment. Atlanta, GA: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. https://www.atsdr.cdc.gov/toxprofiles/tp200.pdf

Bao, WW; Qian, ZM; Geiger, SD; Liu, E; Liu, Y; Wang, SQ; Lawrence, WR; Yang, BY; Hu, LW; Zeng, XW; Dong, GH. (2017). Gender-specific associations between serum isomers of perfluoroalkyl substances and blood pressure among Chinese: Isomers of C8 Health Project in China. Sci Total Environ 607-608: 1304-1312. http://dx.doi.org/10.1016/j.scitotenv.2017.07.124

Batt, AM; Ferrari L. (1995). Manifestations of chemically induced liver damage. Clin Chem 41(12 Pt 2): 1882-7.

Beggs, KM; Mcgreal, SR; McCarthy, A; Gunewardena, S; Lampe, JN; Lau, C; Apte, U. (2016). The role of hepatocyte nuclear factor 4-alpha in perfluorooctanoic acid- and perfluorooctanesulfonic acid-induced hepatocellular dysfunction. Toxicol Appl Pharmacol 304: 18-29. http://dx.doi.org/10.1016/j.taap.2016.05.001

Bjork, JA; Wallace, KB. (2009). Structure-Activity Relationships and Human Relevance for Perfluoroalkyl Acid-Induced Transcriptional Activation of Peroxisome Proliferation in Liver Cell Cultures. Toxicol Sci 111: 89-99. http://dx.doi.org/10.1093/toxsci/kfp093

Blaine, AC; Rich, CD; Hundal, LS; Lau, C; Mills, MA; Harris, KM; Higgins, CP. (2013). Uptake of perfluoroalkyl acids into edible crops via land applied biosolids: field and greenhouse studies. Environ Sci Technol 47: 14062-14069. http://dx.doi.org/10.1021/es403094q

Buhrke, T; Krüger, E; Pevny, S; Rößler, M; Bitter, K; Lampen, A. (2015). Perfluorooctanoic acid (PFOA) affects distinct molecular signalling pathways in human primary hepatocytes. Toxicology 333: 53-62. http://dx.doi.org/10.1016/j.tox.2015.04.004

Buist, SC; Cherrington, NJ; Choudhuri, S; Hartley, DP; Klaassen, CD. (2002). Gender-specific and developmental influences on the expression of rat organic anion transporters. J Pharmacol and Exp Therapuetics 301: 145-515

Burkemper, JL; Aweda, TA; Rosenberg, AJ; Lunderberg, DM; Peaslee, GF; Lapi, SE. (2017). Radiosynthesis and biological distribution of F-18-labeled perfluorinated alkyl substances. Environ Sci Technol Lett 4: 211-215. http://dx.doi.org/10.1021/acs.estlett.7b00042

Butenhoff, JL; Bjork, JA; Chang, SC; Ehresman, DJ; Parker, GA; Das, K; Lau, C; Lieder, PH; van Otterdijk, FM; Wallace, KB. (2012). Toxicological evaluation of ammonium perfluorobutyrate in rats: twenty-eight-day and ninety-day oral gavage studies. Reprod Toxicol 33: 513-530. http://dx.doi.org/10.1016/j.reprotox.2011.08.004

Butenhoff, JL; Kennedy, GL; Frame, S. R.; O'Connor, JC; York, RG. (2004). The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology 196: 95-116. http://dx.doi.org/10.1016/j.tox.2003.11.005

CDC (Centers for Disease Control and Prevention). (2019). Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019, Volume 1. Centers for Disease Control and Prevention, National Health and Nutrition Examination Survey. https://www.cdc.gov/exposurereport/pdf/FourthReport_UpdatedTables_Volume1_Jan2019-508.pdf

Cerrutti, JA; Brandoni, A; Quaglia, NB; Torres, AM. (2002). Sex differences in p-aminohippuric acid transport in rat kidney: role of membrane fluidity and expression of OAT1. Molecular and Cellular Biochem 233: 175-179

Chang, S; Das, K; Ehresman, DJ; Ellefson, ME; Gorman, GS; Hart, JA; Noker, PE; Tan, Y; Lieder, PH; Lau, C; Olsen, GW; Butenhoff, JL. (2008). Comparative pharmacokinetics of perfluorobutyrate in rats, mice, monkeys, and humans and relevance to human exposure via drinking water. Toxicol Sci 104: 40-53. http://dx.doi.org/10.1093/toxsci/kfn057

Crofton, KM. (2004). Developmental disruption of thyroid hormone: Correlations with hearing dysfunction in rats. Risk Anal 24: 1665-1671. http://dx.doi.org/10.1111/j.0272-4332.2004.00557.x

Das, KP; Grey, BE; Zehr, RD; Wood, CR; Butenhoff, JL; Chang, SC; Ehresman, DJ; Tan, YM; Lau, C. (2008). Effects of perfluorobutyrate exposure during pregnancy in the mouse. Toxicol Sci 105: 173-181. http://dx.doi.org/10.1093/toxsci/kfn099

Das, KP; Wood, CR; Lin, MT; Starkov, AA; Lau, C; Wallace, KB; Corton, JC; Abbott, BD. (2017). Perfluoroalkyl acids-induced liver steatosis: Effects on genes controlling lipid homeostasis. Toxicology 378: 37-52. http://dx.doi.org/10.1016/j.tox.2016.12.007

di Masi, A; De Marinis, E; Ascenzi, P; Marino, M. (2009). Nuclear receptors CAR and PXR: Molecular, functional, and biomedical aspects [Review]. Mol Aspects Med 30: 297-343. http://dx.doi.org/10.1016/j.mam.2009.04.002

Espinosa, JS; Stryker, MP. (2012). Development and plasticity of the primary visual cortex [Review]. Neuron 75: 230-249. http://dx.doi.org/10.1016/j.neuron.2012.06.009

Feng, X; Cao, X; Zhao, S; Wang, X; Hua, X; Chen, L; Chen, L. (2017). Exposure of pregnant mice to perfluorobutanesulfonate causes hypothyroxinemia and developmental abnormalities in female offspring. Toxicol Sci 155: 409-419. http://dx.doi.org/10.1093/toxsci/kfw219

Foreman, JE; Chang, SC; Ehresman, DJ; Butenhoff, JL; Anderson, CR; Palkar, PS; Kang, BH; Gonzalez, FJ; Peters, JM. (2009). Differential Hepatic Effects of Perfluorobutyrate Mediated by Mouse and Human PPAR-alpha. Toxicol Sci 110: 204-211. http://dx.doi.org/10.1093/toxsci/kfp077

Forhead, AJ; Fowden, AL. (2014). Thyroid hormones in fetal growth and prepartum maturation [Review]. J Endocrinol 221: R87-R103. http://dx.doi.org/10.1530/JOE-14-0025

Fu, Y; Wang, T; Fu, Q; Wang, P; Lu, Y. (2014). Associations between serum concentrations of perfluoroalkyl acids and serum lipid levels in a Chinese population. Ecotoxicol Environ Saf 106: 246-252. http://dx.doi.org/10.1016/j.ecoenv.2014.04.039

Gebbink, WA; Berger, U; Cousins, IT. (2015). Estimating human exposure to PFOS isomers and PFCA homologues: the relative importance of direct and indirect (precursor) exposure. Environ Int 74: 160-169. http://dx.doi.org/10.1016/j.envint.2014.10.013

Gilbert, ME. (2011). Impact of low-level thyroid hormone disruption induced by propylthiouracil on brain development and function. Toxicol Sci 124: 432-445. http://dx.doi.org/10.1093/toxsci/kfr244

Gilbert, ME; Sanchez-Huerta, K; Wood, C. (2016). Mild thyroid hormone insufficiency during development compromises activity-dependent neuroplasticity in the hippocampus of adult male rats : Supplemental data [Supplemental Data]. Endocrinology 157: 774-787.

Gilbert, ME; Zoeller, RT. (2010). Thyroid hormones--impact on the developing brain: Possible mechanisms of neurotoxicity. In GJ Harry; HA Tilson (Eds.), Neurotoxicology (3rd ed., pp. 79-111). New York, NY: Informa Healthcare.

Gomis, MI; Vestergren, R; Borg, D; Cousins, IT. (2018). Comparing the toxic potency in vivo of long-chain perfluoroalkyl acids and fluorinated alternatives. Environ Int 113: 1-9. http://dx.doi.org/10.1016/j.envint.2018.01.011

Guyatt, G; Oxman, AD; Akl, EA; Kunz, R; Vist, G; Brozek, J; Norris, S; Falck-Ytter, Y; Glasziou, P; DeBeer, H; Jaeschke, R; Rind, D; Meerpohl, J; Dahm, P; Schünemann, HJ. (2011). GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. J Clin Epidemiol 64: 383-394. http://dx.doi.org/10.1016/j.jclinepi.2010.04.026

Haddow, JE; Palomaki, GE; Allan, WC; Williams, JR; Knight, GJ; Gagnon, J; O'Heir, CE; Mitchell, ML; Hermos, RJ; Waisbren, SE; Faix, JD; Klein, RZ. (1999). Maternal thyroid deficiency during pregnancy and subsequent neuropsychological development of the child. N Engl J Med 341: 549-555. http://dx.doi.org/10.1056/NEJM199908193410801

Hall, AP; Elcombe, CR; Foster, JR; Harada, T; Kaufmann, W; Knippel, A; Küttler, K; Malarkey, DE; Maronpot, RR; Nishikawa, A; Nolte, T; Schulte, A; Strauss, V; York, MJ. (2012). Liver hypertrophy: a review of adaptive (adverse and non-adverse) changes--conclusions from the 3rd International ESTP Expert Workshop [Review]. Toxicol Pathol 40: 971-994. http://dx.doi.org/10.1177/0192623312448935

Henrichs, J; Bongers-Schokking, JJ; Schenk, JJ; Ghassabian, A; Schmidt, HG; Visser, TJ; Hooijkaas, H; de Muinck Keizer-Schrama, SM; Hofman, A; Jaddoe, VV; Visser, W; Steegers, EA; Verhulst, FC; de Rijke, YB; Tiemeier, H. (2010). Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: The generation R study. J Clin Endocrinol Metab 95: 4227-4234. http://dx.doi.org/10.1210/jc.2010-0415

Heo, JJ; Lee, JW; Kim, SK; Oh, JE. (2014). Foodstuff analyses show that seafood and water are major perfluoroalkyl acids (PFAAs) sources to humans in Korea. J Hazard Mater 279: 402-409. http://dx.doi.org/10.1016/j.jhazmat.2014.07.004

Hill, AB. (1965). The environment and disease: Association or causation? Proc R Soc Med 58: 295-300.

Hillman, NH; Kallapur, SG; Jobe, AH. (2012). Physiology of transition from intrauterine to extrauterine life. Clin Perinatol 39: 769-783.

Hood, A; Hashmi, R; Klaassen, CD. (1999). Effects of microsomal enzyme inducers on thyroid-follicular cell proliferation, hyperplasia, and hypertrophy. Toxicol Appl Pharmacol 160: 163-170. http://dx.doi.org/10.1006/taap.1999.8752

Hood, A; Klaassen, CD. (2000). Differential effects of microsomal enzyme inducers on in vitro thyroxine (T(4)) and triiodothyronine (T(3)) glucuronidation. Toxicol Sci 55: 78-84.

Hulbert, AJ. (2000). Thyroid hormones and their effects: A new perspective [Review]. Biol Rev Camb Philos Soc 75: 519-631.

Ikeda, T; Aiba, K; Fukuda, K; Tanaka, M. (1985). The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. J Biochem 98: 475-482. http://dx.doi.org/10.1093/oxfordjournals.jbchem.a135302

Jaeschke, H; Lemasters, JJ. (2003). Apoptosis versus oncotic necrosis in hepatic ischemia/reperfusion injury. Gastroenterology 125(4): 1246-57.

Joshi-Barve, S; Kirpich, I; Cave, MC; Marsano, LS; McClain, CJ. (2015). Alcoholic, nonalcoholic, and toxicant-associated steatohepatitis: Mechanistic similarities and differences [Review]. CMGH 1: 356-367. http://dx.doi.org/10.1016/j.jcmgh.2015.05.006

Just, WW; Gorgas, K; Hartl, FU; Heinemann, P; Salzer, M; Schimassek, H. (1989). Biochemical effects and zonal heterogeneity of peroxisome proliferation induced by perfluorocarboxylic acids in rat liver. Hepatology 9: 570-581. http://dx.doi.org/10.1002/hep.1840090411

Kaiser, JP; Lipscomb, JC; Wesselkamper, SC. (2012). Putative Mechanisms of Environmental Chemical-Induced Steatosis. Int J Toxicol 31: 551-563. http://dx.doi.org/10.1177/1091581812466418

Kass, GE. (2006). Mitochondrial involvement in drug-induced hepatic injury. Chem Biol Interact 163(1-2): 145-59

Kato, Y; Kuge, K; Kusuhara, H; Meier, PJ; Sugiyama, Y. (2002). Gender differences in the urinary excretion of organic anions in rats. J Pharmacol and Exp Therapuetics 302: 483-489

Kim, D; Kim, U, nJ; Kim, H; Choi, SD; Oh, J. (2016). Perfluoroalkyl substances in serum from South Korean infants with congenital hypothyroidism and healthy infants - Its relationship with thyroid hormones. Environ Res 147: 399-404. http://dx.doi.org/10.1016/j.envres.2016.02.037

Kim, SK; Kannan, K. (2007). Perfluorinated acids in air, rain, snow, surface runoff, and lakes: relative importance of pathways to contamination of urban lakes. Environ Sci Technol 41: 8328-8334. http://dx.doi.org/10.1021/es072107t

Klaunig, JE; Shinohara, M; Iwai, H; Chengelis, CP; Kirkpatrick, JB; Wang, Z; Bruner, RH. (2015). Evaluation of the chronic toxicity and carcinogenicity of perfluorohexanoic acid (PFHxA) in Sprague-Dawley rats. Toxicol Pathol 43: 209-220. http://dx.doi.org/10.1177/0192623314530532

Kleiner, DE; Makhlouf, HR. (2015). Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. Clin Liver Dis 20(2): 293-312

Korevaar, TIM; Muetzel, R; Medici, M; Chaker, L; Jaddoe, VWV; de Rijke, YB; Steegers, EAP; Visser, TJ; White, T; Tiemeier, H; Peeters, RP. (2016). Association of maternal thyroid function during early pregnancy with offspring IQ and brain morphology in childhood: A population-based prospective cohort study. Lancet Diabetes Endocrinol 4: 35-43. http://dx.doi.org/10.1016/S2213-8587(15)00327-7

Kotthoff, M; Müller, J; Jürling, H; Schlummer, M; Fiedler, D. (2015). Perfluoroalkyl and polyfluoroalkyl substances in consumer products. Environ Sci Pollut Res Int 22: 14546-14559. http://dx.doi.org/10.1007/s11356-015-4202-7

Lau, C. (2015). Perfluorinated compounds: An overview. In JC DeWitt (Ed.), Toxicological effects of perfluoroalkyl and polyfluoroalkyl substances (pp. 1-21). New York: Springer. http://dx.doi.org/10.1007/978-3-319-15518-0_1

Lau, C; Butenhoff, JL; Rogers, JM. (2004). The developmental toxicity of perfluoroalkyl acids and their derivatives [Review]. Toxicol Appl Pharmacol 198: 231-241. http://dx.doi.org/10.1016/j.taap.2003.11.031

Lau, C; Thibodeaux, JR; Hanson, RG; Narotsky, MG; Rogers, JM; Lindstrom, AB; Strynar, MJ. (2006). Effects of perfluorooctanoic acid exposure during pregnancy in the mouse. Toxicol Sci 90: 510-518. http://dx.doi.org/10.1093/toxsci/kfj105

Lau, C; Thibodeaux, JR; Hanson, RG; Rogers, JM; Grey, BE; Stanton, ME; Butenhoff, JL; Stevenson, LA. (2003). Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicol Sci 74: 382-392. http://dx.doi.org/10.1093/toxsci/kfg122

Lavado-Autric, R; Auso, E; Garcia-Velasco, JV; Del Carmen Arufe, M; Escobar Del Rey, F; Berbel, P; Morreale De Escobar, G. (2003). Early maternal hypothyroxinemia alters histogenesis and cerebral cortex cytoarchitecture of the progeny. J Clin Invest 111: 1073-1082. http://dx.doi.org/10.1172/JCI16262

Lemasters, JJ; Qian, T; He, L; Kim, JS; Elmore, SP; Cascio, WE; Brenner, DA. (2002). Role of mitochondrial inner membrane permeabilization in necrotic cell death, apoptosis, and autophagy. Antioxid Redox Signal 4(5): 769-81

Li, M; Zeng, XW; Qian, ZM; Vaughn, MG; Sauvé, S; Paul, G; Lin, S; Lu, L; Hu, LW; Yang, BY; Zhou, Y; Qin, XD; Xu, SL; Bao, WW; Zhang, YZ; Yuan, P; Wang, J; Zhang, C; Tian, YP; Nian, M; Xiao, X; Fu, C; Dong, GH. (2017a). Isomers of perfluorooctanesulfonate (PFOS) in cord serum and

birth outcomes in China: Guangzhou Birth Cohort Study. Environ Int 102: 1-8. http://dx.doi.org/10.1016/j.envint.2017.03.006

Li, Y; Cheng, Y; Xie, Z; Zeng, F. (2017b). Perfluorinated alkyl substances in serum of the southern Chinese general population and potential impact on thyroid hormones. Sci Rep 7: 43380. http://dx.doi.org/10.1038/srep43380

Liu, X; Guo, Z; Krebs, KA; Pope, RH; Roache, NF. (2014). Concentrations and trends of perfluorinated chemicals in potential indoor sources from 2007 through 2011 in the US. Chemosphere 98: 51-57. http://dx.doi.org/10.1016/j.chemosphere.2013.10.001

Ljubjevic, M; Herak-Kramberger, CM; Hagos, Y; Bahn, A; Endou, H, Burckhardt, G; Sabolic, I. (2004). Rat renal cortical OAT1 and OAT3 exhibit gender differences determined by both androgen stimulation and estrogen inhibition. Am J Physiol Renal Physiol 287: F124-F138

Ljubjevic, M; Balen, D; Breljak, D; Kusan, M; Anzai, N; Bahn, A; Burckhardt G; Sabolic, I. (2007). Renal expression of organic anion transporter OAT2 in rats and mice is regulated by sex hormones. Am J Physiol Renal Physiol 292: F361-F372

MDH (Minnesota Department of Health). (2017). PFBA and Drinking Water. https://www.health.state.mn.us/communities/environment/risk/docs/guidance/gw/pfbainfo.pdf

Mellor, CL; Steinmetz, FP; Cronin, MT. (2016). The identification of nuclear receptors associated with hepatic steatosis to develop and extend adverse outcome pathways [Review]. Crit Rev Toxicol 46: 138-152. http://dx.doi.org/10.3109/10408444.2015.1089471

Mirabella, G; Feig, D; Astzalos, E; Perlman, K; Rovet, JF. (2000). The effect of abnormal intrauterine thyroid hormone economies on infant cognitive abilities. J Pediatr Endocrinol Metab 13: 191-194.

Morgan, RL; Thayer, KA; Bero, L; Bruce, N; Falck-Ytter, Y; Ghersi, D; Guyatt, G; Hooijmans, C; Langendam, M; Mandrioli, D; Mustafa, RA; Rehfuess, EA; Rooney, AA; Shea, B; Silbergeld, EK; Sutton, P; Wolfe, MS; Woodruff, TJ; Verbeek, JH; Holloway, AC; Santesso, N; Schünemann, HJ. (2016). GRADE: Assessing the quality of evidence in environmental and occupational health. Environ Int 92-93: 611-616. http://dx.doi.org/10.1016/j.envint.2016.01.004

Morreale de Escobar, G; Obregon, MJ; Escobar del Rey, F. (2004). Role of thyroid hormone during early brain development [Review]. Eur J Endocrinol 151 Suppl 3: U25-U37.

Nian, M; Li, QQ; Bloom, M; Qian, ZM; Syberg, KM; Vaughn, MG; Wang, SQ; Wei, Q; Zeeshan, M; Gurram, N; Chu, C; Wang, J; Tian, YP; Hu, LW; Liu, KK; Yang, BY; Liu, RQ; Feng, D; Zeng, XW; Dong, GH. (2019). Liver function biomarkers disorder is associated with exposure to perfluoroalkyl acids in adults: Isomers of C8 Health Project in China. Environ Res 172: 81-88. http://dx.doi.org/10.1016/j.envres.2019.02.013

Nilsson, H; Kärrman, A; Westberg, H; Rotander, A; van Bavel, B; Lindström, G. (2010). A time trend study of significantly elevated perfluorocarboxylate levels in humans after using fluorinated ski wax. Environ Sci Technol 44: 2150-2155. http://dx.doi.org/10.1021/es9034733

NJDWQI (New Jersey Drinking Water Quality Institute). (2017). Public review draft: Health-based maximum contaminant level support document: Perfluorooctane sulfonate (PFOS) (CAS #: 1763-23-1; Chemical Formula: C8HF17O3S). NJDWQI Health Effects Subcommittee. https://cswab.org/wp-content/uploads/2018/09/New-Jersey-Scientific-Documentation-Supporting-PFOS-13-ppt.pdf

NLM (National Institutes of Health, National Library of Medicine). (2013). TOXNET Toxicology Data Network, Hazardous Substances Database (HSDB): Perfluoro-n-nonanoic acid. Available online at https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+8040

NLM (National Institutes of Health, National Library of Medicine). (2016). TOXNET Toxicology Data Network, Hazardous Substances Database (HSDB): Perfluorohexanoic acid. Available online at https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+8299

NLM (National Institutes of Health, National Library of Medicine). (2017). TOXNET Toxicology Data Network, Hazardous Substances Database (HSDB): Perfluorohexanesulfonic acid. Available online at https://toxnet.nlm.nih.gov/cgi-bin/sis/search/a?dbs+hsdb:@term+@DOCNO+8274

OECD (Organisation for Economic Co-operation and Development). (2018). Toward a new comprehensive global database of per- and polyfluoroalkyl substances (PFAS): Summary report on updating the OECD 2007 list of per- and polyfluoroalkyl substances (PFAS). (ENV/JM/MONO(2018)7). http://www.oecd.org/chemicalsafety/portal-perfluorinated-chemicals/

Pérez, F; Nadal, M; Navarro-Ortega, A; Fàbrega, F; Domingo, JL; Barceló, D; Farré, M. (2013). Accumulation of perfluoroalkyl substances in human tissues. Environ Int 59: 354-362. http://dx.doi.org/10.1016/j.envint.2013.06.004

Permadi, H; Lundgren, B; Andersson, K; Depierre, JW. (1992). Effects of perfluoro fatty acids on xenobiotic-metabolizing enzymes, enzymes which detoxify reactive forms of oxygen and lipid peroxidation in mouse liver. Biochem Pharmacol 44: 1183-1191. http://dx.doi.org/10.1016/0006-2952(92)90383-T

Permadi, H; Lundgren, B; Andersson, K; Sundberg, C; Depierre, JW. (1993). Effects of perfluoro fatty acids on peroxisome proliferation and mitochondrial size in mouse liver: dose and time factors and effect of chain length. Xenobiotica 23: 761-770.

Post, GB; Louis, JB; Lippincott, RL; Procopio, NA. (2013). Occurrence of perfluorinated compounds in raw water from New Jersey public drinking water systems. Environ Sci Technol 47: 13266-13275. http://dx.doi.org/10.1021/es402884x

Puig-Domingo, M; Vila, L. (2013). The implications of iodine and its supplementation during pregnancy in fetal brain development [Review]. Curr Clin Pharmacol 8: 97-109.

Rand, AA; Rooney, JP; Butt, CM; Meyer, JN; Mabury, SA. (2014). Cellular toxicity associated with exposure to perfluorinated carboxylates (PFCAs) and their metabolic precursors. Chem Res Toxicol 27: 42-50. http://dx.doi.org/10.1021/tx400317p

Rosen, MB; Das, KP; Rooney, J; Abbott, B; Lau, C; Corton, JC. (2017). PPARα-independent transcriptional targets of perfluoroalkyl acids revealed by transcript profiling. Toxicology 387: 95-107. http://dx.doi.org/10.1016/j.tox.2017.05.013

Rosen, MB; Das, KP; Wood, CR; Wolf, CJ; Abbott, BD; Lau, C. (2013). Evaluation of perfluoroalkyl acid activity using primary mouse and human hepatocytes. Toxicology 308: 129-137. http://dx.doi.org/10.1016/j.tox.2013.03.011

Rosenmai, AK; Ahrens, L; le Godec, T; Lundqvist, J; Oskarsson, A. (2018). Relationship between peroxisome proliferator-activated receptor alpha activity and cellular concentration of 14 perfluoroalkyl substances in HepG2 cells. J Appl Toxicol 38: 219-226. http://dx.doi.org/10.1002/jat.3515

Ruan, T; Sulecki, LM; Wolstenholme, BW; Jiang, G; Wang, N; Buck, RC. (2014). 6:2 Fluorotelomer iodide in vitro metabolism by rat liver microsomes: comparison with [1,2-(14)C] 6:2 fluorotelomer alcohol. Chemosphere 112: 34-41. http://dx.doi.org/10.1016/j.chemosphere.2014.02.068

Russell, MH; Himmelstein, MW; Buck, RC. (2015). Inhalation and oral toxicokinetics of 6:2 FTOH and its metabolites in mammals. Chemosphere 120: 328-335. http://dx.doi.org/10.1016/j.chemosphere.2014.07.092

Schünemann, H; Hill, S; Guyatt, G; Akl, EA; Ahmed, F. (2011). The GRADE approach and Bradford Hill's criteria for causation. J Epidemiol Community Health 65: 392-395. http://dx.doi.org/10.1136/jech.2010.119933

Sferruzzi-Perri, AN; Vaughan, OR; Forhead, AJ; Fowden, AL. (2013). Hormonal and nutritional drivers of intrauterine growth [Review]. Curr Opin Clin Nutr Metab Care 16: 298-309. http://dx.doi.org/10.1097/MCO.0b013e32835e3643

Song, X; Tang, S; Zhu, H; Chen, Z; Zang, Z; Zhang, Y; Niu, X; Wang, X; Yin, H; Zeng, F; He, C. (2018). Biomonitoring PFAAs in blood and semen samples: Investigation of a potential link between PFAAs exposure and semen mobility in China. Environ Int 113: 50-54. http://dx.doi.org/10.1016/j.envint.2018.01.010

Stahl, LL; Snyder, BD; Olsen, AR; Kincaid, TM; Wathen, JB; Mccarty, HB. (2014). Perfluorinated compounds in fish from U.S. urban rivers and the Great Lakes. Sci Total Environ 499: 185-195. http://dx.doi.org/10.1016/j.scitotenv.2014.07.126

Stenzel, D; Huttner, WB. (2013). Role of maternal thyroid hormones in the developing neocortex and during human evolution. Frontiers in Neuroanatomy 7: 9. http://dx.doi.org/10.3389/fnana.2013.00019

Sun, M; Arevalo, E; Strynar, M; Lindstrom, A; Richardson, M; Kearns, B; Pickett, A; Smith, C; Knappe, DRU. (2016). Legacy and emerging perfluoroalkyl substances are important drinking water contaminants in the Cape Fear River Watershed of North Carolina. Environ Sci Technol Lett 3: 415-419. http://dx.doi.org/10.1021/acs.estlett.6b00398

Sundström, M; Bogdanska, J; Pham, HV; Athanasios, V; Nobel, S; Mcalees, A; Eriksson, J; Depierre, JW; Bergman, Å. (2012). Radiosynthesis of perfluorooctanesulfonate (PFOS) and perfluorobutanesulfonate (PFBS), including solubility, partition and adhesion studies. Chemosphere 87: 865-871. http://dx.doi.org/10.1016/j.chemosphere.2012.01.027

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment. Fed Reg 56: 63798-63826.

U.S. EPA (U.S. Environmental Protection Agency). (1994). Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry [EPA Report]. (EPA/600/8-90/066F). Research Triangle Park, NC: U.S. Environmental Protection Agency, Office of Research and Development, Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office.

U.S. EPA (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment. Fed Reg 61: 56274-56322.

U.S. EPA (U.S. Environmental Protection Agency). (1998). Assessment of thyroid follicular cell tumors [EPA Report] (pp. 1-51). (EPA/630/R-97/002). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-11/documents/thyroid.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2002). A review of the reference dose and reference concentration processes. (EPA/630/P-02/002F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2014-12/documents/rfd-final.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2005). Guidelines for carcinogen risk assessment [EPA Report]. (EPA/630/P-03/001B). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/sites/production/files/2013-09/documents/cancer_guidelines_final_3-25-05.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2011). Recommended use of body weight 3/4 as the default method in derivation of the oral reference dose (pp. 1-50). (EPA/100/R-11/0001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum, Office of the Science Advisor. https://www.epa.gov/sites/production/files/2013-09/documents/recommended-use-of-bw34.pdf

U.S. EPA (U.S. Environmental Protection Agency). (2012a). Benchmark dose technical guidance. (EPA/100/R-12/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. https://www.epa.gov/risk/benchmark-dose-technical-guidance

U.S. EPA (U.S. Environmental Protection Agency). (2013). U.S. Environmental Protection Agency endocrine disruptor screening program SAP review of EDSP tier 1 screening: Assay and battery performance.

U.S. EPA (U.S. Environmental Protection Agency). (2018a). Chemistry Dashboard. Washington, DC. Retrieved from https://comptox.epa.gov/dashboard

U.S. EPA (U.S. Environmental Protection Agency). (2018b). Human health toxicity values for perfluorobutane sulfonic acid and related compound potassium perfluorobutane sulfonate (Public comment draft). (EPA-823-R-18-307).

U.S. EPA (U.S. Environmental Protection Agency). (2019a). ChemView [Database]. Retrieved from https://chemview.epa.gov/chemview

U.S. EPA (U.S. Environmental Protection Agency). (2019b). EPA Actions to Address PFAS. Available online at https://www.epa.gov/pfas/epa-actions-address-pfas

van Cruchten, S; van den Broeck; W. (2002). Morphological and biochemical aspects of apoptosis, oncosis and necrosis. Anat Histol Embryol. 31(4):214-23

van Otterdijk, FM. (2007a). Repeated dose 28-day oral toxicity study with MTDID-8391 by daily gavage in the rat, followed by a 21-day recovery period. (Study Number 06-226). Maplewood, MN: 3M.

van Otterdijk, FM. (2007b). Repeated dose 90-day oral toxicity study with MTDID 8391 by daily gavage in the rat followed by a 3-week recovery period. (Study Number 06-398). Maplewood, MN: 3M.

Venkatesan, AK; Halden, RU. (2014). Loss and in situ production of perfluoroalkyl chemicals in outdoor biosolids-soil mesocosms. Environ Res 132: 321-327. http://dx.doi.org/10.1016/j.envres.2014.04.024

Wahlang, B; Beier, JI; Clair, HB; Bellis-Jones, HJ; Falkner, K; Mcclain, CJ; Cave, MC. (2013). Toxicant-associated steatohepatitis [Review]. Toxicol Pathol 41: 343-360. http://dx.doi.org/10.1177/0192623312468517

Wang, J; Zeng, XW; Bloom, MS; Qian, Z; Hinyard, LJ; Belue, R; Lin, S; Wang, SQ; Tian, YP; Yang, M; Chu, C; Gurram, N; Hu, LW; Liu, KK; Yang, BY; Feng, D; Liu, RQ; Dong, GH. (2019). Renal function and isomers of perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS): Isomers of C8 Health Project in China. Chemosphere 218: 1042-1049. http://dx.doi.org/10.1016/j.chemosphere.2018.11.191

Wang, Z; Cousins, IT; Scheringer, M; Buck, RC; Hungerbühler, K. (2014). Global emission inventories for C4-C14 perfluoroalkyl carboxylic acid (PFCA) homologues from 1951 to 2030, Part I: production and emissions from quantifiable sources [Review]. Environ Int 70: 62-75. http://dx.doi.org/10.1016/j.envint.2014.04.013

Watkins, DJ; Josson, J; Elston, B; Bartell, SM; Shin, HM; Vieira, VM; Savitz, DA; Fletcher, T; Wellenius, GA. (2013). Exposure to perfluoroalkyl acids and markers of kidney function among children and adolescents living near a chemical plant. Environ Health Perspect 121: 625-630. http://dx.doi.org/10.1289/ehp.1205838

Weaver, YM; Ehresman, DJ; Butenhoff, JL; Hagenbuch, B. (2010). Roles of rat renal organic anion transporters in trasnporting perfluorinated carboxylates with differen chain lengths. Toxicol Sci 113(2): 305-314

Weerasinghe, P; Maximilian Buja, L. (2012). Oncosis: an important non-apoptotic mode of cell death. Exp Mol Pathol 93(3):302-8

Wiesel, TN. (1982). The postnatal development of the visual cortex and the influence of environment [Review]. Biosci Rep 2: 351-377. http://dx.doi.org/10.1007/bf01119299

Williams, GM; Iatropoulos, MJ. (2002). Alteration of liver cell function and proliferation: differentiation between adaptation and toxicity. Toxicol Pathol 30(1):41-53

Wolf, CJ; Schmid, JE; Lau, C; Abbott, BD. (2012). Activation of mouse and human peroxisome proliferator-activated receptor-alpha (PPARα) by perfluoroalkyl acids (PFAAs): further investigation of C4-C12 compounds. Reprod Toxicol 33: 546-551. http://dx.doi.org/10.1016/j.reprotox.2011.09.009

Wolf, CJ; Takacs, ML; Schmid, JE; Lau, C; Abbott, BD. (2008). Activation of mouse and human peroxisome proliferator-activated receptor alpha by perfluoroalkyl acids of different functional groups and chain lengths. Toxicol Sci 106: 162-171. http://dx.doi.org/10.1093/toxsci/kfn166

Yang, CH; Glover, KP; Han, X, (2009). Organic anion transporting polypeptide (Oatp)1a1-mediated perflurooctanoate transport and evidence for a renal reabsorption mechanism of Oatp1a1 in renal elimination of perfluorocarboxylates in rats. Toxicol Letters 190: 163-171

Zhang, YM; Dong, XY; Fan, LJ; Zhang, ZL; Wang, Q; Jiang, N; Yang, XS. (2017). Poly- and perfluorinated compounds activate human pregnane X receptor. Toxicology 380: 23-29. http://dx.doi.org/10.1016/j.tox.2017.01.012

Zhao, H; Qu, B; Guan, Y; Jiang, J; Chen, X. (2016). Influence of salinity and temperature on uptake of perfluorinated carboxylic acids (PFCAs) by hydroponically grown wheat (Triticum aestivum L.). 5: 541. http://dx.doi.org/10.1186/s40064-016-2016-9

Zoeller, RT; Rovet, J. (2004). Timing of thyroid hormone action in the developing brain: Clinical observations and experimental findings [Review]. J Neuroendocrinol 16: 809-818. http://dx.doi.org/10.1111/j.1365-2826.2004.01243.x

Zoeller, RT; Tan, SW; Tyl, RW. (2007). General background on the hypothalamic-pituitary-thyroid (HPT) axis [Review]. Crit Rev Toxicol 37: 11-53. http://dx.doi.org/10.1080/10408440601123446