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IRIS Toxicological Review of Perfluorobutanoic Acid (PFBA, CASRN 375-22-4) and Related Salts

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ABBREVIATIONS AND ACRONYMS

ACO	acyl-CoA oxidase
ADME	absorption, distribution, metabolism, and excretion
AFFF	aqueous film-forming foam
AIC	Akaike's information criterion
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
AUC	area-under-the-concentration curve
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDS	Benchmark Dose Software
BMR	benchmark response
BW	body weight
C_{AVG}	average concentration
C_{MAX}	maximum concentration
CA	Cochran-Armitage
CAR	constitutive androstane receptor
CASRN	Chemical Abstracts Service registry number
CDR	Chemical Data Reporting
CI	confidence interval
CL	clearance
CL_A	clearance in animals
CL_H	clearance in humans
CPAD	Chemical and Pollutant Assessment Division
CPHEA	Center for Public Health and Environmental Assessment
CV	constant variance
CYP	cytochrome P450 superfamily
DAF	dosimetric adjustment factor
DNA	deoxyribonucleic acid
DNT	developmental neurotoxicity
DOD	Department of Defense
EPA	Environmental Protection Agency
EOP	Executive Office of the President
ER	extra risk
FLR	full-litter resorption
FTOH	fluorotelomer alcohol
GD	gestation day
GFR	glomerular filtration rate
GGT	γ-glutamyl transferase
GRADE	Grading of Recommendations Assessment, Development, and Evaluation
GSH	glutathione
HAWC	Health Assessment Workspace Collaborative
HED	human equivalent dose
HERO	Health and Environmental Research Online
HISA	highly influential scientific information
HPT	hypothalamic-pituitary-thyroid
IRIS	Integrated Risk Information System
i.v.	intravenous
IQ	intelligence quotient
IQR	interquartile range
ISI	influential scientific information
IUR	inhalation unit risk
LLOQ	lower limit of quantitation
LN	log-normal
LOAEL	lowest-observed-adverse-effect level
MBq	megabecquerel
MOA	mode of action
NCEA	National Center for Environmental Assessment
NCV	nonconstant variance
NIOSH	National Institute for Occupational Safety and Health
NIS	sodium-iodide symporter
NOAEL	no-observed-adverse-effect level
NPL	National Priority List
NTP	National Toxicology Program
OAT	organic anion transporter
OECD	Organisation for Economic Co-operation and Development
OMB	Office of Management and Budget
ORD	Office of Research and Development
OSF	oral slope factor
PC	partition coefficient
PBPK	physiologically based pharmacokinetic
PBTK	physiologically based toxicokinetic
PD	pharmacodynamic
PECO	Populations, Exposures, Comparators, Outcomes
PFAA	perfluoroalkyl acid
PFAS	per- and polyfluoroalkyl substances
PFBA	perfluorobutanoic acid
PFBS	perfluorobutane sulfonate
PFCA	perfluoroalkyl carboxylic acid
PFDA	perfluorodecanoic acid
PFHxA	perfluorohexanoic acid
PFHxS	perfluorohexane sulfonate
PFNA	perfluorononanoic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
PK	pharmacokinetic

PND	postnatal day
POD	point of departure
POD_{HED}	human equivalent dose POD
PPAR	peroxisome proliferator-activated receptor
PQAPP	Programmatic Quality Assurance Project Plan
PT	prothrombin time
PXR	pregnane X receptor
QA	quality assurance
QAPP	Quality Assurance Project Plan
QMP	Quality Management Plan
RBC	red blood cell
RD	relative deviation
RfC	inhalation reference concentration
RfD	oral reference dose
RS	Rao-Scott
SD	standard deviation
S-D	Sprague-Dawley
SE	standard error
TD	toxicodynamic
TH	thyroid hormone
TK	toxicokinetic
TPO	thyroid peroxidase
TRI	Toxic Release Inventory
TSCA	Toxic Substances Control Act
TSCATS	Toxic Substances Control Act Test Submissions
TSH	thyroid-stimulating hormone
TSHR	thyroid-stimulating hormone receptor
UCMR	Unregulated Contaminant Monitoring Rule
UDP-GT	uridine 5′-diphospho-glucuronosyltransferase
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
VOC	volatile organic compound
WOS	Web of Science
Wy-14,643	pirinixic acid

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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:

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Perfluorobutanoic acid (PFBA, CASRN 375-22-4)[1] and its related salts are members of the group of per- and polyfluoroalkyl substances (PFAS). This assessment applies to PFBA as well as salts (including alkali metal salts) of PFBA that would be expected to fully dissociate in aqueous solutions of pH ranging from 4–9 (e.g., in the human body). Thus, while this assessment would not necessarily apply to non-alkali metal salts of PFBA (e.g., silver heptafluorobutyrate; CASRN 3794-64-7) due to the possibility of PFBA-independent contributions of toxicity, it does apply to PFBA salts including ammonium perfluorobutanoate (CASRN 10495-86-0), sodium perfluorobutanoate (CASRN 2218-54-4), potassium heptafluorobutanoate [2966-54-3], and other non-metal or alkali metal salts of PFBA. The synthesis of evidence and toxicity value derivation presented in this assessment focuses on the free acid of PFBA and ammonium perfluorobutanoate given the currently available toxicity data.

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 manmade compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. 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 epidemiological 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; two studies on congenital hypothyroidism and birth weight and gestational duration

[1] The CASRN given is for linear PFBA; the source PFBA used in toxicity studies was reported to be 98% pure (Das et al., 2008)and reagent grade (Butenhoff et al., 2012a). Neither study explicitly states that only the linear form was used. Therefore, there is the possibility that a minor proportion of the PFBA used in the studies were branched isomers and thus observed health effects may apply to the total linear and branched isomers in a given exposure source.

were considered *uninformative*); 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 range across exposure groups. No studies were identified that evaluated the association between PFBA exposure and carcinogenicity.

Animal studies of PFBA exposure in rats and mice have exclusively examined the oral route (i.e., no inhalation or dermal studies were identified during the literature search) and have examined noncancer endpoints only.

Altogether, the available ***evidence indicates*** that developmental, thyroid, and liver effects in humans are likely caused by PFBA exposure in utero or during adulthood (see Sections 3.2.1, 3.2.2, and 3.2.3). There was ***inadequate evidence*** to determine whether reproductive effects might represent a potential human health hazard following PFBA exposure (see Section 3.2.4).

The few epidemiological studies did not inform the potential for effects in the thyroid, liver, reproductive system, or developing offspring, and the evidence integration judgments are based on PFBA studies in animals. Liver effects manifested as increased relative liver weight in adult animals and increased incidence of hepatocellular hypertrophy (see Section 3.2.2 and Tables 3-6 and 3-7). 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 (see Section 3.2.1 and Tables 3-3 and 3-2). Developmental effects in exposed animals were expressed as the loss of viable offspring (total litter resorption), and postnatal delays in postnatal developmental milestones: eye opening, vaginal opening, and preputial separation (see Section 3.2.3 and Table 3-9).

Table ES-1 summarizes the evidence integration judgments for health effects that had enough evidence available to synthesize and draw hazard conclusions, and the toxicity values derived for those health effects.

Table ES-1. Evidence integration judgements and derived toxicity values for PFBA

Health system	Evidence integration judgment	Toxicity value type	Value PFBA (mg/kg-d)	Value NH_4^+ PFB (mg/kg-d)[a]	Confidence in Toxicity Value[b]	UF_C[c]	Basis
Hepatic	*Evidence indicates (likely)*	osRfD	1×10^{-3}	1×10^{-3}	*Medium*	1,000[d]	Increased hepatocellular hypertrophy in adult rats
		Subchronic osRfD	1×10^{-2}	1×10^{-2}	*Medium*	100[e]	Increased hepatocellular hypertrophy in adult rats
Thyroid		osRfD	1×10^{-3}	1×10^{-3}	*Medium-low*	1,000[d]	Decreased total T4 in adult rats

Health system	Evidence integration judgment	Toxicity value type	Value PFBA (mg/kg-d)	Value NH_4^+ PFB (mg/kg-d)[a]	Confidence in Toxicity Value[b]	UF_C[c]	Basis
	Evidence indicates (likely)	Subchronic osRfD	1×10^{-2}	1×10^{-2}	*Medium-low*	100[e]	Decreased total T4 in adult rats
Developmental	*Evidence indicates (likely)*	osRfD	6×10^{-3}	7×10^{-3}	*Medium-low*	100[e]	Developmental delays in mice[f]
		Subchronic osRfD	6×10^{-3}	7×10^{-3}	*Medium-low*	100[e]	Developmental delays in mice[f]
Reproductive	*Evidence inadequate*	osRfD	Not derived	Not derived	NA	NA	NA
		Subchronic osRfD	Not derived	Not derived	NA	NA	NA
RfD			1×10^{-3}	1×10^{-3}	*Medium*	1,000[d]	Hepatic and thyroid effects
Subchronic RfD			6×10^{-3}	7×10^{-3}	*Medium-low*	100[e]	Developmental effects[f]

See Section 5.2.1 for full details on study and dataset selection, modeling approaches (including BMR selection), uncertainty factor application, candidate value selection, and characterization of confidence in the osRfDs and RfDs.

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

[a] See Tables 5-7 and 5-10 for details on how to calculate candidate values for salts of PFBA. The osRfDs presented in this table have been rounded to 1 significant digit from the candidate values presented in Tables 5-7 and 5-10.

[b] The overall confidence in the derived toxicity values is synthesized from confidence judgments regarding confidence in the study used to derive the toxicity value, confidence in the evidence base supporting the hazard, and confidence in the quantification of the point of departure; see Table 5-8 for full details for these confidence judgments.

[c] See Table 5-5 for an explanation of the uncertainty factors applied to derive the osRfD and subchronic osRfD values.

[d] UF_C = 1000 comprised of UF_A = 3, UF_H = 10, UF_S = 10, UF_L = 1, and UF_D = 3.

[e] UF_C = 100 comprised of UF_A = 3, UF_H = 10, UF_S = 1, UF_L = 1, and UF_D = 10.

[f] The point of departure 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 (liver, thyroid, developmental toxicity), increased liver hypertrophy and decreased T4 in adult male rats after subchronic exposure, as reported in Butenhoff et al. (2012a), were selected as the basis for the oral reference dose (RfD) (see Section 5.2.1). A no-observed-adverse-effect level (NOAEL) of 6 mg/kg-day NH_4^+PFB was identified for increased liver hypertrophy, and a NOAEL of 6 mg/kg-day NH_4^+PFB was identified for decreased T4 (see Table 5-4). These values were used as the points of departure (PODs). After converting the PODs from units of mg/kg-day NH_4^+PFB to units of mg/kg-day PFBA (by multiplying by the ratio of the molecular weights of the free acid and the ammonium salt), the ratio of serum clearance values between rats and humans was used to account for pharmacokinetic differences between species (see Table 5-3), resulting in the human equivalent doses (POD_{HED}) of

1.15 mg/kg-day and 1.27 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 pharmacokinetic and pharmacodynamic uncertainty in the extrapolation from rats to humans (UF_A = 3), interindividual differences in human susceptibility (UF_H = 10), extrapolation from a subchronic-to-chronic exposure duration (UF_S = 10), and deficiencies in the toxicity database (UF_D = 3) (see Table 5-5). The selected overall RfD for PFBA derived based on liver and thyroid effects is 1×10^{-3} mg/kg-day.[2],[3]

Confidence in the Oral Reference Dose (RfD)

The overall confidence in the RfD is *medium*, based on the confidence in the principal study, confidence in the quantification of the PODs, and confidence in the evidence bases supporting the thyroid and liver effects (see Table 5-8). The subchronic exposure toxicity study conducted by Butenhoff et al. (2012a) reported on administration of NH_4^+PFB 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]).[4] Confidence in the oral toxicity database for derivation of the RfD is *medium* because consistent and coherent effects occurred 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 (1) use of a NOAEL roughly equivalent to BMDL (suggesting that this POD might not be more substantially more uncertain than a BMD-based POD); (2) use of a NOAEL roughly equivalent with a decrease of one standard deviation for thyroid effects (demonstrating that the NOAEL would be roughly equivalent to the BMD, but higher than the BMDL, if BMD modeling had been conducted); and (3) dosimetric adjustments using PFBA-specific pharmacokinetic information (see Table 5-8).

[2] See Table 5-7 for details on how to calculate candidate values for salts of PFBA; briefly, the candidate values for different salts of PFBA would be calculated by multiplying the candidate value for the free acid of PFBA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{231}{214} = 1.079$. This same method of conversion can be applied to other salts of PFBA, such as the potassium or sodium salts, using the corresponding molecular weights.

[3] Note that the RfD for the free acid presented in this document and an RfD for the anion of PFBA (perfluorobutanoate, $C_3F_7CO_2^-$, CASRN 45048-62-2) would be practically identical given the molecular weights between the two compounds differ by less than 0.5%, (i.e., by the weight of a single hydrogen atom).

[4] HAWC is a modular content management system designed to store, display, and synthesize multiple data sources for the purpose of producing human health assessments of chemicals. This online application documents the overall workflow of developing an assessment from literature search and systematic review to data extraction (human epidemiology, animal bioassay, and in vitro assay), dose-response analysis, and finally evidence synthesis and visualization. In order to view HAWC study evaluation results, visualizations, etc., users must create first create a free account; see https://hawcprd.epa.gov/about for more details.

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 (see Section 5.2.3).

Evidence for Carcinogenicity

Under EPA's *Guidelines for Carcinogen Risk Assessment (*U.S. EPA, 2005*).* EPA concluded there is *inadequate information to assess carcinogenic potential* for PFBA by either oral or inhalation routes of exposure (see Section 3.2.5). This conclusion precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure (see Section 5.3).

Subchronic Oral Reference Dose (RfD) for Noncancer Effects

In addition to providing organ/system-specific RfDs for lifetime exposures in multiple systems, less-than-lifetime (subchronic) RfDs also were derived (see Section 5.2.2 and Tables 5-9 and 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., the use of a UF_S = 1 rather than 10 for subchronic studies given there is no extrapolation to a chronic exposure duration). Thus, the individual organs and systems for which specific subchronic RfD values were derived were the liver, thyroid, and developing fetus. The value for the developing fetus was selected for the subchronic RfD. A BMDL of 3.8 mg/kg-day NH_4^+PFB for increased time to vaginal opening in neonatal female mice was used as the basis for the POD. After converting the PODs from units of mg/kg-day NH_4^+PFB to units of mg/kg-day PFBA (by multiplying by the ratio of the molecular weights of the free acid and the ammonium salt), the HED was calculated by multiplying the POD for the free acid by the ratio of serum clearance values between mice and humans. The subchronic RfD for PFBA was calculated by dividing the POD_{HED} of 0.62 mg/kg-day PFBA by a composite uncertainty factor of 100 to account for extrapolation from rats to humans (UF_A = 3), for interindividual differences in human susceptibility (UF_H = 10), and deficiencies in the toxicity database (UF_D = 3). The subchronic RfD derived from the effects on delayed time to vaginal opening, as representative of general developmental delays, was 6×10^{-3} mg/kg-day[5].

[5] See Table 5-10 for details on how to calculate subchronic candidate values for salts of PFBA; briefly, the candidate values for different salts of PFBA would be calculated by multiplying the candidate value for the free acid of PFBA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{231}{214} = 1.079$. This same method of conversion can be applied to other salts of PFBA, such as the potassium or sodium salts, using the corresponding molecular weights.

1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSMENT METHODS

A series of five PFAS assessments (PFBA, perfluorohexanoic acid [PFHxA], perfluorohexane sulfonate [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 on the basis of 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, Section 12).

1.1. BACKGROUND INFORMATION ON PERFLUOROBUTANOIC ACID (PFBA)

Section 1.1 provides a brief overview of aspects of the physicochemical properties, human exposure, and environmental fate characteristics of perfluorobutanoic acid (PFBA, CASRN 375-22-4) and its related salt ammonium perfluorobutanoate (NH_4^+ PFB, CASRN 10495-86-0) that might 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 salts 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^+PFB is $C_4H_4F_7NO_2$. More specifically, these PFAS are classified as perfluoroalkyl carboxylic acids [PFCAs; OECD (2018)]. Because PFBA and NH_4^+PFB are PFCAs containing less than seven perfluorinated carbon groups, they are considered short-chain PFAS. The specific chemical formula of PFBA is $C_4HF_7O_2$ and the chemical formula of NH_4^+PFB is $C_4H_4F_7NO_2$. More specifically,

these PFAS are classified as perfluoroalkyl carboxylic acids [PFCAs; OECD (2018)]. Because PFBA and NH_4^+PFB 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^+PFB are presented in Figure 1-1, and select physicochemical properties are provided in Table 1-1.

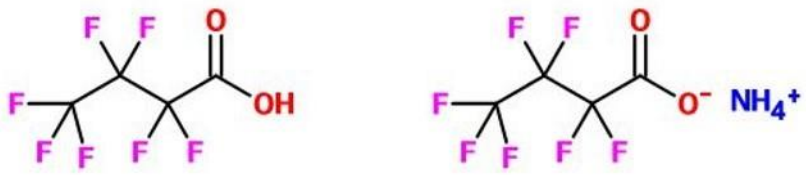

Figure 1-1. Chemical structures of perfluorobutanoic acid (PFBA) and ammonium perfluorobutanoate (NH_4^+PFB).

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

Property (unit)	Value	
	PFBA (free acid)	NH_4^+PFB
Molecular weight (g/mol)	214[a]	231[a]
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
Octanol-water partition coefficient (Log Kow)	1.43[a]	ND
Soil adsorption coefficient (L/kg)	88.9[a, b]	ND
Bioconcentration factor (BCF)	6.67[a,b]	ND

ND = no data.

[a]U.S. EPA (2018a) Chemicals Dashboard (PFBA DTXSID: 4059916): [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 to depend 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, 2018a). They are synthetic compounds that are or 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 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, carpets, 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 long-chain PFAS [ATSDR (2018a) https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS]. The production and use of these chemicals, however, 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 could continue to be a source of environmental contamination due to disposal or breakdown in the environment (Kim and Kannan, 2007).

No Chemical Data Reporting (CDR) on production volume for PFBA or its salts are available in EPA's ChemView (U.S. EPA, 2019a). Also, because facilities manufacturing, processing, or otherwise using PFAS are not required to report on releases to the environment, no quantitative information on PFBA is available in EPA's Toxic Release Inventory [TRI (U.S. EPA, 2019a)].[6]

Wang et al. (2014) estimated global emission estimates of PFBA from direct and indirect (i.e., 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 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, 2018a; NLM, 2017, 2016, 2013) and many are found worldwide in the air, soil, groundwater, and 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 released to air exist in the vapor phase in the atmosphere and resist photolysis, but particle-bound concentrations also have been measured (NLM, 2017, 2016, 2013; Kim and Kannan,

[6]As part of the National Defense Authorization Act for Fiscal Year 2020 (Section 7321), 172 per- and polyfluoroalkyl substances will be added to the TRI list; however, neither PFBA nor its ammonium salt is on the list of PFAS subject to TRI reporting requirements for Reporting Year 2022.

2007). Wet and dry deposition are potential removal processes for particle-bound PFAS in air (ATSDR, 2018b; Barton et al., 2007; Prevedouros et al., 2006; Hurley et al., 2004). Vapor intrusion may be a concern for PFBA given its vapor pressure and Henry's law constant, although no data currently exist measuring inhalation exposures due to vapor intrusion of PFBA.

PFBA would not 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 loss of PFBA from soils following biosolid application was estimated to be 385 days. PFBA would not be expected to be mobile in soil based on its soil adsorption coefficient (see Table 1-1).

The potential for PFAS to bioconcentrate in aquatic organisms depends on their bioconcentration factors (see Table 1-1), with longer chain PFAS accumulating to a greater degree. Thus, the potential for PFBA to bioconcentrate 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 (e.g., PFOA, PFOS, PFHxA, PFHxS, PFDA, and PFNA) with bioaccumulation factors of 28.4–56.8 for lettuce and 68.4 for corn. PFBA had a bioaccumulation factor of 12.2–18.2 for tomatoes, which was higher than all other PFAS studied except perfluoropentanoic acid (bioaccumulation factor of 14.9–17.1).

PFBA has not been evaluated under the National Air Toxics Assessment program (https://www.epa.gov/national-air-toxics-assessment). Likewise, although 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.

PFBA can be detected in most dust samples obtained from U.S. homes and vehicles, however, 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, Section 2.1). PFBA also has 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, Section 2.1).

PFBA also can be detected in food. PFBA has been found in fish at 16% of sites sampled in the U.S. Great Lakes (maximum concentration 1.3 ng/g) (Stahl et al., 2014). Additionally, although most of the available data are from samples from outside the U.S., PFBA has been detected in

grocery items including dairy products, meats and seafood, fruits and vegetables, food packaging, and spices (see Appendix A, Section 2.1).

Specifically, regarding drinking water, PFBA concentrations ranged from 0.0855 to 2.04 µg/L in seven municipal wells in Oakdale, Minnesota (U.S. EPA, 2019a). In a study of 23 public water systems in New Jersey (out of over 1,000), 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]. 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, 2017).

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

Media	Value	Number of NPL sites with detections
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 (2017).

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

The general population could be exposed to PFAS via inhalation of indoor or outdoor air (with PFAS possibly being released to the atmosphere via manufacturing processes or via disposal, i.e., incineration), ingestion of drinking water and food, and dermal contact with PFAS-containing products (ATSDR, 2018a). Exposure might also occur via hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018a). The oral route of exposure has been considered the most important one among the general population, however (Klaunig et al., 2015). Contaminated drinking water is likely to be a significant source of exposure. Due to the moderate water solubility and mobility of PFAS in groundwater (and general lack of remediation technology at water treatment facilities), populations consuming drinking water from any contaminated watershed could be exposed to PFAS (Sun et al., 2016). Use of powdered granulated carbon is more efficient in

removing longer-chain PFAS (Sun et al., 2016). Gebbink et al. (2015) modeled exposure to PFBA among the adult general population using 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. PFBA has also been detected in breastmilk and baby food products, indicating a potential additional route of exposure for infants. Antignac et al. (2013) reports that PFBA was detected in 17% (8 of 48) of breastmilk samples in a population of French mothers, with a mean concentration of 0.081 µg/L. Lorenzo et al. (2016) further reported that PFBA was detected in breastmilk, infant formulas, dry cereal baby food, and processed baby food in Valencia, Spain.

Although PFBA-specific exposure information is sparse, populations that might experience exposures greater than those of the general population could include individuals in occupations that require frequent contact with materials containing PFAS that break down 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 break down into PFBA has occurred might also be more highly exposed.

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 (see Table 1-3), are provided in Appendix A, Section 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)[7]
- 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). In addition, studies included in ongoing IRIS PFAS assessments (PFHxA, PFHxS, PFNA, PFDA) were also scanned for any studies that met PFBA PECO criteria.
- 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, epidemiological studies relevant to PFBA sometimes were 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 (e.g., 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 (see Table 1-3) identify the evidence that addresses the specific aims of the assessment and to focus the literature screening, including study inclusion/exclusion.

[7] Toxline has recently been moved into PubMed as part of a broad National Library of Medicine reorganization. Toxline searches can now be conducted within PubMed.

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 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 PFBA alone. (Note: Other PFBA mixture studies will be tracked as potential supplemental material.) Studies must address exposure to the following: PFBA (CASRN 375-22-4), or the ammonium salt NH_4^+PFB (CASRN 10495-86-0). [Note: Although PFBAs are not metabolized or transformed in the body, precursor compounds known to be bio-transformed to a PFAS are of interest, e.g., 6:2 fluorotelomer alcohol is metabolized to multiple analytes including PFHxA and PFBA (Russell et al., 2015b). 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 toward 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, Section 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 pharmacokinetic studies (excluding models)[8]
- Exposure assessment or characterization (no health outcome) studies
- Human case reports or case series studies

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 epidemiological and animal toxicological studies used in the PFBA assessment are provided in the systematic review protocol (see Appendix A, Section 6). The general approach for evaluating PECO-relevant health effect studies is the same for epidemiological and animal toxicological studies, although the specifics of applying the approach differ; thus, they are described in detail in Appendices A, Sections 6.2 and 6.3, respectively. Approaches for evaluating mechanistic evidence are described in detail in Appendix A, Section 6.5.

The key concerns for the review of epidemiological 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 ability of a study to detect a true effect; low sensitivity is a bias toward the null when an effect

[8]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, Section 9.2 for details).

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, Section 6 for definitions) related to each evaluation domain representing the different characteristics of the study methods that were evaluated on the basis of the criteria outlined in HAWC.

Once all 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, Section 8. Briefly, data extraction and content management were 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 could 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 Appendix A, Sections 9 and 10 for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis 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; processes for synthesizing mechanistic information are covered in detail in Appendix A, Section 9.2.

The syntheses of the human and animal health effects evidence focus 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 could be used if few or no studies with higher confidence are available to help evaluate consistency, or if the study designs of the *low* confidence studies address notable uncertainties in the set of *high* or *medium* confidence studies on a given health effect. If *low* confidence studies are used, a careful examination of 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 two-step 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 terms associated with the different strength of evidence judgments within evidence streams are *robust*, *moderate*, *slight*, *indeterminate*, and *compelling evidence of no effect*.

The animal, human, and mechanistic evidence judgments are then combined to draw an overall judgment that incorporates 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. The final output is a summary judgment of the evidence base for each potential human health effect across evidence streams. The terms associated with these summary judgments are *evidence demonstrates*, *evidence indicates (likely)*, *evidence suggests*, *evidence inadequate*, and *strong evidence of no effect*. The decision points within the structured evidence integration process are summarized in an evidence profile table for each considered health effect.

As discussed in the protocol (see Appendix A), the methods for evaluating the potential carcinogenicity of PFAS follow processes laid out in the EPA cancer guidelines U.S. EPA (2005) and that the judgements described here for different cancer types are used to inform the evidence integration narrative for carcinogenicity and selection of one of EPA's standardized cancer descriptions. These are: (1) *carcinogenic to humans*, (2) *likely to be carcinogenic to humans*, (3) *suggestive evidence of carcinogenic potential*, (4) *inadequate information to assess carcinogenic potential*, or (5) *not likely to be carcinogenic to humans*. However, for PFBA, data relevant to cancer were sparse and did not allow for such an evaluation (see Section 3.3).

1.2.5. Dose-Response Analysis

The details for the dose-response employed in this assessment can be found in Appendix A, Section 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 a reference value like the RfD depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer outcomes, a dose-response assessment was conducted for evidence integration conclusions of *evidence demonstrates* or *evidence indicates (likely)*. In general, toxicity values are not developed for noncancer hazards with *evidence suggests* conclusions (see Appendix A, Section 10.2 for exceptions).

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, 2012, 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 Appendix A, Section 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. Although 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/bmds) that can be applied to typical data sets, including those that are

nonlinear. In situations where alternative models with significant biological support are available (e.g., pharmacodynamic 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, 2012)]. Additional judgment or alternative analyses are used if the procedure fails to yield reliable results; for example, if the fit is poor, modeling might be restricted to the lower doses, especially if competing toxicity at higher doses occurs. When alternative approaches fail or are not applicable, the NOAEL/LOAEL approach is used for POD estimation. 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

As summarized in Section 1.2.1, the assessment used PECO criteria (see Table 8, Appendix A) to identify the evidence that addresses the specific aims of the assessment and focuses the literature screening, including study inclusion. In addition to those studies meeting the PECO criteria, studies containing supplemental material potentially relevant to the specific aims of the assessment were tagged 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 and other major scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as "potentially relevant supplemental material" included the following:

- In vivo mechanistic or mode-of-action studies, including non-PECO routes of exposure (e.g., intraperitoneal injection) and non-PECO populations (e.g., nonmammalian models);
- In vitro and in silico models;
- Absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic (PK) studies (excluding models);
- Exposure assessment or characterization (no health outcome) studies; and
- Human case reports or case-series studies.

The last literature search update prior to release of the draft Toxicological Review for public comment was April 2020. As shown in Figure 2-1, the searches through 2020 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, 2021). 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 PECO criteria. This included eight epidemiological studies, nine animal studies (including one published study (Butenhoff et al., 2012a) that reported on two unpublished industry reports (van Otterdijk, 2007a) and (van Otterdijk, 2007b), and one in vivo genotoxicity study. No high-throughput screening data on perfluorobutanoic acid (PFBA) were identified from ToxCast or Tox21. Additional literature searches were conducted in April 2021 and 2022. Those studies were screened as described in the protocol, resulting in the identification of two additional studies that met PECO criteria and are

included in this revised assessment (Grandjean et al., 2020; Zeng et al., 2020). In addition, a table compiling the published literature submitted in public comments received through the EPA docket (https://www.regulations.gov/docket/EPA-HQ-ORD-2020-0675) was provided to the external peer review panel and posted to the docket. That table includes the full text screening decisions for those studies (ultimately, none of the studies submitted to the docket that were not identified through the literature search updates through 2022 were incorporated into this Toxicological Review).

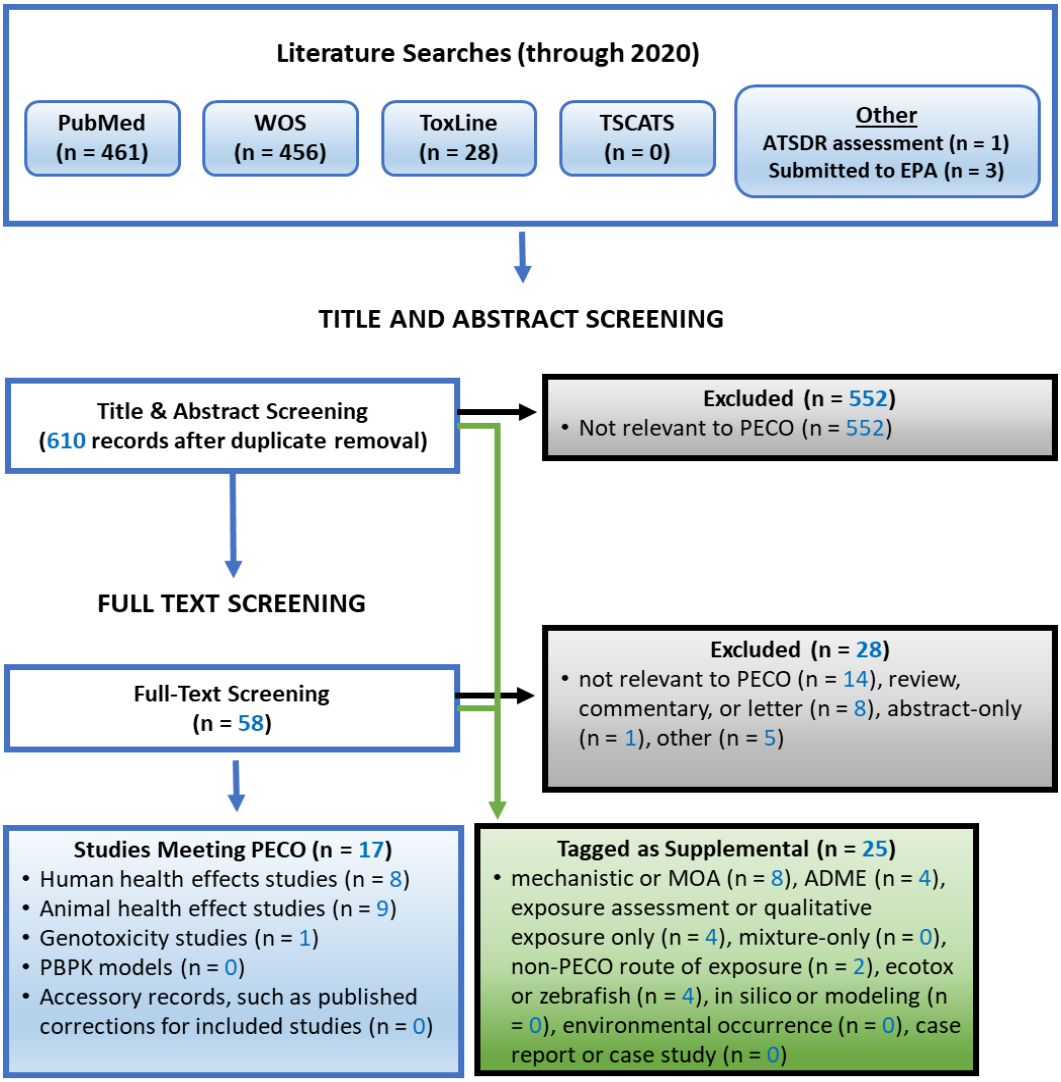

Figure 2-1. Literature search and screening flow diagram for perfluorobutanoic acid (PFBA).

TheButenhoff et al. (2012a) study reported the findings of two unpublished industry reports: a 28-day and 90-day gavage study fully reported in(van Otterdijk, 2007a, b). All three of these references are listed here as separate studies, but Figure 2-2 below only provides study quality determinations for (Butenhoff et al., 2012a).

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 is presented in Sections 3.2.1–3.2.5.

The evidence base of all repeated-dose oral toxicity studies for PFBA and the related compound ammonium perfluorobutanoate (NH_4^+PFB) 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., 2012a; Foreman et al., 2009; van Otterdijk, 2007a), one subchronic-duration study in rats (Butenhoff et al., 2012c; van Otterdijk, 2007b), and one gestational exposure study in mice (Das et al., 2008). In addition, eight epidemiological studies were identified that report on the association between PFBA and human health effects (Nian et al., 2019a; Wang et al., 2019; Song et al., 2018; Bao et al., 2017a; Li et al., 2017a; Li et al., 2017b; Kim et al., 2016; Fu et al., 2014). The available animal studies were generally well conducted (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 epidemiological studies are summarized in Figure 2-3, and rationales for each domain and overall confidence rating are available in HAWC (see link in Figure 2-3). Based on the study evaluations, one human epidemiological study was considered *uninformative* due to critical deficiencies in exposure measurement (Kim et al., 2016) this study is not discussed further in this assessment except to point out in more detail its critical deficiencies in the relevant health effects section.

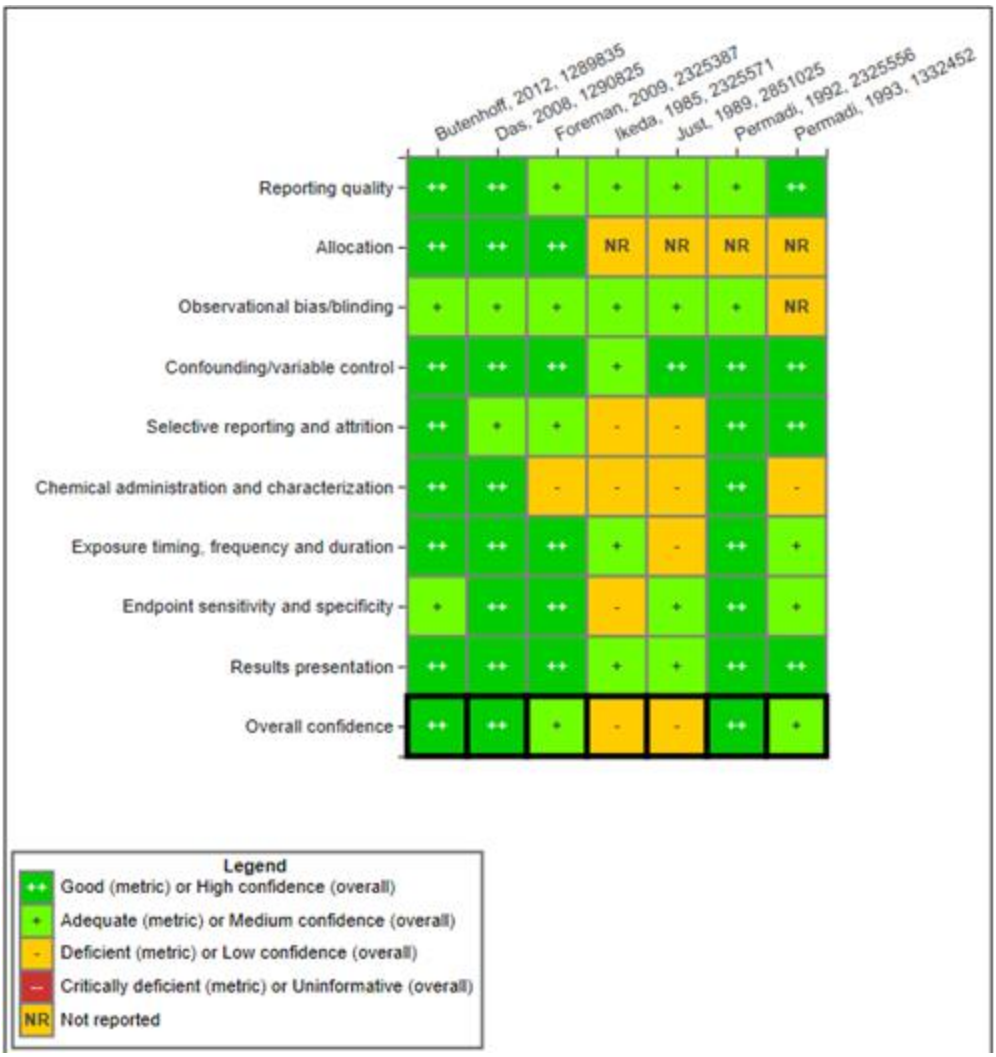

Figure 2-2. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA; interactive data graphic for rating rationales).

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

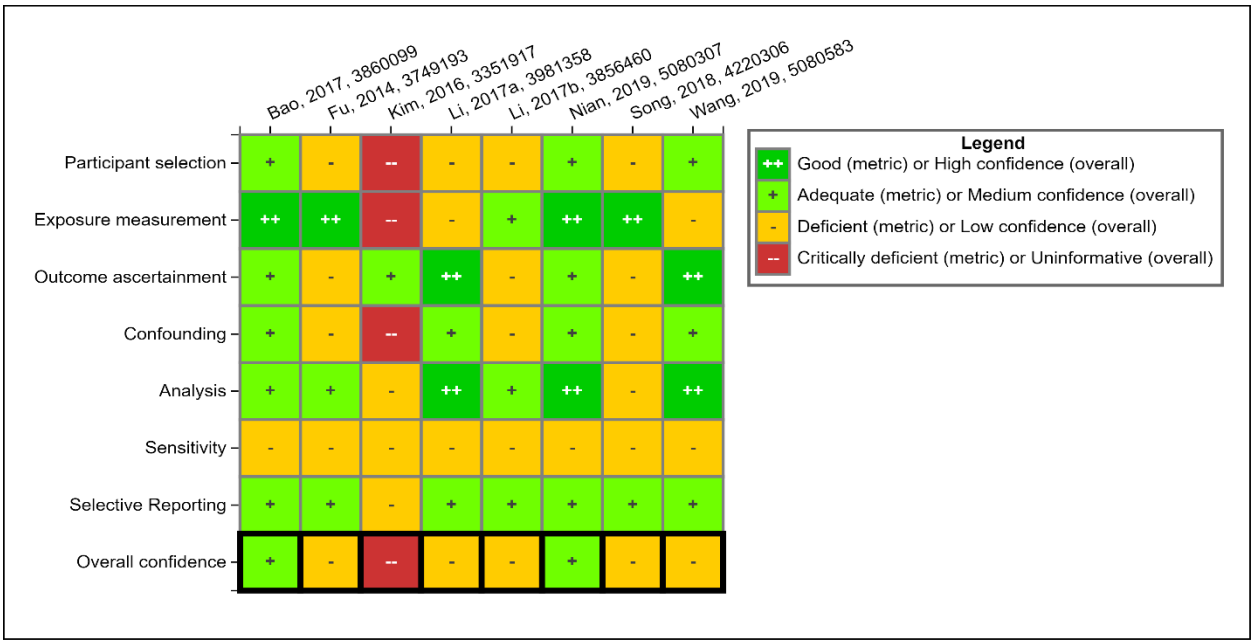

Figure 2-3. Evaluation results for epidemiological studies assessing effects of perfluorobutanoic acid (PFBA; interactive data graphic for rating rationales).

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., 2019a), developmental effects (Li et al., 2017a) reproductive effects (Song et al., 2018), blood lipids (Fu et al., 2014) hypertension/blood pressure (Bao et al., 2017b) and renal function (Wang et al., 2019).

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND EVIDENCE INTEGRATION

3.1. PHARMACOKINETICS

Pharmacokinetic studies have been conducted with dosing solutions prepared from PFBA [e.g., (Burkemper et al., 2017)] and the ammonium and potassium salts [e.g., (Chang et al., 2008)]. Some of the results evaluated below are semi-qualitative (e.g., that distribution is to all tissues of the body), hence are described with reference to the acidic form, since given PFBA's pKa of 0.08 the salts immediately dissociate after dissolution and analytic measurements are of the perfluorobutanoate ion. These results are applicable independent of the form used to prepare dosing solutions.

The one study for which quantitation of the pharmacokinetic parameters might depend on the form is (Chang et al., 2008). Chang et al. (2008) was careful to identify the form used for each part of their study so it is also clear that the chemical analysis used to measure concentrations in serum used to determine pharmacokinetic parameters is that of the acid, PFBA. However, calculation of the volume of distribution and clearance also involves the administered dose and Chang et al. (2008) does not specify whether or not the doses were converted to dose of the acid form. In a subsequent paper by the same research group evaluating the pharmacokinetics of PFHxS, Sundström et al. (2012) explicitly state, "concentrations in serum, liver, urine and feces are reported as PFHxS anion, and percent recoveries of administered dose in those matrices are corrected for the potassium salt." Hence, we will presume that Chang et al. (2018) similarly corrected either the applied dose or the serum concentrations to consistent units before reporting their pharmacokinetic parameters. Since the key parameters, volume of distribution and clearance, effectively involve the ratio of dose to serum concentration (or the area-under-the-concentration curve), resulting in measures of volume per kg BW or volume per time that are independent of the molecular weight, these results can be applied to analysis of PFBA per se, i.e., the acid or anion. Conversion to corresponding doses of a given salt is applied before or after pharmacokinetic analysis then provides the appropriate human equivalent doses for each form.

Animal evidence has shown that PFBA, like other perfluorinated chemicals, is well absorbed following oral administration and distributes to all tissues examined (Burkemper et al., 2017). A study evaluating the volume of distribution concluded, however, that the empirical volume of distribution is in the range typically associated with extracellular distribution (Chang et al., 2008). Because of its chemical resistance to metabolic degradation, PFBA appears to be primarily eliminated unchanged in urine and feces.

Pharmacokinetic 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. (2015a) 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 pharmacokinetics (PK) after inhalation and oral exposure of rats to 6:2 FTOH. The distribution of PFBA in human tissues also has been investigated (Pérez et al., 2013). Information on the absorption and distribution of PFBA to the serum and liver specifically has been investigated in several toxicological studies (Gomis et al., 2018; Butenhoff et al., 2012a; Foreman et al., 2009; Das et al., 2008).

3.1.1. Absorption

Chang et al. (2008) conducted a set of pharmacokinetic experiments in which Sprague-Dawley (S-D) rats three male and three female) were given either a single intravenous (i.v.) or oral dose (30 mg/kg body weight via gavage) of ammonium perfluorobutanoate (NH_4^+PFB). 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 might be a statistical result from the small sample size (n = 3/group) or due to a problem in dosing. The result, however, indicates 100% oral absorption.

In other experiments, Chang et al. (2008) orally administered 3–300 mg/kg to male and female S-D rats via gavage. 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 exposed to PFBA via oral gavage also were 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 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 [1]8F]-PFBA (~0.074 MBq/µL). At 4 hours postinjection, the [1]8F]-PFBA was detected in every tissue investigated, with most of the dose found in the stomach (~7.5% injected dose/g). All concentrations in the blood, lung, liver, kidney, intestines, and skin

were 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^+PFB 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^+PFB 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 measured only in the liver and blood serum), these studies demonstrated consistently that PFBA does distribute to the liver compartment in both species. Butenhoff et al. (2012a) observed that liver concentrations of PFBA (µg/g) were higher in male and female S-D rats exposed to PFBA for 28 days vs. rats exposed for 90 days. The ratio between liver concentrations (µg/g) and serum concentrations (µg/mL) ranged from 26% to 47% in the 28-day rats and 16% to 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 mice and in postnatal day (PND) 1 and PND 10 pups. Serum levels and liver levels of PFBA appeared to be lower in nonpregnant mice compared to pregnant mice in the lowest two dose groups, with mean serum concentrations approximately twofold higher in pregnant mice compared to nonpregnant mice in the 35 mg/kg-day and 175 mg/kg-day dose groups (see Table 3-1). This pattern also was observed for liver concentrations where pregnant animals had approximately two to three times the liver concentration of PFBA compared to nonpregnant animals in the 35 mg/kg-day and 175 mg/kg-day dose groups. However, these differences were not statistically significant and are based on only two or three nonpregnant mice at each dose level, and serum and liver levels were essentially identical between pregnant and nonpregnant mice at the high dose (350 mg/kg) level. Additionally, the serum and liver concentrations of PFBA were attenuated in high-dose (350 mg/kg) animals. Possible explanations for this pattern (with both liver and serum levels being lower in non-pregnant than pregnant animals) would be higher oral absorption, lower clearance, or higher distribution to other tissues (including fetuses and placenta) at the intermediate doses but not at the higher doses in the pregnant mice. However, serum concentrations in PND 1 pups were about 7-fold lower than the pregnant dams (end of gestation) and liver concentrations were 6–7 fold lower, indicating that distribution to the fetuses was not higher than distribution to other maternal tissues. Since PFBA absorption data (see Section 3.1.1) are consistent with close to 100% absorption, similar to other PFAS, it is not possible for absorption to be increased during pregnancy. It is possible that clearance is reduced during pregnancy due to hormonal changes affecting renal transporters, increasing resorption and hence internal doses, with this effect being neutralized by saturation of the transporters at the highest doses. Pharmacokinetic data from

Chang et al. (2008) (see Section 3.1.4) are consistent with saturation of renal resorption in the range of 3-100 mg/kg doses in female mice, which supports this possible explanation. However, given the small sample-size of the non-pregnant animals in Das et al. (2008) and the fact that the animals were dosed for 17 days, compared to the single doses used in PK studies, additional experiments would be needed to validate and more accurately quantify any pregnancy-related differences.

As would be expected, both the serum and liver concentrations in PND 1 pups were much greater than those in PND 10 pups, since dosing ceased on GD 18 (Das et al., 2008). Das et al. (2008) corroborated the observations by Butenhoff et al. (2012a) 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, 20%–23% in male mice, and 15%–17% in female mice. Interestingly, minimal differences in liver/serum concentrations also were observed in various genetic strains of mice exposed to 35–350 mg/kg PFBA: 34%–47% in wild-type mice, 19%–37% in peroxisome proliferator-activated receptor alpha (PPARα) null mice, and 22%–37% in humanized PPARα mice (Foreman et al., 2009). These results suggest that PPARα status has minimal effect on the distribution of PFBA between liver and serum.

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 mice 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 mice Das et al. (2008)		PD10 male and female mice 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. (2012a)		90-d male rats Butenhoff et al. (2012a)	
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	–	–

Dose group (mg/kg-d)	Serum (µg/mL)	Liver (µg/g)	Serum (µg/mL)	Liver (µg/g)
	Pregnant mice Das et al. (2008)		Nonpregnant female mice Das et al. (2008)	
	28-d female rats Butenhoff et al. (2012a)		90-d female rats Butenhoff et al. (2012a)	
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	–	–
	28-d PPARα +/+ mice Foreman et al. (2009)*		28-d PPARα -/- mice Foreman et al. (2009)*	
35	87 ± 27	30 ± 1.6	67 ± 14	13 ± 1
175	108 ± 7	51 ± 5	99 ± 16	36 ± 7
350	115 ± 11	46 ± 4	81 ± 20	28 ± 5
	28-d hPPARα mice Foreman et al. (2009)*			
35	59 ± 12	21 ± 4		
175	146 ± 20	32 ± 4		
350	35 ± 5	9 ± 2		

* Foreman et al. (2009) analyzed tissue concentrations in male wild-type (PPARα +/+), PPARα -/- and humanized PPARα (hPPARα) mice on an Sv/129 genetic background.* Foreman et al. (2009) analyzed tissue concentrations in male wild-type (PPARα +/+), PPARα -/- and humanized PPARα (hPPARα) mice on an Sv/129 genetic background.

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) analyzed. The observation that PFBA was observed in the greatest quantities in kidney samples could be related to kidney reabsorption. Chang et al. (2008) observed that rats given 300 mg/kg PFBA orally excreted substantially greater amounts of PFBA in the urine than did rats given 100 mg/kg (90.16% ± 2.75% vs. 50.99% ± 4.35%), and the authors suggested this as evidence of saturation of a renal tubular reabsorption process.

Abraham et al. (2021) analyzed PFBA levels in lung and tissue samples collected from tumor patients in France and observed concentrations approximately three to four orders of magnitude lower than Pérez et al. (2013): 0.08–0.24 ng/g in lung (n = 7) and 0.04–0.19 ng/g in kidney (n = 9). These were different individuals living in a different country, so some difference in exposure levels is expected. Additionally, tissue samples were obtained from cancer patients versus people that died from trauma or ischemic heart disease, further complicating the comparison. But given the relatively rapid clearance of PFBA compared to other PFAS, one would expect its tissue levels to be lower than other PFAS, not the highest, and one would have to assume that exposure to

the subjects of Abraham et al. (2021) to be thousands of times lower than the subjects of Pérez et al. (2013) order to otherwise explain the difference. Bangma et al. (2021) determined that a saturated oxo-fatty acid as an analytic interferent with PFBA in the placenta, indicating that it could also have given falsely high measurements in the Pérez et al. (2013) lung samples. It is noted that Pérez et al. (2013) describe careful and fairly comprehensive quality-assurance (QA) methods employed, such as use of matrix-matched calibration, while Abraham et al. (2021) does not report what QA/quality-control (QC) methods were used. Given the QA of (Pérez et al., 2013) an interferent would need to be present in human lung but not pig lung (species source of tissues used for QA) to result in large over-estimates. Resolution of this discrepancy is beyond the scope of this review and likely requires additional tissue samples from a larger population, preferably one with known PFBA exposure levels in the weeks immediately prior to sampling, given the short half-life.

Data are not available that can be used reliably to estimate the volume of distribution (V_d) in humans, which effectively provides the total body burden based on observed blood or serum concentrations. An estimation of human body distribution for other PFAS is provided by the PBPK models for PFOA and PFOS of Loccisano et al. (2011) which assume identical tissue:blood partition coefficients (PCs) in humans and monkeys, equal to the values measured using tissues from rats (PFOA) and mice (PFOS). This assumption is common to many PBPK models, based on the expectation that the biochemical properties of a given tissue, muscle for example, which determines the relative affinity of a chemical for that tissue compared to blood, are similar across mammalian species: mouse, rat, monkey, and human muscle are all similar in composition and the difference in chemical distribution to muscle as a whole is determined by the difference in the volume of muscle per kg BW between species.

PCs are the effective tissue specific V_d values because they determine the ratio of the amount in a tissue vs. blood concentration at equilibrium. Based on this PBPK model Loccisano et al. (2011), the V_d for PFOA predicted in monkeys and humans is 0.210 and 0.195 L/kg, respectively, and for PFOS is 0.333 and 0.322 L/kg, respectively. These predictions are obtained by summing the tissue fractions (ratios of tissue volumes/BW) multiplied by the corresponding PCs. In comparison, based on the Loccisano et al. (2011) model for adult rats, the corresponding V_d values in that species, for PFOA and PFOS, are 0.290 and 0.398, respectively. The difference between these rat values and the human and monkey values is primarily due to the difference in physiology, specifically the proportion of BW that is liver, kidney, and other tissues. Because of the physiological similarities between humans and monkeys (more similar tissue fractions), the predicted V_d values are within 7% of each other, although the difference between human and rat V_d values is predicted to be 49% for PFOA and 24% for PFOS. They are much more similar between humans and monkeys than between humans and rats, but the difference between humans and rats is still less than a factor of 1.5.

Li et al. (2020) evaluated the transplacental transfer of multiple PFAS, including PFBA, in human preterm vs. full-term births, and evaluated the data for correlation with the expression of

nine placental transporters. The transplacental transfer efficiency (TTE) was calculated as the ratio of PFAS concentration in cord serum, collected at the time of birth, to the concentration in maternal serum collected within 1 week of (prior to) birth. The median TTE for preterm births was 0.48, with first and third quartiles of Q1 = 0.27 and Q3 =1.06 (n = 33) Li et al. (2020), hence the distribution in the preterm fetus was predominantly less than one though it may not be significantly so. This result is qualitatively consistent with the observations of Das et al. (2008) in mice, described above. However, the human TTE was observed to increase to a median value of 1.06 in full-term deliveries, with the difference between preterm and full-term indicated as significant (Li et al., 2020). This result is consistent with a possible loss of integrity of the placenta as a passive barrier to PFAS transport occurring towards the end of pregnancy, as discussed by the authors, since the TTE did not show a significant correlation with any of the transporters evaluated (Li et al., 2020).

The extent to which the volume of distribution may change during pregnancy in humans has not been evaluated. Based on data reported by Kuczmarski et al. (2000) an average woman gains about 25% of her initial body-weight during pregnancy. If the data of Li et al. (2020) can be interpreted as showing that distribution to this additional mass is about one half of distribution to other maternal tissues, then the total volume of distribution in the pregnant mother (L) would increase about 12.5% while her mass increases 25%, leading to V_d in late pregnancy of 112.5/125 = 90% of non-pregnant V_d, which is not a significant change. Even if distribution to the fetus was much lower in the human fetus than the mother, her V_d would decrease by no more than 20%. While such a change may be marginally significant, it is still well within the overall uncertainty for estimates of V_d. Another factor during human pregnancy is the decrease in serum proteins, with the decrease in albumin concentration being consistent with dilution of the protein into an increased total plasma volume (Paaby, 1960). Such a decrease could lead to both an increased V_d of PFBA, since a smaller fraction would be bound in blood, and an increase in clearance. Since the reduction in protein concentration is on the order of 10%–20% (Paaby, 1960) like the potential change due to the growth of the fetus discussed just above, the impact is not expected to be large, and it is in the opposite direction of that effect. Hence, while pregnancy-related factors specific to distribution may cause some change in distribution, this change is not expected to be significant. On the other hand, if hormonal changes increase renal resorption during pregnancy, as is suggested in mice (discussion above), that could significantly increase maternal body burden during that time. Measurements of clearance in pregnant vs. non-pregnant women (i.e., using matched blood and urine samples) would be needed to determine if such a difference exists.

Based on this analysis for PFOA and PFOS, the most reasonable choice for estimation of V_d for PFBA in humans is to assume that it is similar to the V_d estimated for PFBA in monkeys, rather than values estimated for mice or rats.

It is recognized that the distribution of PFAS depends on the extent of binding to various proteins and partitioning into phospholipid membranes. Chen and Guo (2009) measured the binding of PFBA to human serum albumin and obtained a binding constant of $(1.1 \pm 0.1) \times 10^6$ M^{-1}

for albumin site I with no observed binding to the Trp site or site II. However, corresponding measures of phospholipid partitioning and binding to cellular proteins are not available, so it is not possible to estimate the extent to which these contribute to tissue partitioning.

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., 2015b; Ruan et al., 2014). No evidence of biotransformation for PFBA, however, was found. PFBA, a short-chain (C4) of perfluoroalkyl acids (PFAAs), is expected to be metabolically inert because its chemical stability is the same as longer chain PFAA chemicals, including perfluorohexane sulfonate (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. For rats and monkeys, three animals per sex were used (rats: three animals each for i.v. and oral dosing) at the single dose given to each. For mice, three animals per sex *per time point* were used at each dose, or 15–18 animals/dose. OECD guidelines state that a minimum of four animals per sex per dose should be used (OECD, 2010). Thus, the rat and monkey studies fall short of this standard. For rats, however, the average clearance from the two routes of exposure is proposed to best represent males and females of that species (details below), which is then based on data from six animals per sex. For monkeys, the average volume of distribution for both males and females are used as an estimate for that value in humans, again incorporating data from six animals. Therefore, these data are presumed sufficient for the specific parameters being estimated. In S-D rats exposed orally to 30 mg/kg PFBA, a marked difference was noted 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 intravenous exposure (see Appendix C for a complete discussion on whether the calculated elimination constants in various species are mono- or biphasic). The difference in oral λ resulted in half-lives ($t_{1/2}$) of 9.22 and 1.76 hours, respectively, for males and females. Chang et al. (2008) reported clearance (CL) values as mL/hour, not normalized to BW, but the normalized average CL can be calculated as dose/AUC, using the AUC values they reported. For oral doses in male and female rats, the result CLs are 0.38 and 1.6 L/kg-day, respectively, while for i.v. doses they are 0.66 and 3.0 L/kg-day, respectively.

Russell et al. (2015b) 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 for 24 hours after start of

exposure. The decline in PFBA blood concentration was negligible, however, 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 PK model resulted in estimation of a 27.7-hour half-life for that sex, which could explain the inability to estimate a half-life from the single-day exposures. Both estimates depend on the estimated yield (percent of 6:2 FTOH metabolized to PFBA), however, 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. Thus, the results of Chang et al. (2008) is used to represent excretion in rats.

In male CD-1 mice, the clearance was similar in mice exposed to 10 mg/kg (0.35 ± 0.09 mL/hour) and 30 mg/kg PFBA (0.37 ± 0.80 mL/hour); however, clearance at 100 mg/kg was much higher (0.98 ± 0.14 mL/hour) (Chang et al., 2008). Although the fit of the simple one-compartment model used to describe the kinetic data appeared adequate for the two lower doses, it underpredicted the data at 24 and 48 hours for the 100 mg/kg dose, indicating it was not sufficient for this highest exposure. In female mice clearance showed a similar, but less strong pattern, with values of 0.76 ± 0.03, 0.87 ± 0.04, and 1.67 ± 0.08 mL/hour at 10, 30 and 100 mg/kg doses, respectively (Chang et al., 2008). Unlike the data for male mice, the female mouse data were fit well by the one-compartment PK model. For female data, the possible dose-dependence can be resolved by using the average clearance for the lower two doses, which are closer to the doses evaluated for point-of-departure (POD) determination. Because male mouse endpoints are not considered for POD determination, an alternative PK analysis of these data is not supported.

Using dose/AUC, the corresponding CL values are 0.23, 0.25, and 0.66 L/kg-day in male mice at 10, 30, and 100 mg/kg, respectively, and 0.62, 0.72, and 1.36 L/kg-day in female mice, respectively.

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 (Chang et al., 2008). Notably, the β phase began at around 24 hours and was observed because samples also were taken at 2, 4, 7, and 10 days, while in rodents, samples were reported only to 24 hours (rats and female mice) or 48 hours (male mice). Whereas serum levels in female rats and mice dropped to less than 3% of peak concentration by 24 hours, indicating minimal longer-term elimination, the levels in male mice and rats did not drop as quickly and are more suggestive of a β phase. Also noted is that the mouse and rat PK plots in Chang et al. (2008) use a linear y-axis, while the monkey PK plots use a log y-axis. That a β phase would have been clearly observed in male mice and rats is possible had serum sampling been continued for a longer duration, and possibly in female mice and rats had the data simply been plotted with a log y-axis. 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; $t_{1/2}$ values in female monkeys were 2.28 ± 0.14 hours and 41.04 ± 4.71 hours, respectively.

Excretion of PFBA from the serum in humans also was investigated by Chang et al. (2008). In the initial occupational study, baseline PFBA serum concentration was determined in male workers (n = 3) exposed to either PFBA or related fluorinated compounds. Following voluntary removal from the workplace, workers had blood samples taken over 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 nine 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). Because these workers had been exposed previously for a significant duration and the PK study was conducted over periods ranging from 7 to 11 days, the observed elimination is reasonably presumed to represent β-phase elimination, rather than the initial distribution phase. Although only two female subjects were included in the second study (and their final PFBA serum concentrations fell below the limit of detection), their estimated $t_{1/2}$ values (118 hours and 56 hours) fell within the range of $t_{1/2}$ values reported for males (44–152 hours). Therefore, although sex differences in serum excretion in rodent species appear strong, the data in cynomolgus monkeys and humans do not indicate such a difference.

Measurements for four of the subjects evaluated by Chang et al. (2008) fell below the lower limit of quantification (LLOQ) when the second blood sample was taken, requiring the authors to assume a value of LLOQ/$\sqrt{2}$ for those values. This approach introduces considerable uncertainty, so the population half-life excluding those individuals was estimated as described in Appendix C.2 to obtain a half-life of 67.9 hours (rather than the author-reported arithmetic mean value of 72 hours). This revised estimate will be used for subsequent analysis.

Using an assumed $BW^{0.75}$ scaling and standard species BWs of 0.25 kg in rats and 80 kg in humans, the half-life in humans is predicted to be 4.2 times greater than in rats. Given half-lives of 9.22 and 1.76 hours, respectively, in male and female rats (oral dose values), one would then predict half-lives of 37.8 hours in men and 7.2 hours in women. Although 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), $BW^{0.75}$ scaling is not based on data for this class of chemicals (i.e., serum binding and clearance mechanisms are known to occur for PFAS). For example, EPA's *Recommended Use of Body Weight 3/4 as the Default Method in Derivation of the Oral Reference Dose (*U.S. EPA, 2011*)* does not mention serum binding; it does include references related to VOCs, drugs, and overall metabolism (with metabolism a significant component in the clearance of many other toxic chemicals) but does it cite papers evaluating the pharmacokinetics of PFAS. These results for PFBA indicate that $BW^{0.75}$ scaling would lead to a lower prediction of human health risk at a given exposure than dosimetric scaling

based on the empirical data. Further, although 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 $BW^{0.75}$ is an unlikely occurrence—even given the small sample size—and using of $BW^{0.75}$ scaling (applied to the half-life in female rats) could underpredict the risk of exposure by an order of magnitude. Therefore, use of $BW^{0.75}$ 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. Female rodents (rats: 100.68%–112.37%; mice: 65.44%–67.98%) are observed to have higher percentages of the dose excreted in urine at 24 hours compared to male rodents (rats: 50.99%–90.16%; mice: 34.58%–35.16%). This is consistent with evidence that organic anion transporters (OAT) expressed in the kidneys of rodents reabsorb PFAS (Weaver et al., 2010; Yang et al., 2009) and are more highly expressed in male rodents (Ljubojevic et al., 2007; Ljubojevic et al., 2004; Buist et al., 2002; Cerrutti et al., 2002; Kato et al., 2002). Both Yang et al. (2009) and Weaver et al. (2010), however, observe that PFBA is not an active substrate of organic anion transporters OAT1, OAT2, or OATP1a1. Therefore, although 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 known. 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

PFBA clearance (CL) data, which can be used to estimate the average blood concentration for a given dose, are available for mice and rats. For mice, the average CL from PK experiments at 10 and 30 mg/kg is suggested for use in animal-human extrapolation. For rats, the average of values estimated from i.v. and oral exposure to 30 mg/kg is suggested.

Direct comparison of animal and human data requires consideration of observed half-lives because such data are available in humans, but CL cannot be directly estimated in humans. Collectively, although the PFBA excretion half-lives for male and female rats appear shorter than for male and female mice, respectively, data suggest a strong sex-specific pharmacokinetic difference for both species (i.e., females appear to have a much faster excretion rate than males). Humans have a longer serum excretion half-life (~day) than rodents (~hour). Although data in male mice and rats might indicate a longer β phase elimination, the lower dose data in male mice are reasonably fit using a single half-life (one-compartment model) as are the i.v. and oral data at the single dose given to rats (30 mg/kg); the female mouse and rat data are likewise fit well by a one-compartment model (Chang et al., 2008). Therefore, although a longer elimination phase might be evident if additional data were available, the estimated total clearance is unlikely to differ substantially from

the estimates provided here. The α-phase half-lives in monkeys (1.6–2.3 hours) are similar to the half-life obtained for female mice (2.8–3.1 hours) and female rats (1–1.8 hours) but are substantially shorter than the half-life observed in male mice (13–16 h at lower doses) and male rats (6–9 hours). The β-phase half-life in monkeys (1.7 days) is considerably longer than any of these rodent values but is comparable to the lower end of the range for human subjects (1.8–2 days), although roughly one-half the average among humans (3 days). As noted above, these human half-lives are expected to represent β-phase, considering the period of observation vs. exposure.

Human CL can be estimated using the PK relationship, $CL = V_d \cdot ln(2)/t_{0.5}$. Because human data do provide a value of $t_{1/2}$, only a value of V_d is needed to determine CL. As discussed above, however, one can reasonably anticipate that V_d in humans is similar to that in other primates based on the similarity in physiology and assumptions common to PBPK modeling. This similarity is illustrated on the basis of PBPK models for PFOA and PFOS Loccisano et al. (2011) from which V_d in humans is predicted to be within 7% of the value for monkeys for those two PFAS. Thus, this choice seems appropriate for estimating human clearance of PFBA. Using the average human half-life of 67.9 hours (2.8 days) from Appendix C.2 and average of male and female monkey V_d of 0.485 L/kg from Chang et al. (2008) the resulting human clearance is 0.12 L/kg-day.

Table 3-2 provides a summary of PFBA pharmacokinetics.

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

Species/ sex	Study design	Excretion half-life (h)	AUC (µg-h/mL)	Clearance (mL/h)	Clearance (L/kg-d)[a]	Volume of distribution (mL/kg)
Rats						
Male	30 mg/kg i.v. dose	6.38 ± 0.53	1,090 ± 78	7.98 ± 0.57	0.661	253 ± 6
	30 mg/kg oral dose	9.22 ± 0.75	1,911 ± 114	4.63 ± 0.28	0.377	209 ± 10
Female	30 mg/kg i.v. dose	1.03 ± 0.03	239 ± 5	27.65 ± 0.55	3.01	187 ± 3
	30 mg/kg oral dose	1.76 ± 0.26	443 ± 42	14.32 ± 1.36	1.63	173 ± 21
Mice						
Male	10 mg/kg oral dose	13.34 ± 4.55	1,026 ± 248	0.35 ± 0.09	0.234	152
	30 mg/kg oral dose	16.25 ± 7.19	2,869 ± 6,116	0.37 ± 0.80	0.251	296
	100 mg/kg oral dose	5.22 ± 2.27	3,630 ± 530	0.98 ± 0.14	0.661	207
Female	10 mg/kg oral dose	2.87 ± 0.30	387 ± 14	0.76 ± 0.03	0.620	107
	30 mg/kg oral dose	3.08 ± 0.26	999 ± 42	0.87 ± 0.04	0.720	134
	100 mg/kg oral dose	2.79 ± 0.30	1,760 ± 88	1.67 ± 0.08	1.36	207
Monkeys						
Male	10 mg/kg i.v. dose	1.61 ± 0.06 (α)	112 ± 6	494 ± 61	2.14	526 ± 68

		40.32 ± 2.36 (β)				
Female	10 mg/kg i.v. dose	2.28 ± 0.14 (α) 41.04 ± 4.71 (β)	159 ± 8	224 ± 19	1.51	443 ± 59
Humans						
Males and females	NV	Study 1: 28.6–109.71 Study 2: 72 (mean)	NV	NV	NV	NV

AUC = area-under-the-concentration-curve, NV = not available.
All data from Chang et al. (2008).All data from Chang et al. (2008).
[a]Calculated as dose (mg/kg) x (1,000 µg/mg) × (24 h/d) / ((AUC µg-h/mL) × (1,000 mL/L)).

The mouse PK data of Chang et al. (2008) clearly indicate nonlinear elimination, with more rapid clearance at higher concentrations consistent with a mechanism of saturable renal resorption. Since there is only a modest difference in clearance between the lowest two doses (10 and 30 mg/kg-day) it is reasonable to assume first-order elimination around and below these dose levels in mice. However, use of the low-dose clearance for effects associated with higher doses is likely to over-predict the corresponding HED, since mouse clearance is higher at higher exposures.

Unfortunately, the single dose used for PK in rats is not sufficient to demonstrate when saturation might occur in that species. The data and model fits shown by Chang et al. (2008), particular for the i.v. administration, appear quite consistent with first-order elimination assumed in their analysis. Hence, for the purposes of the current analysis, it is assumed that the estimated CL is applicable to 30 mg/kg-day doses or below and to avoid extrapolation above that dose.

Some mechanistic insight can be gained by comparing the clearance values described above with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding. Davies and Morris (1993) summarized GFR for multiple species. Considering the time period when those data were collected, it seems appropriate to use the species average BW values listed in Table III of Davies and Morris (1993): 0.02 kg for the mouse, 0.25 kg for the rat, and 70 kg for the human. Using those, the GFR/BW for these species are 20.2 L/kg-day in mice, 7.55 L/kg-day in rats, and 2.57 L/kg-day in humans. which are, respectively, 83 and 32 times higher than PFBA clearance in male and female mice (average of values at lowest two doses), 14.5 and 3.3 times higher than the average for male and female rats from Chang et al. (2008), and 21 times higher than the human PFBA clearance estimated above.

Binding to serum proteins plays a likely role in these very large differences. Chen and Guo (2009) measured the binding of PFBA to human serum albumin and obtained a binding constant of $(1.1 \pm 0.1) \times 10^6$ M^{-1} for albumin site I with no observed binding to the Trp site or site II. Using a representative serum albumin concentration of 40 mg/mL = 6×10^{-4} M, the predicted free fraction of PFBA is $f_{free} = 0.0015$. This binding may play a role in the limiting the rate of the renal excretion of PFBA, in addition to the role played by renal transporters. Using this value, GFR × f_{free} = 0.03 L/kg-day in mice, 0.01 L/kg-day in rats, and 0.004 L/kg-day in humans. The measured CL for male mice

(0.23–0.66 L/kg-day) is 8- to 22-fold higher than GFR× f_{free} and the CL for female mice (0.62–1.36 L/kg-day) is 21- to 45-fold above GFR× f_{free}. Even more significantly, CL in rats is as much as 300-fold higher than the corresponding GFR× f_{free}, and the estimated CL in humans (0.12 L/kg-day) is 30-fold higher than the corresponding GFR× f_{free}. The source of these apparent discrepancies is unclear. It is reasonable to expect that plasma protein binding will limit the clearance of PFBA. However, these results indicate that either f_{free} is significantly under-estimated or that clearance is not strictly limited to the free fraction (estimated from an in-vitro binding constant). Binding and dissociation are dynamic processes, and it may be that as blood passes through the glomerulus and filtration occurs, some portion of the albumin-bound PFBA is sufficiently labile to dissociate and also be cleared. A mathematical model that incorporates the kinetics of plasma binding and release to describe uptake of drugs by the brain has been previously described by Robinson and Rapoport (1986), but adaptation of this model to renal clearance of PFBA would require measurement of the separate rates of association and dissociation, data which have not been reported.

Another possible explanation is from imperfect filtering of albumin by the glomerulus, leading to some urinary excretion of albumin which may carry bound PFBA. Van Camp et al. (1990) observed an albumin excretion rate in female rats on normal diets (i.e., control animals) of about 1 mg/day, which corresponds to a clearance of 0.025 mL/day given a serum albumin concentration of 40 mg/mL. The urine samples were collected at the mid-point of the experiment. Based on the BW reported on the first and final days of the experiment, the rats at this time were around 0.14 kg, hence had an albumin CL of 1.8×10^{-4} L/kg-day; i.e., about four orders of magnitude lower than the PFBA CL in female rats (see Table 3-2). While kidney damage is known to increase albumin excretion (for example, a high phosphate diet increased albumin excretion in female rats about 50-fold (Matsuzaki et al., 2002)), an increase of 10,000-fold occurring within the 24-hour time-frame of the PK experiments, when kidney toxicity has not been reported for PFBA exposure in rats, seems rather unlikely. However, if only 5% of the bound PFBA is sufficiently labile to be available for clearance, that would be consistent with the empirical data and estimated clearance rates.

3.2. NONCANCER EVIDENCE SYNTHESIS AND INTEGRATION

For each potential health effect discussed below, the synthesis describes the database of available studies and the array of the experimental animal study results (the primary evidence available for this PFAS) across studies. Effect levels presented in these arrays are based on statistical significance[9] or biological significance, or both. Examples relevant to interpretations of biological significance include directionality of effect (e.g., statistically significantly decreased cholesterol/triglycerides are of unclear toxicological relevance) and tissue-specific considerations for magnitude of effect (e.g., statistically nonsignificant increase of ≥10% in liver weight might be considered biologically significant). A significant finding at a single, lower dose level but not at

[9]In this review, "statistical significance" indicates a p-value < 0.05, unless otherwise noted.

multiple, higher dose levels might be interpreted as potentially spurious. For this section, evidence to inform organ/system-specific effects of PFBA in animals following developmental exposure is discussed in the individual organ/system-specific sections (e.g., liver effects after developmental exposure are discussed in the liver effects sections). 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[10] 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, inverse associations with thyroxine (T4), free triiodothyronine (T3), and thyroid-stimulating hormone (TSH) were reported. Among the thyroid hormones measured, only TSH demonstrated a statistically significant association (Pearson correlation coefficient = −0.348, $p < 0.01$).

Animal Studies

Two *high* confidence studies reported in two unpublished reports and one publication from the same research group evaluated the effects of PFBA exposure on the thyroid, specifically hormone levels, histopathology, and organ weight (Butenhoff et al., 2012c; Butenhoff et al., 2012a; van Otterdijk, 2007a, b) following oral exposure (via gavage) of SD rats.[11] Some outcome-specific considerations for study evaluations were influential on the overall study rating for thyroid effects, but none of these individual domain-specific limitations were judged likely to be severe or to have a notable impact on the study results; all studies considered further in this section were rated as *high* or *medium* confidence (see Figure 3-1). For more information on outcome-specific considerations for study evaluations, please refer to the study evaluations in the HAWC PFBA project page.

[10]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.

[11]The Butenhoff et al. (2012a) study reported the findings of two unpublished industry reports: a 28-day and 90-day gavage study fully reported in (van Otterdijk, 2007a, b). These industry reports were conducted at the same facility and largely by the same staff but 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 Toxicological Review, both (Butenhoff et al., 2012c; Butenhoff et al., 2012a) and the relevant industry report are cited when discussing effects observed in these reports. Although only 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.

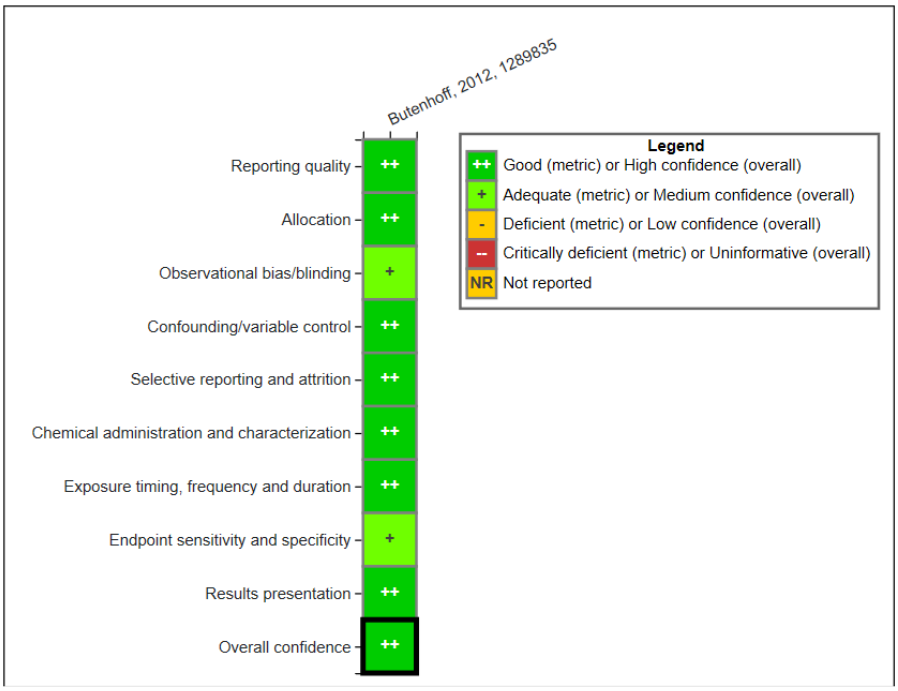

Figure 3-1. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA) exposure on the thyroid (see interactive data graphic for rating rationales).

Organ weight

Absolute and relative thyroid weights were statistically significantly ($p < 0.01$) increased (~2-fold) at the end of treatment in male rats exposed to 6 or 30 mg/kg-day via oral gavage for 28 days compared with controls. Organ weights, however, were increased only ~50% at 150 mg/kg-day, and this difference was not statistically significant. 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 the rats exposed to NH_4^+PFB for 90 days (Butenhoff et al., 2012a; van Otterdijk, 2007b).

Thyroid hormones

Male rats exposed to NH_4^+PFB for 28 days via gavage exhibited significantly 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., 2012a; 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%). TSH levels were not affected by NH_4^+PFB at any exposure level. No treatment-related effects on any of the thyroid hormone measures were observed in female rats exposed for 28 days (Butenhoff et al., 2012a; 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. (2012a)		**−46**	**−50**	**−66**
28 d; female S-D rats Butenhoff et al. (2012a)		−0.5	+18	−25
90 d; male S-D rats Butenhoff et al. (2012a)	[a]	−9[b]	**−30**[b]	
90 d; female S-D rats Butenhoff et al. (2012a)	−6	+27	−15	
Total T4				
28 d; male S-D rats Butenhoff et al. (2012a)		**−59**	**−66**	**−79**
28 d; female S-D rats Butenhoff et al. (2012a)		−8	+27	−31
90 d; male S-D rats Butenhoff et al. (2012a)	+13	−15	**−39**	
90 d; female S-D rats Butenhoff et al. (2012a)	+16	+14	−21	

Bolded cells indicate statistically significant changes compared to controls (except for the 6 mg/kg-d and 30 mg/kg-d dose groups for free T4 in male rats exposed for 90 d, tests for statistical significance in those cases were made to the 1.2 mg/kg-d group [see footnote b]); shaded cells represent doses not investigated in the individual studies.

[a]No sample for the control group was available due to insufficient sample volume for assay.

[b]Comparison is made to the 1.2 mg/kg-d dose group.

Decreased total T4 and free T4 levels also were observed in male rats exposed to NH_4^+PFB via gavage for 90 days (Butenhoff et al., 2012a; van Otterdijk, 2007b). Total T4 increased 13% and decreased 15% following 1.2 and 6 mg/kg-day, respectively. In male rats exposed to the highest dose tested (30 mg/kg-day NH_4^+PFB), total T4 was significantly reduced by 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. The decrease in free T4, however, 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^+PFB for 90 days, although total T4 was nonsignificantly decreased at the highest dose [30 mg/kg-day; (Butenhoff et al., 2012a; van Otterdijk, 2007b)].

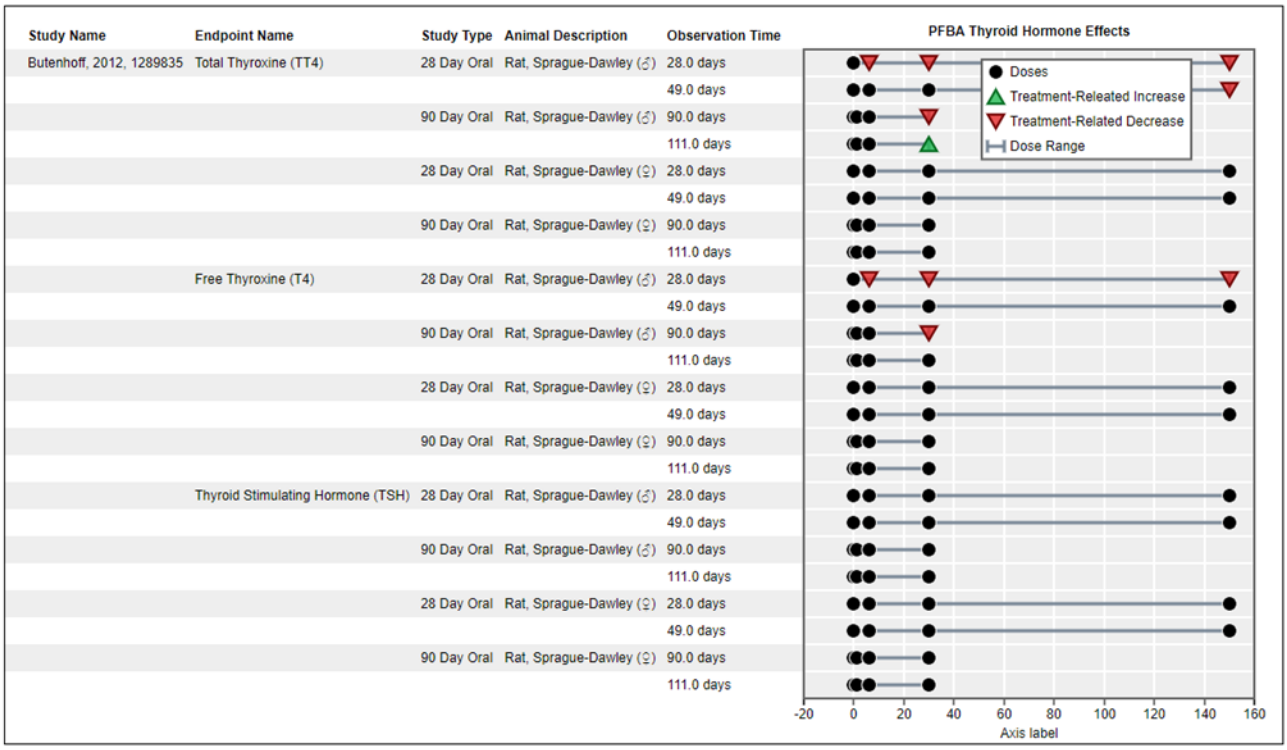

Figure 3-2. Thyroid hormone response to ammonium perfluorobutanoate (NH_4^+PFB) exposure (see interactive data graphic and rationale for study evaluations for thyroid hormone effects in Health Assessment Workspace Collaborative [HAWC]).

Histopathology

Butenhoff et al. (2012a); van Otterdijk (2007a, 2007b) also investigated thyroid histopathological and histomorphological effects in male and female rats resulting from NH_4^+PFB 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 in the 30 mg/kg-day dose group graded by the study authors as "minimal" severity (trend test p = 0.0498; Cochran-Armitage test, performed by EPA). In the 150 mg/kg-day dose group, three of the seven affected animals were observed to have lesions graded as "slight," a severity level greater than "minimal"; the remaining four affected animals were graded as having "minimal" lesions. Female rats treated for 28 days with 150 mg/kg-day NH_4^+PFB had 40% incidence (4/10) of minimal lesions compared with 3/10 minimal lesions observed in the control group. Thyroid histopathology was not examined in the 30-mg/kg-day females and no effects were noted in the 6-mg/kg-day group (although the thyroid of only one animal was available for testing in this group). No treatment-related effects were observed in the recovery groups. In contrast to the histopathological examination, the histomorphometric analysis reported no effects on thyroid cell height or colloidal area in either the treatment or recovery groups. Follicular hypertrophy/hyperplasia also was 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 were 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. (2012a)	3 (min)		3 (min)	**9 (min)**	7 (4 min, 3 mild)
90 d; male S-D rats Butenhoff et al. (2012a)	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 points scaled as follows: min = minimal severity; mild = mild/slight severity; mod = moderate severity; sev = marked severity.

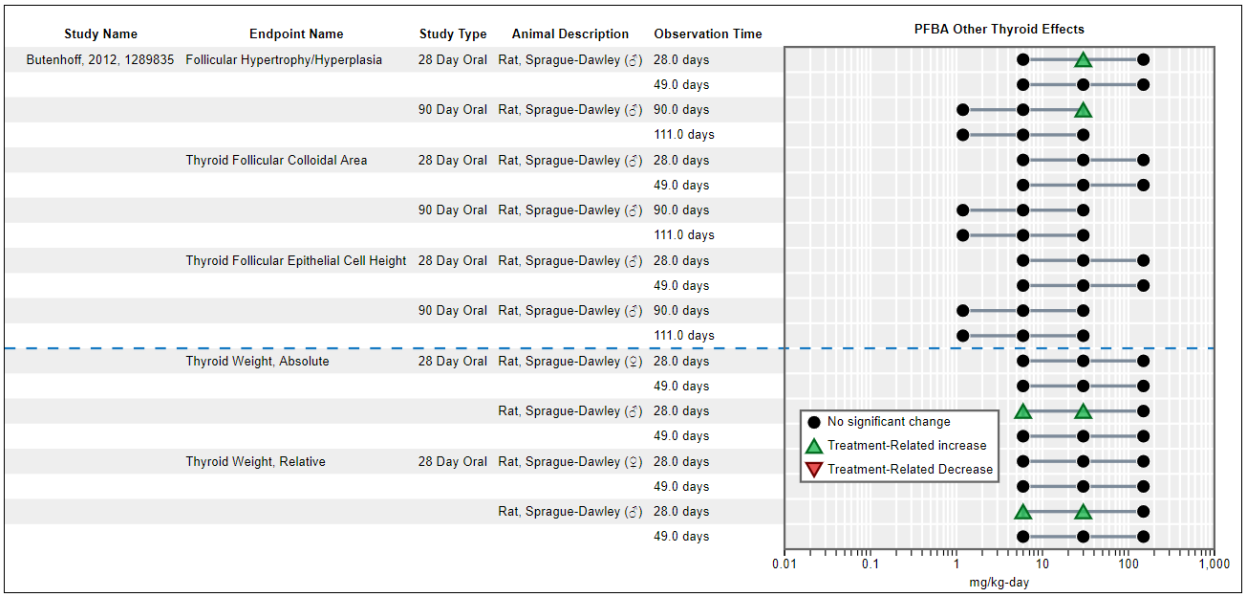

Figure 3-3. Thyroid histopathology and organ-weight responses to ammonium perfluorobutanoate (NH_4^+PFB) exposure (see interactive data graphic and rationale for study evaluations for other thyroid effects in Health Assessment Workspace Collaborative [HAWC]).

Mechanistic Evidence and Supplemental Information

Thyroid effects observed in the PFBA database consist of increased thyroid weight, increased incidence of follicular hypertrophy/hyperplasia, and decreased levels of thyroxine (total and free T4). Overall, a pattern of decreased hormone levels with corresponding alterations in tissue weight and histopathology in the absence of an increase in TSH was observed. However, the

coefficient of variation for TSH in controls in the 90-day study (Butenhoff et al., 2012a; van Otterdijk, 2007a, b) ranged from 40%–55%, compared to 13%–25% for free T_4. The lack of an observation of increased TSH may be due to difficulties in detecting relatively small changes in TSH given the assay used in the study. While there is uncertainty in the reliability of the TSH measurements and patterns of TH changes in animals may not translate perfectly to human clinical definitions, decreases in T4 alongside normal levels of TSH is consistent with the human clinical condition referred to as hypothyroxinemia [see additional discussion in (U.S. EPA, 2018b)]. Although the PFBA database is limited to two adult exposure studies (28- and 90-d) (Butenhoff et al., 2012a; van Otterdijk, 2007a, b) in rats the observed thyroid hormone effects are supported by supplemental information from structurally related PFAS (PFBS and PFHxA).

Decreases in thyroid hormones (total T3, total T4, and free T4) were observed in PFBS-exposed pregnant mice and gestationally exposed female mouse offspring at ≥200 mg/kg-day (Feng et al., 2017) and in adult female and male rats following short-term exposures of ≥62.6 mg/kg-day (NTP, 2019). Increased TSH was reported in mouse dams and in offspring during development of the reproductive system (PND 30) following gestational exposure Feng et al. (2017), but no changes were noted in rats exposed to PFBS as adults . Increased TSH was reported in mouse dams and in offspring during development of the reproductive system (PND 30) following gestational exposure Feng et al. (2017), but no changes were noted in rats exposed to PFBS as adults (NTP, 2019), a pattern consistent with the observed changes following adult PFBA exposure. Thyroid weight and histopathology were not changed after short-term exposure to PFBS in adult male or female rats (NTP, 2019).

Although the available evidence for PFHxA appears to provide weaker support for endocrine effects than studies on PFBA or PFBS (see public comment draft for PFHxA; (U.S. EPA, 2021b), the only study in the PFHxA database of animal toxicity studies to examine thyroid hormone levels observed that short-term oral exposure to PFHxA altered thyroid hormone levels in male but not female rats (NTP, 2018). Dose-dependent decreases in free and total T4 (25%–73% and 20%–58%, respectively) and to a lesser degree T3 (18%–29%) were observed with no concomitant increase in TSH (NTP, 2018).

Decreased serum T4 or T3 is a key event preceded by disrupted TH synthesis (via multiple possible mechanisms, including thyroid stimulating hormone receptor [TSHR] binding and thyroid peroxidase [TPO] or sodium-iodide symporter [NIS] inhibition) and results in a myriad of downstream neurodevelopmental outcomes, including altered hippocampal anatomy/function and hearing deficit. Thyroid hormones are critically important for proper brain development (Bernal, 2015; Miller et al., 2009; Williams, 2008; Crofton, 2004; Morreale de Escobar et al., 2004; Zoeller and Rovet, 2004; Howdeshell, 2002) because they directly influence neurodevelopmental processes, such as neurogenesis, synaptogenesis, and myelination (Puig-Domingo and Vila, 2013; Stenzel and Huttner, 2013; Patel et al., 2011). Early in gestation, TH is delivered to the developing fetal brain via placental transfer from the mother to the fetus (Calvo et al., 1990). The mother

imparts TH as its sole source until the fetal thyroid gland begins functioning. The fetal gland is completely nonfunctional until late gestation (gestation day [GD] 17), having only minimal functionality until near parturition (GD 22 (Bernal, 2015; Obregon et al., 2007; Morreale de Escobar et al., 2004)), at this point, in rats, approximately 17% of fetal T4 is still derived from the maternal source despite the presence of a newly functioning thyroid gland (G et al., 1990). In humans, these maternal-derived fetal T4 estimates range from 30% to 50% (Obregon et al., 2007; Morreale de Escobar et al., 2004; Vulsma et al., 1989).

Recent mechanistic data in human fetal tissue demonstrates the presence of thyroid receptors and transporters in the brain which suggests the fetal brain has a direct sensitivity to thyroid hormones and supports the decades of observational, genetic, and animal research (Diez et al., 2021; López-Espíndola et al., 2019). In addition, given the importance of thyroid hormones in neurodevelopment in humans and animals, low thyroid hormone status is associated with adverse neurological effects (Stagnaro-Green and Rovet, 2016; Zoeller and Rovet, 2004), and is likely associated with effects in numerous other organ systems, including the heart, bone, lung and intestine (Mullur et al., 2014; Bassett et al., 2007; Mochizuki et al., 2007; Wexler and Sharretts, 2007; Bizzarro and Gross, 2004). Butenhoff et al. (2012a) observed that PFBA not only reduced thyroid function via decreased serum total and free T_4 but also increased thyroid hormone action in the liver. This pattern of the effects has been seen following exposure to polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs). For instance, increased TH gene expression in the liver has been shown with a corresponding, inverse reduction in serum total and free T_4 (Giera et al., 2011) and (Bansal et al., 2014). Following PCB exposure, this complex pattern also occurred with changes in thyroid hormone action in the brain (Bansal and Zoeller, 2008; Zoeller et al., 2000; Zoeller and Crofton, 2000) and (Mullur et al., 2014). Increased thyroid hormone activation in the liver is known to reduce serum cholesterol (Mullur et al., 2014). Butenhoff et al. (2012a) reported decreased serum cholesterol following PFBA exposure; these effects are described in section 3.2.2 "Hepatic Effects".

Cases of severe maternal and fetal hypothyroidism, which results from iodine deficiency, Hashimoto's disease, or premature birth, further underscore the importance of maintaining thyroid hormone homeostasis during pregnancy. Several human epidemiological studies have demonstrated key relationships between decreased circulating levels of thyroid hormones, such as T4 in pregnant women and in utero and early postnatal life neurodevelopmental status. For example, neurodevelopmental and cognitive deficits have been observed in children who experienced a 25% decrease in maternal T4 during the second trimester in utero (Haddow et al., 1999). Children born euthyroid but 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 disorder) or concomitant abnormalities in brain imaging (Korevaar et al., 2016; Henrichs et al., 2010; Lavado-Autric et al., 2003; Mirabella et al., 2000). This level of T4 insufficiency (<10th percentile), defined as mild-to-moderate thyroid insufficiency, has

been shown to correspond to a 15%–30% decrease in T4 serum levels compared to median levels (Finken et al., 2013; Julvez et al., 2013; Román et al., 2013; Henrichs et al., 2010). Animal toxicity studies also have shown that decreases in mean maternal T4 levels of ~10%–17% during pregnancy and lactation elicit neurodevelopmental toxicity in rat offspring (Gilbert et al., 2016; Gilbert, 2011). Human studies also observe that thyroid hormone insufficiency is associated with cognitive deficits in children (Crofton and Zoeller, 2005; Crofton et al., 2005).

There are very few human studies available to inform what percent decrease in T4 might lead to other adverse outcomes. This is mainly due to the nature of epidemiological studies, typically with representative samples analyzed post hoc; many also bin data by "hypothyroid, euthyroid, hypothyroxinemic" based on reference ranges, and then correlate to adverse outcomes. Specifically, three human studies Jansen et al. (2019); Levie et al. (2018); Korevaar et al. (2016) were identified that had sample sizes large enough to capture a wide range of TSH and/or T4 values, which were then correlated to various neurodevelopmental outcomes that could be quantified. However, these studies still do not make direct comparisons from a percent decrease in hormones that would lead to an adverse effect; rather, they stratify their hormone samples by standard deviation to the mean/median, quartiles, etc. Therefore, it's difficult to make a conclusion in humans regarding what percent of hormone dysfunction is adverse, as those kinds of data are not generated. Additionally, in experimental animal models, there are no definitive values regarding to what degree of T4 reduction is adverse. This is due to several factors, including the existence of multiple thyroid-dependent processes in the brain, which likely have differing spatiotemporal sensitivities. But there are studies that show how graded reductions in T4 can lead to neuronal heterotopia (Gilbert et al., 2014), synaptic transmission defects (Gilbert and Sui, 2008), now and differential gene expression (O'Shaughnessy et al., 2018) and (Sharlin et al., 2010).

There are data gaps in the PFBA developmental toxicity database, including a lack of information on the thyroid and nervous system following gestational exposure. Although short-term PFBA exposure did not appear to alter thyroid hormone levels in nonpregnant adult female rats, thyroid hormone levels fluctuate throughout normal gestation (O'Shaughnessy et al., 2018; Hassan et al., 2017; Pérez et al., 2013; Calvo et al., 1990; Fukuda et al., 1980) as maternal demands to provide the fetus with adequate thyroid hormones. Specifically, serum T4 and T3 normally decline over the course of pregnancy and then rise during the postnatal period (O'Shaughnessy et al., 2018). Thus, although no changes in thyroid hormone levels occurred in nonpregnant rats, that PFBA influences hormone homeostasis differently in pregnant rats during the perinatal period is possible as maternal and fetal hormone demands fluctuate.

Overall, animal studies specific to PFBA and other potentially relevant PFAS provide support for thyroid hormone disruptions which can potentially lead to other effects of concern (e.g., neurodevelopmental effects).

Evidence Integration Summary

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

The animal evidence comes from two *high* confidence experiments conducted by the same laboratory (Butenhoff et al., 2012a; 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 or free T4; T3 was not measured) were consistent, dose dependent, and associated with increasing absolute and relative thyroid weights and histopathology (follicular hypertrophy/hyperplasia). These decreases were large in 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 (U.S. EPA, 2021b). 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, some effects across durations of exposure are inconsistent: some effects occur in the 28-day study but not in the 90-day study, and the magnitude of change of some effects is larger in the short-term than in the subchronic study. Also, in male rats, for free T4 only, the lack of a control group in animals exposed for 90 days complicates the interpretation of that endpoint.

Although the organ-weight increases and histopathological effects (follicular hypertrophy) observed in Butenhoff et al. (2012a) are consistent with a scenario where serum T4 levels are low but TSH levels are normal, the mechanism by which these changes occurred unclear. Rodents are more sensitive to these histopathological changes (follicular cell hypertrophy), which then can develop into follicular cell tumors (U.S. EPA, 1998a). Increased thyroid follicular cell hypertrophy supports the finding that the thyroid hormone economy is perturbed. The observed changes are likely due to increased metabolism or competitive displacement of T4. That no thyroid effects (e.g., hormone or histopathological changes) were observed in adult nonpregnant females at any dose or treatment duration might be related to PFBA pharmacokinetics because clearance rates in

rats are faster in females (compared to males, see Section 3.1.4). Taken together, the available animal studies provided *moderate* evidence for thyroid effects.

Rodents and humans share many similarities in the production, regulation, and functioning of thyroid hormones. Although differences exist, including the timing of in utero thyroid development and hormone turnover rates, rodents are considered a good model for evaluating the potential for thyroid effects in humans (Zoeller et al., 2007). More specifically, the observed decreases in total or free T4 in the absence of increases in TSH are considered biologically relevant to humans (Crofton, 2004; Lau et al., 2003). TSH is an indicator that the thyroid system has been perturbed, but it does not always change when serum T4 is decreased (Hood et al., 1999). Adverse neurological outcomes have been demonstrated following decreased T4 levels during the early neonatal period with no changes in T3 or TSH (Crofton, 2004). 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). Rodents have been shown to have a unique sensitivity to thyroid follicular hyperplasia (leading to development of follicular tumors), however, that is considered less relevant to humans (U.S. EPA, 1998a). Nevertheless, the coherent and consistent perturbations to thyroid hormone economy and the resultant increased thyroid histopathology indicates that PFBA is exerting some effect on the thyroid 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 might be even more sensitive to change in humans compared to rodents (U.S. EPA, 1998a).

A notable data gap exists for fuller interpretation of the reported thyroid effects. Studies evaluating PFBA effects on neurodevelopment or thyroid measures after developmental exposure (see Section 3.2.3 "Developmental Effects") were not identified, thus leaving uncertainty on the potential for more sensitive developmental effects of PFBA exposure on the thyroid and 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). That thyroid hormones are at sufficient levels is essential during times critical to brain development and functioning and in the growth, development, and functioning of numerous organ system processes, including basal metabolism and reproductive, hepatic, sensory (auditory, visual) and immune systems (Forhead and Fowden, 2014; Gilbert and Zoeller, 2010; Hulbert, 2000) (see Mechanistic Evidence and Supplemental Information subsection above). Mammals are more susceptible during perinatal and

postnatal lifestages 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). Although the PFBA database lacks information on thyroid hormone levels in exposed pregnant animals or offspring exposed during gestation, these effects have been observed following exposure of mice to the structurally related PFAS, PFBS (U.S. EPA, 2018b). Decreases in total T4 and T3 were observed in dams at GD 20 and offspring at PND 1, 30, and 60, clearly indicating that thyroid hormone levels were perturbed during periods of neurological development. Further, given the evidence is consistent with PFBA, the PFBS assessment identifies developmental neurotoxicity as a database limitation due to the known association between thyroid hormone insufficiency during gestation and developmental neurotoxicity outcomes (U.S. EPA, 2018b). Accordingly, given that developmental neurotoxicity (due to thyroid hormone insufficiency) is a concern following exposure to PFBS, it follows that this concern is relevant to exposure to PFBA during development because of the similarities in thyroid effects across the two PFAS.

Taken together, the ***evidence indicates*** that PFBA exposure is likely to cause thyroid toxicity in humans, given relevant exposure circumstances (see Table 3-5). This judgment is based primarily on consistent and biologically coherent results from two *high confidence* studies (short-term and subchronic study design) in male rats that indicate effects on thyroid hormone levels (T4 without compensatory effects on TSH). These effects on thyroid hormone levels generally occurred 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

<table>
<tr><th colspan="5">Evidence Stream Summary and Interpretation</th><th>Inferences and Summary Judgment</th></tr>
<tr><td colspan="5">Evidence from studies of exposed humans (see Section 3.2.1: Human Studies)</td><td rowspan="6">⊕⊕⊙
Evidence indicates (likely)

Primary basis:
Two high confidence studies in rats ranging from short-term to subchronic exposure; effects observed at ≥6 mg/kg-d PFBA; similar effects for related PFAS

Human relevance:
Effects in rats are considered potentially relevant to humans based on conserved biological processes, and the observed pattern of changes is consistent with potential neurological outcomes following decreased T4 during development (see Section 3.2.1: Mechanistic Evidence and Supplemental Information)

Cross-stream coherence:
N/A (human evidence indeterminate)</td></tr>
<tr><td>Studies and confidence</td><td>Summary of key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Judgments and rationale</td></tr>
<tr><td>Thyroid Hormones
1 low confidence study</td><td>• Single study reporting inverse associations with free T4, free T3, and TSH; only TSH was statistically significant</td><td>• No factors noted</td><td>• Lack of coherent associations across hormones
• Imprecision</td><td>⊙⊙⊙
Indeterminate</td></tr>
<tr><td colspan="5">Evidence from in vivo animal studies (see Section 3.2.1: Animal Studies)</td></tr>
<tr><td>Studies and confidence</td><td>Summary of key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Judgments and rationale</td></tr>
<tr><td>Thyroid Hormones
2 high confidence studies in adult rats:
• 28-d
• 90-d</td><td>• Decrease in free and total T4 in male rats at >6 mg/kg-d
• Decrease in T4 with no increase in TSH</td><td>• Consistent increases in males across all studies
• Dose-response gradient
• Coherence of decreased T4 with histopathology
• Magnitude of effect, up to 79%
• High confidence studies</td><td>• Potential lack of expected coherence (no compensatory TSH increase to T4 decrease)</td><td>⊕⊕⊙
Moderate

Findings considered adverse based on consistent and biologically coherent results for thyroid hormone levels, organ weights, and</td></tr>
</table>

Evidence Stream Summary and Interpretation					Inferences and Summary Judgment
Histopathology *2 high* confidence studies in adult rats: • 28-d • 90-d	• Follicular hypertrophy/hyperplasia observed in male rates at 30 mg/kg-d • No histopathological effects at 150 mg/kg-d (after short-term exposure)	• *Consistent* follicular hypertrophy/hyperplasia in male rats across studies • *Coherence* of hypertrophy with T4 decreases • *High* confidence studies	• Potential *lack of expected coherence* (no change in TSH levels) • Unexplained lack of significant effects at highest tested dose	histopathology. The observation of effects only in males might be explained by pharmacokinetics. Uncertainties remain as to how organ weights and histopathology are affected in the absence of TSH increases.	*Susceptible populations and lifestages:* The developing fetus and children are susceptible to altered thyroid hormone status; the lack of data on thyroid or nervous system effects following gestational exposure is a data gap.
Organ Weight *1 high* confidence study in adult rats: • 28-d	• Increase in thyroid weight (absolute and relative) at 6 and 30 mg/kg-d • No change in thyroid weight at 150 mg/kg-d	• *Magnitude of effect*, >2-fold increases • *High* confidence study	• Potential *lack of expected coherence* (no change in TSH levels) • Unexplained lack of significant effects at highest tested dose		
Mechanistic evidence and supplemental information (see subsection above)					
Summary of key findings, interpretation, and limitations				**Evidence stream judgment**	
Key findings and interpretation: • PFBA-induced thyroid changes similar to those for related PFAS (i.e., PFBS and, although the evidence is weaker, PFHxA) • Findings for PFBS indicate the potential for effects of concern during development *Limitations*: No PFBA-specific mechanistic evidence informing thyroid effects				Findings for related PFAS support the plausibility of findings for PFBA, and the potential for effects of concern with PFBA exposure during development	

3.2.2. Hepatic Effects

Human Studies

One epidemiological study reported on the relationship between PFBA exposure and serum biomarkers of liver injury. This study Nian et al. (2019a)This study Nian et al. (2019a) 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 limited exposure contrast for PFBA (detected in 70%, median [interquartile range (IQR)] = 0.15 ng/mL [0.01–0.51 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 [IQR] = 0.1 [0.03–0.2] 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 studies in rats and mice (Butenhoff et al., 2012a; 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 likely 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). For more information on outcome-specific considerations for study evaluations, please refer to the study evaluations in the HAWC PFBA database.

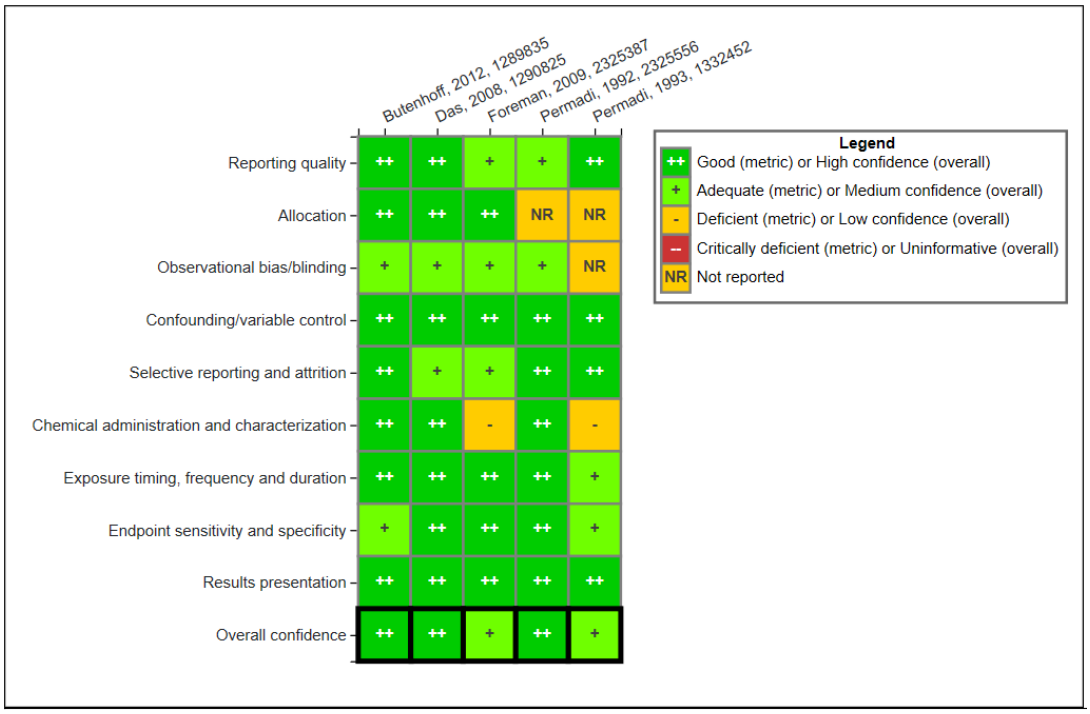

Figure 3-4. Evaluation results for animal studies assessing effects of perfluorobutanoic acid (PFBA) exposure on the liver (see interactive data graphic for rating rationales).

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. (2012a); van Otterdijk (2007a)		5	**24**		**48**		
28 d; female S-D rats Butenhoff et al. (2012a); van Otterdijk (2007a)		−1	0		−3		
90 d; male S-D rats Butenhoff et al. (2012a); van Otterdijk (2007a)	9	7	**33**				
90 d; female S-D rats Butenhoff et al. (2012a); 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). This study was judged *low* confidence, however, on the basis of concerns over reporting, exposure characterization, and endpoint sensitivity/selectivity. Conversely, following 10 days of dietary exposure to 0.02% PFBA, relative liver weight was 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 in these same dose groups in humanized PPARα (hPPARα) 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 also was

observed in male S-D rats exposed to ≥30 mg/kg-day NH_4^+PFB via oral gavage for 28 days (Butenhoff et al., 2012a; 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 experienced no increases in relative liver weights (1%–3% decrease).

Similar to increases following 28-day exposures, relative liver weights also were observed to increase in male S-D rats exposed to NH_4^+PFB via oral gavage for 90 days (Butenhoff et al., 2012a; 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 termination of subchronic exposure. As observed in the short-term study, exposure to NH_4^+PFB 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^+PFB via oral gavage increased relative (to body weight) liver weights in pregnant (measured on GD 18) and nonpregnant P_0 females at ≥175 mg/kg-day (Das et al., 2008). 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 weight increase also were observed in F_1 animals at PND 1: 30% and 41% at 175 and 350 mg/kg-day, respectively. In animals at PND 10, however, 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 could 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 no effect following a recovery period is consistent with the findings of the subchronic and short-term exposures in adult animals (Butenhoff et al., 2012a; van Otterdijk, 2007a, b).

Although not an oral toxicity study, Weatherly et al. (2021) also observed statistically significant increases in relative liver weight (up to 60% increases) in mice dermally exposed to PFBA.

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 observed only 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., 2012a; van Otterdijk, 2007b) so whether these animals would exhibit the same effects at the exposure levels used in the developmental toxicity study Das et al. (2008) is unclear. Regardless, the data for relative liver weight seem to indicate that male animals are more susceptible to this effect than female animals, possibly because 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 NH4+PFB, respectively (Butenhoff et al., 2012a; 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 NH4+PFB for 90 days (Butenhoff et al., 2012a; 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 NH4+PFB for 90 days did not increase liver weights in female rats (~3%–8% increases). In a developmental toxicity study in CD-1 mice (Das et al., 2008), exposure to NH4+PFB increased absolute liver weights in pregnant and nonpregnant P0 females 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 P0 females. Similar magnitudes of absolute liver weights increase (27% and 32%) also were observed in F1 animals at PND 1 at 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 in pregnant P0 animals at postweaning (PND 22).

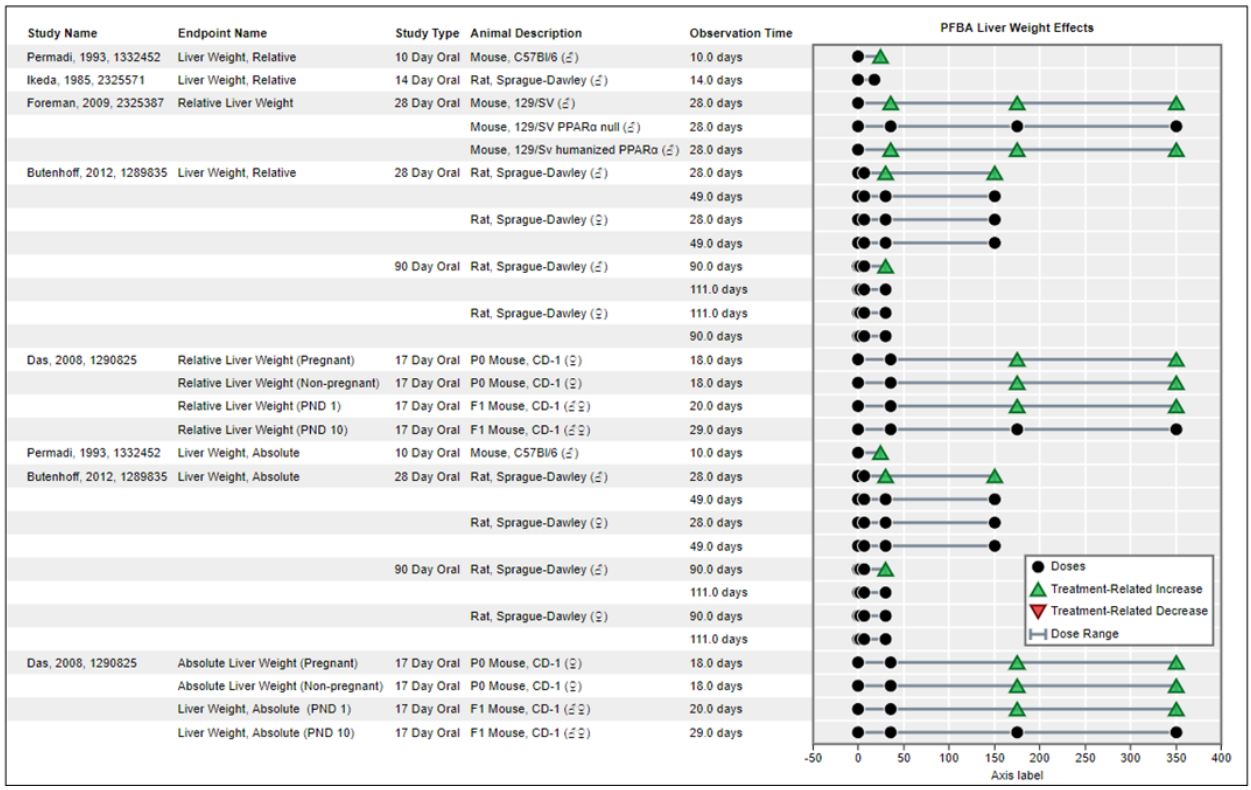

Figure 3-5. Liver-weight response to ammonium perfluorobutanoate (NH_4^+PFB) or perfluorobutanoic acid (PFBA) exposure (see interactive data graphic and rationale for study evaluations for liver-weight effects in Health Assessment Workspace Collaborative [HAWC]).

Histopathology

Histopathological examination of the livers of mice and rats across three separate gavage studies of 28-day (Butenhoff et al., 2012a; Foreman et al., 2009; van Otterdijk, 2007a) or 90-day (Butenhoff et al., 2012a; 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 (incidences of 100% in all doses), 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 were 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 also was observed in 6/10 S-D rats exposed to 150 mg/kg-day PFBA for 28 days (Butenhoff et al., 2012a; van Otterdijk, 2007a) and 9/10 rats exposed to 30 mg/kg-day PFBA for 90 days (Butenhoff et al., 2012a; 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 35 mg/kg-day (1/10), 175 mg/kg-day (6/10) and 350 mg/kg-day (9/10), whereas focal necrosis was observed in only 1/10 (35 and 175 mg/kg-day) and 2/10 (350 mg/kg-day) hPPARα mice (Foreman et al., 2009). PPARα null mice only developed focal necrosis in the 175 mg/kg-day (1/10) and 350 mg/kg-day (2/10) dose groups. For all strains, most of the necrotic lesions were judged mild in severity. By comparison, in rats exposed to PFBA for 28 days, no increase in hepatocellular coagulative necrosis (Butenhoff et al., 2012a; van Otterdijk, 2007a) was observed. No effects on hepatocellular necrosis in rats were observed following 90-day exposures to PFBA (Butenhoff et al., 2012a; 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). Whether these effects occurred at lower doses was not mentioned. Further, no quantitative data were 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 (Butenhoff et al., 2012b; 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.

All mice in all exposure groups were observed to develop hepatocellular hypertrophy (characterized by increased cytoplasmic eosinophilia, decreased glycogen content, and increased cellular volume) following dermal exposures of up to 15% v/v (Weatherly et al., 2021). Necrotic lesions were not consistently observed following dermal exposure to PFBA, although genes associated with necrosis were increased following exposure.

Although the number of studies was small, mice did seem more sensitive to development of hepatocellular lesions compared to rats, possibly owing to the observed differences in pharmacokinetics between the two species: Mice are observed to have serum excretion half-lives approximately two 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. (2012a); van Otterdijk (2007b)	0		0	0		**6 (min)**		
90 d; male rats Butenhoff et al. (2012a); 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. (2012a); 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								
	None reported							
28 d; hPPARα male mice Foreman et al. (2009)	Periportal vacuolation reported to increase at 350 mg/kg-d, compared to controls (responses at 35 mg/kg-d or 175 mg/kg-d were not reported by study authors)							
28 d; PPARα null male mice Foreman et al. (2009)	Centrilobular vacuolation reported to increase at 350 mg/kg-d, compared to controls (responses at 35 mg/kg-d or 175 mg/kg-d were not reported by study authors)							

Bolded cells indicate statistically significant changes compared to controls; shaded cells represent doses not investigated in the individual studies. Severity normalized to four points 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/γ activator) was 3 total (1 minimal, 2 mild) at 50 mg/kg-d exposure.

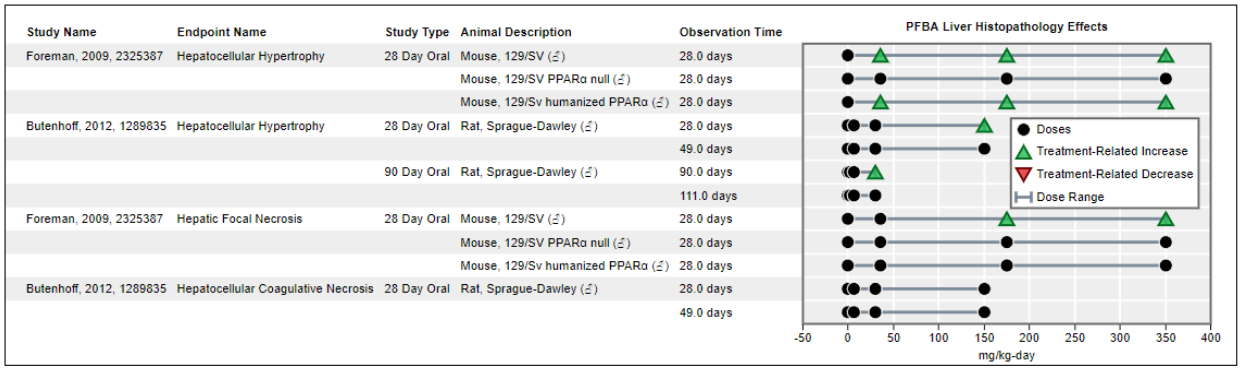

Figure 3-6. Liver histopathology response to ammonium perfluorobutanoate (NH_4^+PFB) or perfluorobutanoic acid (PFBA) exposure (see interactive data graphic and rationale for study evaluation for liver histopathology effects in Health Assessment Workspace Collaborative [HAWC]).

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., 2012a; 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 in males and at 6 and 30 mg/kg-day in females (but not at 150 mg/kg-day), although decreases were small (~5%–9% relative to control) and were reported to be within the concurrent reference range for S-D rats. Prothrombin time, however, was statistically significantly decreased ($p < 0.01$) in all dose groups in females after the 21-day recovery period. 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., 2012a; van Otterdijk, 2007a). ALT was not statistically significantly increased by PFBA exposure in wild-type, PPARα null, or hPPARα mice (Foreman et al., 2009), although it did increase almost 3-fold at 350 mg/kg-day (20.28 U/I) compared to controls (7.39 U/I). Cholesterol levels were significantly ($p < 0.01$) decreased 20% and 27% in male rats exposed to 30 and 150 mg/kg-day PFBA, respectively, for 28 days (Butenhoff et al., 2012a; van Otterdijk, 2007a). Cholesterol levels returned to control values following recovery, and no effects on cholesterol were observed 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.

In mice exposed to PFBA dermally (up to 15% v/v), several serum biomarkers including serum cholesterol, glucose, and ALP were increased, and urea nitrogen was decreased, relative to controls (Weatherly et al., 2021). Other serum biomarkers (ALT, total protein, albumin, or globulin) were not increased due to 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) states the panel recommendations from Hall et al. (2012) can be used to judge whether observed hepatic effects are adverse or adaptive in nature. Given that Hall et al. (2012) was focused on framing noncancer liver effects in the context of progression to liver tumors, however, 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." For PFBA, the "additional relevant information" consists of multiple in vitro mechanistic studies, an in vivo study investigating PFBA-induced liver effects in wild-type humanized PPARα mice, and PPARα-null mice (Foreman), as well as evidence from other PFAS that help elucidate possible MOAs of PFBA liver toxicity.

Many of the hepatic effects caused by exposure to perfluoroalkyl acids such as PFBA have been attributed to activation of the peroxisome proliferator-activated receptor alpha (PPARα[12]) (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 as it relates to differences in PPARα activation and activity across species. 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.

Although 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). Although PFAS generally have been shown to activate PPARα, however, shorter chain PFAS such as PFBA appear to be weak activators. For example, Bjork and Wallace (2009) showed PFBA is a weaker activator of PPARα in primary rat and human hepatocytes than is either the six-carbon PFHxA or the 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

[12]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 is also associated with cell proliferation, oxidative stress, and inflammation (NJDWQI, 2017; Angrish et al., 2016; Mellor et al., 2016; Hall et al., 2012).

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) observed 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 α/γ 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. Although 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). Although PFAS generally have been shown to activate PPARα, however, shorter chain PFAS such as PFBA appear to be weak activators. For example, Bjork and Wallace (2009) showed PFBA is a weaker activator of PPARα in primary rat and human hepatocytes than is either the six-carbon PFHxA or the 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) observed 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 α/γ 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. Although 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 also occurred in positive controls treated with Wy-14,643.

Liver enlargement is 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 could include accumulation of lipid material (e.g., micro- or macrovesicular 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 in the absence of additional indicators of cell status Williams and Iatropoulos (2002), such as reduced glutathione (GSH) levels, mitochondrial integrity, receptor-dependent or independent signal transduction pathway activity (e.g., pro-survival vs. pro-cell death balance), or redox state, for example. Although hepatocellular hypertrophy is commonly attributed to receptor-dependent organellar growth and proliferation (e.g., 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 also has been associated with cell death processes, in particular oncosis or oncotic necrosis (Kleiner et al., 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 on 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 or primary necrotic cell death (Malhi et al., 2006; 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 might not be accurate.

One study investigated the activation of PPARα and pregnane X receptor (PXR) in the livers of exposed neonatal mice (Das et al., 2008). This study showed the expression of genes associated

with either PPARα or PXR was not increased in the livers of neonatal male and female mice, possibly indicating that the increased liver weights in these animals were associated with a non-PPARα or PXR MOA. No other 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]); however, 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 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 plays an important role in regulating responses to xenobiotics and 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 might 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) and in 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 et al. (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. Although no statistically significant increases in focal necrosis were observed at any dose in PPARα null or humanized mice, necrosis did increase slightly in the highest dose compared to controls (2/10 vs. 0/10) in both strains; that exposure to higher doses of PFBA would elicit increased necrotic effects in hPPARα or PPARα null mice is possible. Foreman et al. (2009) suggest that, given the differences in pharmacokinetics between the strains (see Section 3.1) and lower liver concentrations of PFBA in humanized and null mice, that higher levels of exposure could possibly elicit a similar phenotype in these strains. 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 statistically significant increases in liver necrosis in wild-type mice, but not PPARα null mice, suggests that PPARα is required for the development of this lesion. The observation that the positive control for PPARα activation, Wy-14,643 also did not result in statistically significant increase in this lesion (in this study) further supports that a PPARα-independent, complementary, or multifaceted MOA could 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 provided only for the control and high-dose groups for the PPARα null and hPPARα mice. This result is consistent with Das et al. (2017) reported PFAS increased accumulation and oxidation of lipids in the liver of exposed mice, with accumulation occurring faster than oxidation. Thus, although vacuolation occurs in humanized PPARα mice, oxidation is also induced (as evidenced by the upregulation of ACO), limiting lipid accumulation to a degree. In PPARα null mice, however, accumulation of lipids in the liver 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 although 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 wild-type 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 macrovesicular vacuolation (considered nonadverse) and microvesicular vacuolation. Microvesicular vacuolation is described by the presence of hepatocytes partially or completely filled with multiple small vacuoles without displacement of the nucleus (Kleiner and Makhlouf, 2016). This pattern of vacuolation is precisely what Foreman et al. (2009) observed in hPPARα mice exposed to PFBA. Additionally, focal necrosis is observed in wild-type mice in Foreman et al. (2009). Thus, according to the Hall recommendations, observation of liver weight increases, hypertrophy, microvesicular vacuolation, and necrosis across wild-type 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 occurring in null PPARα mice indicates a PPARα-independent mechanism for lipid accumulation 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.

Disrupted lipid metabolism due to PFBA exposure is supported by the findings of a dermal toxicity study Weatherly et al. (2021) that observed significant upregulation of genes associated with steatosis (*Cd36*, *Fasn*, *Lpl*, *Scd1*), cholestasis (*Abcd4*, *Abcc2*, *Abcc3*), and phospholipidosis (*Fabp1*, *Hpn*, *Lss*).

Overall, evidence specific to PFBA and from other potentially relevant PFAS provides 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)Additionally, application of the recommendations from Hall et al. (2012) clearly supports 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 epidemiological study with reduced sensitivity (Nian et al., 2019b). These null findings from a single study with low sensitivity did not influence the evidence integration judgments, providing *indeterminate* evidence.

Hepatic effects associated with oral exposures to PFBA have been consistently observed in high or medium confidence short-term and subchronic oral studies in adult mice or rats of both sexes (Butenhoff et al., 2012a; Foreman et al., 2009; van Otterdijk, 2007a, b; Permadi et al., 1993; Permadi et al., 1992) and in an oral developmental toxicity study in mice (Das et al., 2008). Although there are hepatic effects observed in a single dermal toxicity study Weatherly et al. (2021), concerns over characterizing how dermal exposures relate to oral exposures preclude the use of this study in evidence synthesis judgments. 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), as can be seen in this interactive graphic on HAWC. Although uncertainties remain, given the consistency, coherence, and inferred adversity (see below) of these findings, there is moderate animal evidence for hepatic effects of PFBA exposure.

Increased liver weights were consistently observed in male, but not female, adult rats following 28- or 90-day exposures (Butenhoff et al., 2012a; van Otterdijk, 2007a, b) and 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 did not differ appreciably across species, but

wild-type PPARα mice seemed to exhibit greater magnitudes of effect vs. 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., 2012a; Foreman et al., 2009; van Otterdijk, 2007a, b), although differences in the type or severity of lesions differed somewhat across species and durations of exposure. Wild-type and hPPARα mice were both observed to develop statistically significantly increased hepatocellular hypertrophy following 28 days of oral exposure to PFBA, whereas only wild-type mice developed statistically significantly increased hepatic focal necrosis (Foreman et al., 2009). PPARα null mice were not observed to develop statistically significant increases in either of these lesions in response to exposure. Adult male rats also were observed to develop hepatocellular hypertrophy, but not coagulative necrosis, following 28 or 90 days of exposure (Butenhoff et al., 2012a; van Otterdijk, 2007a, b). Again, differences in pharmacokinetics might 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). In wild-type mice, however, liver weight increases occurred at lower doses than did focal necrosis in the same study Foreman et al. (2009) although 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 occurred 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., 2012a; van Otterdijk, 2007a, b).

Changes in serum biomarkers of liver function 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 statistically significant changes in ALT, AST, or ALP were observed. Although 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, the observed decreased bilirubin 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 as a marker of liver injury. Lastly, cholesterol levels were decreased in a dose-dependent manner following the 28-day, but not the 90-day, exposure. Although ALT was also not statistically significantly increased in wild-type, hPPRAα, or PPARα mice following exposure to PFBA, ALT was increased almost 3-fold in PPARα null mice in the high dose group (350 mg/kg-day). As a whole, the various clinical chemistry endpoints, as measurements of liver toxicity, were

incoherent across some endpoints and durations of exposure. Thus, these data (i.e., on serum biomarkers of liver function) were considered too uncertain and not influential to the overall evidence integration judgments (i.e., the coherent, consistent, and biologically plausible liver weight and histopathology endpoints were strong enough on their own to support 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, Exposure of wild-type and hPPARα mice to PFBA increased both liver weights and hepatocellular hypertrophy. Only wild-type mice were observed to have statistically significantly increased focal necrosis following exposure, possibly indicating that activation of PPARα was a necessary step in the MOA for developing this lesion. Hepatic focal necrosis, however, was not statistically significantly increased in any group (wild-type, hPPARα, or PPARα null mice) exposed to the positive control (the PPARα/γ activator Wy-14,643). Further, increased vacuolation was reported only 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 occurs, at least in part, through a PPARα-independent mechanism, and that either the lack, or attenuated activity, of PPARα-induced lipid catabolism is not sufficient to overcome the increased accumulation. This strongly suggests a complementary or multifaceted MOA for development of PFBA-induced hepatic effects. Indeed, based on evidence from other PFAS chemicals, non-PPARα mechanisms relevant to hepatic effects are apparent. 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 MOA, 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 the PPARα-mediated components of the undefined MOA(s) appear relevant to human toxicity, given 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 occurring 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, although 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 indicates that PPARα alone cannot be identified as the exclusive MOA for PFBA-induced liver effects. Lastly, independent of conclusions regarding PPARα as the MOA, 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) indicates coincident histological evidence of liver injury/damage can be used to support the conclusion that liver weight/hypertrophic effects are adverse. That PFBA induces a constellation of effects in the liver, including increased liver weight, hypertrophy, vacuolation, and necrosis is clear from the in vivo evidence in rodents. Therefore, according to Hall et al. (2012), these coincident effects are 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 on liver weight and, separately, histopathology 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 ***evidence indicates*** that PFBA exposure is likely 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 Stream Summary and Interpretation					Evidence Integration Summary Judgment
Evidence from studies of exposed humans (see Section 3.2.2: Human Studies)					⊕⊕⊙ ***Evidence indicates* (likely)** *Primary basis:* Three *high and one medium* confidence studies in male adult rats and mice and maternal and neonatal mice (short-term, subchronic, and gestational exposures) at ≥30 mg/kg-d PFHxA *Human relevance:* Effects in rats are considered relevant to humans (**see Section 3.2.2: Mechanistic Evidence and Supplemental Information**) *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and lifestages:* None identified, although those with preexisting liver disease could be at greater risk
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Serum Biomarkers 1 *medium* confidence study; 1 *low* confidence study	• No association between PFBA and liver biomarkers or blood lipids in studies with poor sensitivity	• No factors noted	• No factors noted	⊙⊙⊙ *Indeterminate*	
Evidence from in vivo animal studies (see Section 3.2.2: Animal Studies)					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Organ Weight *4 high*, 2 *medium*, and 1 *low* confidence studies in adult rats and maternal and neonatal mice: • 14-d (×3) • 28-d (×2) • 90-d • Gestational	• Increased liver weight observed in: ○ male adult rats at ≥30 mg/kg-d ○ female mice and PND1 neonates at ≥175 mg/kg-d ○ male wild-type PPARα and hPPARα mice at ≥35 mg/kg-d (no effects in PPARα null mice) • Reduced effects in female rats could be attributable to pharmacokinetics	• *Consistent* increases, across most studies (one null study) • *Dose-response* in most studies (one null study) • *Coherence* with histopathology in male rats and mice (especially at high dose) • *Magnitude of effect*, up to 112% • *High* and *medium* confidence studies	• No factors noted	⊕⊕⊙ *Moderate* Findings were considered adverse (as determined using Hall et al. (2012) criteria (see **Section 3.2.2: Mechanistic Evidence and Supplemental Information**), consistent, dose dependent, and biologically coherent	

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Histopathology *2 high and 1 medium* confidence studies in adult rats and mice: • 28-d (×2) • 90-d	• Hepatocellular hypertrophy observed in: ○ male adult rats at 30 mg/kg-d (subchronic) ○ male wild-type PPARα and hPPARα mice at ≥35 mg/kg-d (short-term) • Focal necrosis observed in male wild-type PPARα mice exposed to ≥175 mg/kg-d (short-term) • Vacuolation observed in male PPARα-null and hPPARα mice at 350 mg/kg-day (short-term) • Reduced effects in female rats could be attributable to pharmacokinetics	• *Consistent* cellular hypertrophy or focal necrosis across studies and species • *Coherence* with liver weight effects (especially at high doses) • *Dose-response* • *High* and *medium* confidence studies	• No factors noted	across multiple measures of hepatic toxicity (i.e., liver weight and histopathological changes). PPARα-dependence appears likely for some effects (focal necrosis) but not others (vacuolation) Findings were considered adverse (as determined using Hall et al. (2012) criteria (see **Section 3.2.2: Mechanistic Evidence and Supplemental Information**), consistent, dose dependent, and biologically coherent across multiple measures of hepatic toxicity (i.e., liver weight and histopathological changes). PPARα-dependence appears	*Other inferences:* the MOA for liver effects is not fully established, although available evidence indicates that multiple pathways are likely involved

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Serum Biomarkers *2 high* confidence studies in adult rats: • 28-d • 90-d	• Increased ALP and decreased bilirubin in male or male and female rats, respectively, at 30 mg/kg-d	• *High* confidence studies	• *Incoherent* observations (e.g., increased ALP but with no clear increases in ALT or AST, and bilirubin decreased not increased as expected)	likely for some effects (focal necrosis) but not others (vacuolation)	
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Summary of key findings, interpretation, and limitations**			**Evidence stream judgment**	
Molecular Initiating Events—PPARα	*Key findings and interpretation:* • In vitro increased expression of PPARα-responsive genes in primary rata and human hepatocytes and cells transfected with rat or human PPARα. • In vivo increased expression of PPARα-responsive genes in wild-type and hPPARα mice. *Limitations*: small database investigating PPARα activation, some inconsistencies regarding the strength of activation 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 Initiating Events—Other Pathways	*Key findings and interpretation*: • Indirect evidence of alternative pathways following observation of effects in humanized PPARα and PPARα null mice exposed to PFBA. • Direct evidence from other PFAS (PFOA, PFOS, PFDA, PFHxA, PFHxS) that multiple non-PPARα pathways (PPARγ, CAR, PXR) activated following exposure. *Limitations*: No PFBA-specific in vitro data; only one in vivo study providing indirect evidence.				
Organ Level Effects	*Key findings and interpretation*: • Observation of increased liver weight and increased hepatocellular hypertrophy/vacuolation in humanized PPARα mice.				

Evidence Stream Summary and Interpretation			Evidence Integration Summary Judgment
	• Concurrent observation that a known PPARα/γ activator (Wy-14,643) did not elicit the same statistically significant increased effects (focal necrosis) as PFBA exposure in wild-type mice. *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 and effects attributable to developmental exposure. The latter includes all studies where exposure is limited to gestation 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 Effects" and "Reproductive Effects," respectively). Synthesis descriptions of studies across sections can vary in detail, depending on the impact the data have 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 epidemiological study that investigated developmental effects (birth weight, gestational age) Li et al. (2017a) was cross-sectional study based on umbilical cord PFBA exposure deemed low confidence primarily due to concerns over participant selection and exposure measurement. Li et al. (2017a) reported a mean birth weight deficit of −46 grams (95%CI: −111, 19) in the overall population per each unit (ng/mL) PFBA increase; this was driven by the association in boys (−86 grams; 95%CI: −180, 9) as the results were null in girls. The exposure range in this study, however, is quite small and a one-unit increase is beyond the bounds of the exposure range in this population. Thus, when expressed on an IQR unit change, they reported small birth weight deficits (-4 grams (95%CI: −10, 2) per each PFBA IQR unit change (0.09 ng/mL) and in boys (−8 grams; 95%CI: −16, 1). No association was observed with gestational age in weeks.

Animal Studies

A standardized suite of potential developmental effects was evaluated 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 developmental effects, but none of these individual domain-specific considerations were judged 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 via gavage from GD 1 to 17 of CD-1 mice (male and female offspring were evaluated) to NH_4^+PFB 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). Significantly increased full litter resorptions also occurred at 350 mg/kg-day (28% vs. 7% in controls), although no effects were observed 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 reproductive developmental landmarks (preputial separation and vaginal opening, respectively) were delayed. Preputial separation was delayed by 2.3 days at 350 mg/kg-day although 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)			
	0	35	175	350
Full-litter resorptions; pregnant P_0 female CD-1 mice on GD 18 Das et al. (2008)	2/29	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)	91.7 ± 2.1	90.2 ± 2.4	92.9 ± 1.6	87.9 ± 2.6
Survival to PND 7 (%); F_1 male and female CD-1 mice on PND 7 Das et al. (2008)	90.9 ± 2.3	90.0 ± 2.3	90.0 ± 3.1	86.4 ± 2.7
Survival to PND 14 (%); F_1 male and female CD-1 mice on PND 14 Das et al. (2008)	90.9 ± 2.3	89.7 ± 2.4	89.6 ± 3.2	85.7 ± 3.0
Survival to PND 21 (%); F_1 male and female CD-1 mice on PND 21 Das et al. (2008)	90.9 ± 2.3	88.7 ± 2.4	89.6 ± 3.2	85.7 ± 3.0
Delayed eye opening (d); F_1 male and female CD-1 mice Das et al. (2008)	16.28 ± 1.19	**17.38 ± 0.79**	**17.69 ± 0.68**	**17.8 ± 0.83**
Delayed vaginal opening (d); F_1 female CD-1 mice Das et al. (2008)	31.25 ± 2.62	33.71 ± 2.59	**34.57 ± 2.59**	**34.92 ± 2.23**
Delayed preputial separation (d); F_1 male CD-1 mice Das et al. (2008)	29.55 ± 1.14	30.21 ± 1.99	30.56 ± 1.84	**31.88 ± 1.72**

Bolded cells indicate statistically significant changes compared to controls.

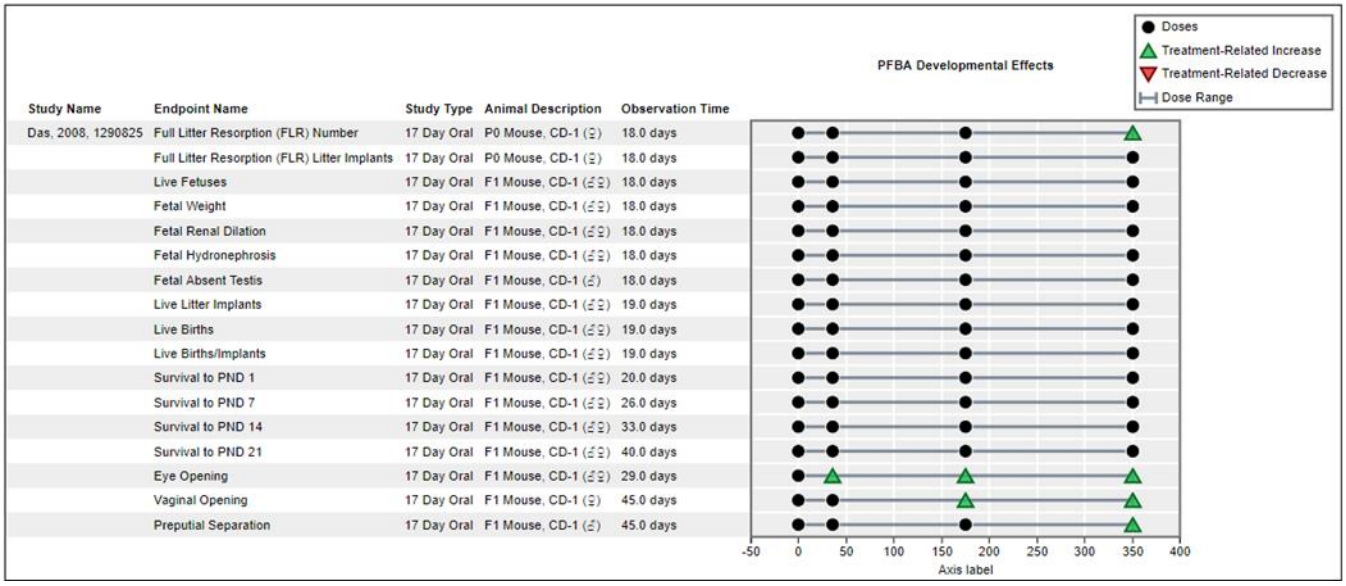

Figure 3-8. Pre- and postnatal developmental responses to gestational ammonium perfluorobutanoate (NH_4^+PFB) exposure (see interactive data graphic and rationale for study evaluations for developmental effects in Health Assessment Workspace Collaborative [HAWC]).

Evidence Integration Summary

One *low* confidence human study reported lower birth weight in boys with higher PFBA exposure. No association was observed with gestational age. The lack of additional studies with lower risk of bias reduces the interpretability of these findings. Overall, the evidence on potential developmental effects from studies of humans exposed to PFBA was *indeterminate*.

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 offspring (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 (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 reach reproductive milestones was also altered in male rodents exposed to PFOA: preputial separation accelerated 2–4 days in mice exposed to doses up to 10 mg/kg-day and delayed ~1.5 days in mice exposed to 20 mg/kg-day (Lau et al., 2006). In rats exposed to 30 mg/kg-day PFOA, preputial separation was delayed ~3.5 days (Butenhoff et al., 2004). Thus, qualitatively, a consistent pattern of altered reproductive milestones is observed following exposure to other PFAS, including the structurally related PFBS, increasing certainty in the similar findings available for PFBA.

The onset of puberty in humans is driven by surges in the levels of estrogen in females and testosterone in males, so the timing of puberty can be altered by exposure to endocrine disrupting chemicals that mimic or antagonize these hormones. In female rodents, pubertal markers include vaginal opening (indicative of the first ovulation in rats, but not mice) and the subsequent first estrus and onset of regular estrous cyclicity (rats and mice) (Prevot, 2014). Since vaginal opening isn't indicative of first ovulation in mice, the delayed vaginal opening in mice reported by Das et al. (2008) , not a direct correlate to puberty in humans. However, it is assuredly a marker of sexual and/or reproductive development. As the EPA's Reproductive Guidelines (U.S. EPA, 1996) state that both accelerations and delays in the timing of puberty should be considered adverse, it is reasonable to extend this conclusion to developmental milestones that are more broadly indicative of sexual and/or reproductive developmental, Further, the absence of effects on body weight in

PFBA-exposed offspring or maternal toxicity strengthens the confidence that the observed developmental delays are biologically significant, adverse effects. Taken together, the available animal studies provided *moderate* evidence of potential developmental effects.

Data gaps in the developmental toxicity database include a lack of information on the thyroid and nervous system following gestational exposure. Given that PFBS alters thyroid hormone levels following gestational exposure and that PFBA induces changes in thyroid hormone levels in exposed adult animals, PFBA also might alter normal thyroid hormone action in the developing fetus. As both PFBA and PFBS evidence bases lack studies on developmental neurotoxicity, a potential consequence of altered thyroid hormone action during development, this represents an important unknown.

Thus, considering the coherent suite of developmental effects, primarily developmental delays, observed following PFBA exposure in one *high* confidence study, and similar effects observed following exposure to multiple other PFAS (including the structurally similar PFBS), the ***evidence indicates*** PFBA exposure is likely 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 hormone action, the available PFBA-specific developmental effects alone support this judgment.

Table 3-10. Evidence profile table for developmental effects

Evidence Stream Summary and Interpretation					Evidence Integration Summary Judgment
Evidence from studies of exposed humans (see Section 3.2.3: Human Studies)					⊕⊕⊙ ***Evidence indicates* (likely)** *Primary basis:* One *high* confidence gestational study in mice, with effects observed at ≥35 mg/kg-d PFBA *Human relevance:* In the absence of evidence to the contrary, the developmental effects observed in mice are considered relevant to humans based on conserved biological processes *Cross-stream coherence:* N/A (human evidence *indeterminate*) *Susceptible populations and lifestages:* Pregnancy and early life *Other inferences:* PFBA-induced developmental effects are consistent with effects seen for other PFAS (see Section 3.2.3: Evidence Integration Summary
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Birth Weight 1 *low* confidence study	• Birth weight deficit with higher PFBA exposure in boys (nonstatistically significant)	• No factors noted	• *Low* confidence study • *Imprecision*	⊙⊙⊙ *Indeterminate*	
Evidence from in vivo animal studies (see Section 3.2.3: Animal Studies)					
Studies, outcomes, and confidence	**Summary of key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Judgments and rationale**	
Developmental Milestones *1 high* confidence gestational study in mice	• Dose-dependent delays in developmental milestones in: ○ Eye opening in males and females at ≥ 35 mg/kg-d ○ Preputial separation in males at 350 mg/kg-d ○ 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	• *Dose-response* gradient • *Coherence* across developmental milestones • *Magnitude of effect*, up to 12% increase in time to milestone and 4-fold increase in full litter resorptions • *High* confidence study	• No factors noted	⊕⊕⊙ *Moderate* Coherent delays in developmental milestones, with multiple alterations observed at ≥35 mg/kg-d	

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. No evidence of an association between PFBA exposure and decreased semen quality was found (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 due to the small sample size (n = 58) and risk of outcome misclassification, which makes the null finding difficult to interpret. Other study deficiencies including the potential for selection bias and confounding were noted in the study evaluation, but the direction of these biases is unknown.

Animal Studies

Two *high* confidence studies reported in three publications from the same research group Butenhoff et al. (2012a); van Otterdijk (2007a, 2007b) 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 in mice. 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 d) 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. (2012a); van Otterdijk (2007a)]. In a separate cohort, this effect was not observed 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 none arose during the recovery period (Butenhoff et al., 2012a; van Otterdijk, 2007a).

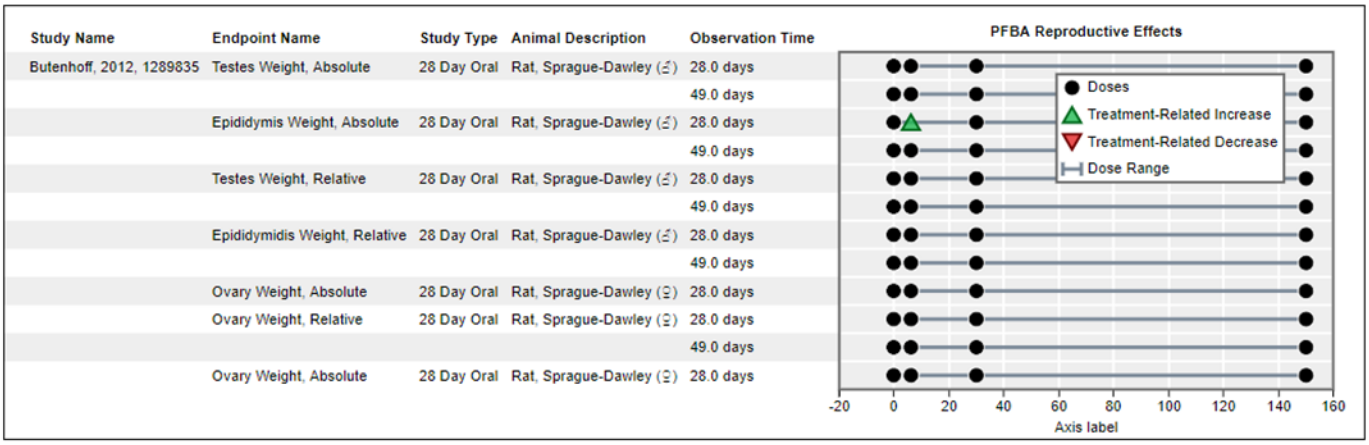

Figure 3-9. Reproductive responses to ammonium perfluorobutanoate (NH_4^+PFB) exposure (see interactive data graphic and rationale for study evaluations for reproductive effects in Health Assessment Workspace Collaborative [HAWC]).

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. There is evidence for 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) where the human relevance of delayed development of the reproductive system observed in mice are outlined 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 toward the null) are not interpreted to influence the evidence integration judgments, and thus the human evidence was *indeterminate*.

The available animal evidence is sparse, limited to evaluations of reproductive organ-weight measurements in a *high* confidence short-term experiment reported in three publications from the same research group (Butenhoff et al., 2012a; van Otterdijk, 2007a, b). Specifically, the authors evaluated reproductive organ weights in a cohort of rats immediately after exposures ended and another cohort 21 days postexposure, both of which were largely null. Given the limited interpretability of these data, the animal evidence was *indeterminate*.

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 ***inadequate 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 311). This determination is consistent with the lack of convincing evidence for reproductive effects for

several other PFAS, including Gen X, PFOA, PFOS, and PFBS (U.S. EPA, 2021a, 2018b, 2016a, b) and (U.S. EPA, 2021b).

Table 3-11. Evidence profile table for reproductive effects

<table>
<tr><th colspan="5">Evidence Stream Summary and Interpretation</th><th>Evidence Integration Summary Judgment</th></tr>
<tr><td colspan="5">Evidence from studies of exposed humans (see Section 3.2.4: Human Studies)</td><td rowspan="6">⊙⊙⊙
Insufficient Evidence

Primary basis:
One high confidence study in rats

Human relevance:
Organ weight changes in rats are considered relevant to humans in the absence of evidence to the contrary

Cross-stream coherence:
N/A (human evidence indeterminate)

Susceptible populations and lifestages:
None identified</td></tr>
<tr><td>Studies, outcomes, and confidence</td><td>Summary of key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Judgments and rationale</td></tr>
<tr><td>Birth Weight
1 low confidence study</td><td>• No association between PFBA exposure and semen quality</td><td>• No factors noted</td><td>• Low confidence study</td><td>⊙⊙⊙
Indeterminate</td></tr>
<tr><td colspan="5">Evidence from in vivo animal studies (see Section 3.2.4: Animal Studies)</td></tr>
<tr><td>Studies, outcomes, and confidence</td><td>Summary of key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Judgments and rationale</td></tr>
<tr><td>Organ weights
1 high confidence 28-d study in rats</td><td>• Increased epididymal weight in rats at 6 mg/kg-d but not higher doses
• No changes in testis or ovary weights</td><td>• No factors noted</td><td>• Lack of dose-response
• Lack of coherence across reproductive organ weights</td><td>⊙⊙⊙
Indeterminate

Largely null findings in in the only available study that examined reproductive organ weights</td></tr>
</table>

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 immunosuppression, blood pressure, and renal function, while several 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 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

Two studies examined the association between PFBA exposure and immunosuppression. One *medium* confidence study examined severity of COVID-19 illness in Denmark using biobank samples and national registry data (Grandjean et al., 2020). There was some concern for selection bias in this study due to the expectation that biobank samples were more likely to be available for individuals with chronic health concerns. In addition, severity of COVID-19 is not a direct measure of immune suppression as other factors may contribute to illness severity. This study reported higher odds of severe disease (based on hospitalization, admission to intensive care and/or death) with higher exposure to PFBA (OR = 1.57, 95% CI = 0.96, 2.58 for >LOD vs. LOD in all participants; OR = 2.10, 95% CI = 1.02, 4.33 in only participants with exposure measured at the time of diagnosis, which reduces concern for selection bias). In addition, one *low* confidence cross-sectional study in China analyzed hepatitis B surface antibody (Zeng et al., 2020). This study was downgraded due to concerns for exposure misclassification resulting from lack of temporality between the exposure and outcome. There is no way to determine when participants were exposed to hepatitis B; additionally, vaccination status was not considered. This study reported an inverse association between PFBA exposure and hepatitis B surface antibody (mean difference = -0.18 log mIU/mL, 95% CI = -0.28, -0.08). Overall, both available studies report findings consistent with immune suppression with greater PFBA exposure. However, there is residual uncertainty in both studies, and, in the absence of additional corroboration (see animal evidence discussion below), they do not support a stronger judgment than concluding that there i**s inadequate evidence** of immunosuppression. In addition, neither study is suitable for dose-response modeling due to dichotomous exposure modeling Grandjean et al. (2020) and study limitations Zeng et al. (2020).In addition, neither study is suitable for dose-response modeling due to dichotomous exposure modeling Grandjean et al. (2020) and study limitations Zeng et al. (2020).

One *medium* confidence cross-sectional study 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). Although this was a *medium* confidence study, potential for bias remains; this includes outcome misclassification resulting from the volatility of blood pressure and its measurement at a single time point and the cross-sectional design. In the absence of additional confirmatory epidemiological 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 estimated 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). There is potential for reverse causation in this association, however. 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 the conclusions are somewhat uncertain 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., 2012a; Foreman et al., 2009; Das et al., 2008; van Otterdijk, 2007a, b). In general, no PFBA-related effects on body weight were observed 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 during the study, indicating the lower measured body weights were not treatment related. The change in body weight across the duration of the study was not changed at any dose in any group of animals, however, indicating 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., 2012a; van Otterdijk, 2007a, b). PFBA appears not to affect body weight across multiple species, exposure durations, or lifestages.

Some evidence of effects on the hematological system was observed in S-D rats exposed to PFBA. Following 28 days of exposure, no effects other than on 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 found in male rats, but consistent statistically significant 7%–8% decreases in PT were observed in all exposed female dose groups ($p < 0.01$). 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. Although the number of RBCs and the 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. These effects returned 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 returned to control levels following a recovery period, and no consistency of effects across exposure durations or sexes were found.

Immunotoxicity were observed in mice dermally exposed to up to 15% v/v PFBA (Weatherly et al., 2021). Following 28 days of dermal exposure, the frequency of CD4+ and CD8+ T-cells were decreased in the draining lymph nodes, whereas B-cells, dendritic cells, and CD11b+ cell numbers were increased. Cell frequencies were also changed in the ear pinna following dermal exposure: CD45+, CD4 T-cell, CD8 T-cell, NK cell, eosinophils, neutrophils, and dendritic cell numbers were all increased. Relatively fewer changes were noted in the spleen, however, where total cells, B-cells, CD4 T-cells, neutrophils, and CD11b dendritic cells were all decreased. It is not clear that these data relate to the uncertain evidence of potential immunosuppressive effects observed in the two human studies, and thus they do not strengthen that evidence. Further, no oral PFBA toxicity studies that investigated immunotoxicity were available. Overall, this single animal study does not provide evidence supporting a stronger evidence integration judgment and overall, there is **inadequate evidence** to determine whether PFBA exposure can cause immunological effects in humans.

Ocular effects also were 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. Although 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 also 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, not consistent across the treatment period, and low incidence. No ocular histopathological results were observed in the 90-day subchronic study. Thus, although some ocular effects were observed following PFBA exposure,

effects across durations were somewhat inconsistent, 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 cancer. Only one study Crebelli et al. (2019) investigated PFBA-induced genotoxicity: No evidence of DNA damage or micronucleus formation was observed in male mice exposed to PFBA via drinking water for 5 weeks. As shown in Table 4-2, EPA's carcinogenicity conclusion for the closely related PFAS, PFBS, is ***inadequate information to assess carcinogenic potential***. While there is some evidence of carcinogenicity for PFOA and PFOS, the ability to relate the findings for these longer chain PFAS to PFBA is currently too uncertain to influence the carcinogenicity judgment for PFBA.

Overall, there is ***inadequate information to assess the carcinogenic potential*** of PFBA exposure.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

The currently available ***evidence indicates*** hazards likely exist 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 relevant to the identified 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, organized by health effect, and further summarized in Table 4-1.

The hazard identification judgment that the ***evidence indicates*** PFBA exposure is likely to cause thyroid toxicity in humans (given relevant circumstances) 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 on 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. Additionally, increased thyroid weights and increases in thyroid follicular hypertrophy were observed. Although the observed thyroid histopathological changes support the potential for PFBA to disrupt the thyroid hormone homeostasis, however, rodents are uniquely sensitive to the development of thyroid follicular hypertrophy and tumor development U.S. EPA (1998b) compared with humans. Because of 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 hazard identification judgment that the ***evidence indicates*** PFBA exposure is likely 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 (rats and mice), in males and females, and 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 MOAs could explain some of the observed effects in exposed rodents and that observed effects might 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 hazard identification judgment that the ***evidence indicates*** PFBA exposure is likely 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) 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, PFOA, and PFOS.

There was ***inadequate 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, potential for PFBA exposure to affect the immune system, 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, PFOS, and GenX (U.S. EPA, 2021a; MDH, 2020, 2019, 2018; U.S. EPA, 2018b, 2016a, b). See Table 4-2 for a comparison of the noncancer hazard judgments drawn for PFBA with the judgments in the final EPA assessments for PFBS, PFOA, PFOS, and GenX.

Table 4-1. Evidence integration summary for health effects for which *evidence indicates* a hazard exists

Evidence stream scenarios	Evidence in studies of humans[a]	Evidence in animal studies[a]	Evidence basis
No Studies, or Low Confidence or Conflicting Evidence	**Developmental** **Hepatic** **Thyroid**		**Developmental** • No human studies • Coherent observations of delays in developmental milestones (eye opening, vaginal opening, preputial separation) and fetal mortality in one *high confidence* study of mice exposed gestationally • Consistent with findings for related PFAS • No MOA information • Human relevance presumed **Thyroid** • Single *low confidence* study in humans • Consistent and biologically coherent results for thyroid hormone levels (T4 without compensatory changes in TSH), organ weights, and histopathology from two *high confidence* studies (short-term, subchronic) in male rats • Consistent with findings for related PFAS • No MOA information • Human relevance presumed **Hepatic** • Two null studies (one *medium* and one *low confidence*) in humans with poor sensitivity • Consistent, dose-dependent, and biologically coherent effects on liver weights and histopathology from seven *high* or *medium confidence* studies in adult male rats and mice (short-term and subchronic) and adult and female mice exposed as adults or gestationally • PPARα-dependence observed for some effects (focal necrosis) but other effects (vacuolation) occur in animals lacking PPARα activity (null mice) or in animals with human PPARα (humanized mice) • Involvement of both PPARα-dependent and independent mechanisms, including hypertrophic responses in humanized PPARα mice • MOA information supports human relevance
Strong Mechanistic Evidence Alone			
One High or Medium Confidence Apical Study without Supporting or Conflicting Evidence		**Developmental**	
Multiple High or Medium Confidence Apical Studies with Some Inconsistency or Important Uncertainties		**Thyroid** **Hepatic**	
Multiple High or Medium Confidence Apical Studies with Strong Support (e.g., MOA understanding supporting biological plausibility)			

Stronger Evidence Stream Scenarios

[a]Can 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.

Table 4-2. Hazard conclusions across published EPA PFAS human health assessments

Health Outcome	EPA PFAS Assessments[a,b,c]				
	PFBA	PFBS	GenX Chemicals	PFOA[d]	PFOS[d]
Thyroid	+	+	-[e]	Human: + Animal: +/-	Human: +/- Animal: +/-
Liver	+	-	+	Human: + Animal: +	Human: - Animal: +
Developmental	+	+	+/-	Human: + Animal: +	Human: + Animal: +
Reproductive	-	-	+/-	Human: - Animal: +/-	-[e]
Immunotoxicity	-	-	+/-	Human: + Animal: +	Human: +/- Animal: +
Renal	-	+	+/-	Human: +/- Animal: +/-	-[e]
Hematological	-	-[e]	+/-	-[e]	-[e]
Ocular	-	-[e]	-[e]	-[e]	-[e]
Serum Lipids	-[e]	-	-[e]	Human: + Animal: +	Human: +
Hyperglycemia	-[e]	-[e]	-[e]	Human: - Animal: -	Animal: +/-
Nervous System	-[e]	-[e]	-[e]	Human: - Animal: -	Animal: +/-
Cardiovascular	-[e]	-	-[e]	-[e]	-[e]
Cancer	-	-	+/-	+/-	+/-

[a] Assessments used multiple approaches to summarizing their non-cancer hazard conclusions; for comparison purposes, the conclusions are presented as follows: '+' =evidence demonstrates or evidence indicates (e.g., PFBA), or evidence supports (e.g., PFBS); '+/-' =suggestive evidence; '-' = inadequate evidence (e.g., PFBA) or equivocal evidence (e.g., PFBS); and '-/-' = sufficient evidence to conclude no hazard (no assessment drew this conclusion).

[b] The assessments all followed the EPA carcinogenicity guidelines (2005) U.S. EPA (2005) a similar presentation to that used to summarize the noncancer judgments is applied for the cancer hazard conclusions, as follows: '+' = carcinogenic to humans or likely to be carcinogenic to humans; '+/-' = suggestive evidence of carcinogenic potential; '- ' = inadequate information to assess carcinogenic potential; and '-/-' = not likely to be carcinogenic to humans (no assessment drew this conclusion).

[c] The hazard conclusions for the various EPA PFAS assessments presented in this table were not considered during evidence integration and thus did not inform the evidence integration conclusions presented in the PFBA assessment.

[d] The U.S. EPA PFOA U.S. EPA (2016b) and PFOS U.S. EPA (2016a) assessments did not use structured language to summarize the noncancer hazard conclusions. The presentation in in this table was inferred from the hazard summaries found in the respective assessments; however, this is for comparison purposes only and should not be taken as representative of the conclusions from these assessments. Those interested in the specific noncancer hazard conclusions for PFOA and PFOS must consult the source assessments. Note that new assessments for PFOA and PFOS are currently being finalized to support a National Primary Drinking Water Regulation; hazard conclusions in these updated assessments may differ from those presented in this table.

[e] No data available for this outcome for this PFAS, so '- 'entered by default.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

No human or animal studies were available to inform the potential for PFBA exposure to cause cancer and the single study of genotoxicity did not observe effects. Overall, there is ***inadequate information to assess the carcinogenic potential*** of PFBA exposure. See Table 4-2 for a comparison of the carcinogenicity conclusion drawn for PFBA with the carcinogenicity conclusions in the final EPA assessments for PFBS, PFOA, PFOS, and GenX.

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 pharmacokinetics between males and females. The serum half-life of PFBA following a single oral dose of 30 mg/kg-day is approximately 9 hours in male rats, compared to 2 hours in female rats (see Table 3-2). Differences in half-life values is similar in mice, with male mice having much longer half-lives than females at 30 mg/kg-day (16 hours vs. 3 hours). 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 (OATs). However, as noted in Section 3.1.4, PFBA is not an active substrate of OAT1, OAT2, or OATP1a1 which are expressed in the kidney and active towards other PFAS, and as described at the end of Section 3.1.5, it seems unlikely that urinary excretion of albumin (which is not sex-dependent in control rats (Matsuzaki et al., 2002)) could explain the observation.

Further, and specifically relevant to hepatic effects, the liver concentrations of PFBA following subchronic exposure to 30 mg/kg-day is approximately 16-fold higher in male rats than in female rats [16.09 vs. 0.91 mg/kg-day; Butenhoff et al. (2012a); van Otterdijk (2007a, 2007b)], and responses for liver weight are 11-fold higher (33% vs 3% increase, see Table 3-6). 0.91 mg/kg-day; Butenhoff et al. (2012a); van Otterdijk (2007a, 2007b)], and responses for liver weight are 11-fold higher (33% vs 3% increase, see Table 3-6). On the other hand, the estimated clearance in male rats is only 4.5-fold lower than in female rats (see Table 3-2). Thus, PFBA clearance (primarily in the urine) partly explains the sex difference in internal dose and liver weight effect, suggesting that additional sex-related differences that impact the distribution to the liver contribute to the overall difference in response for this endpoint. However, reductions in free and total T4 at 150 mg/kg-day after 28 days and at 30 mg/kg-day after 90 days were 2- to 3-fold less severe in female rats compared to male rats (see Table 3-3), which is somewhat less than the relative clearance (4.5-fold), but sufficiently similar to suggest that the difference in clearance is a primary factor in the difference in response.

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 vs. 2.28 hours in females (Chang et al., 2008). Also, although quantitative data were not provided, serum excretion half-lives were reported not to 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 also were 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, although 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 pharmacokinetic 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 observed 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), that pregnancy and early life represent two sensitive lifestages to PFBA exposure is possible.

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

The available ***evidence indicates*** that oral exposure to PFBA is likely to cause adverse thyroid, hepatic, and developmental effects in humans based on multiple *high* and *medium* confidence animal toxicity studies (Butenhoff et al., 2012a; Foreman et al., 2009; Das et al., 2008; van Otterdijk, 2007a, b; Permadi et al., 1993; Permadi et al., 1992).

No human or animal toxicity studies are available to inform the potential for PFBA to cause adverse effects via inhalation. Likewise, no human or animal studies are 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 lifestages) that is likely to be without an appreciable risk of adverse health effects over a lifetime. The RfD derived in Section 5.2.1 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 might be useful in some contexts (e.g., when assessing the potential cumulative effects of multiple chemical exposures occurring 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 between 30 days and 10% of the life span in humans. These subchronic toxicity values are presented because they might be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures). Section 5.2.3 discusses that no information exists to inform the potential toxicity of inhaled PFBA.

5.2.1. 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. (2012a) 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^+PFB (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., 2012a; Foreman et al., 2009; van Otterdijk, 2007a). Although these studies were used for qualitative hazard identification purposes (they supported the final evidence integration judgments for these endpoints and thus were critical for identifying these endpoints for dose-response analysis), they ultimately were not considered for use as the basis for 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. Although short-term studies were not used for the identification of points of departure (PODs), however, they were deemed relevant to decisions regarding the application of uncertainty factors for deriving 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. (2012a); Das et al. (2008); van Otterdijk (2007b) in conjunction with histopathological lesions [i.e., hepatocellular hypertrophy; Butenhoff et al. (2012a); van Otterdijk (2007b)]. As discussed in Section 3.2.2, the observed effects in the livers of exposed experimental animals are judged 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 multifaceted mode of action (MOA) 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 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 in addition to raw incidence was available. Therefore, both total incidence of lesions and incidence of "slight" severity lesions were considered for dose-response analysis.

A pattern of adverse effects in the thyroid also is 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., 2012a; van Otterdijk, 2007b). Decreased thyroid hormone levels are

judged 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, on the basis of 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 observed at 6 mg/kg-day in rats exposed to PFBA for 28 days, but not in rats exposed for 90 days (where it was observed only at 30 mg/kg-day). This discrepancy can be explained, however, 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) vs. 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 vs. 52.2 μg/mL, respectively. Because serum concentrations following chronic exposures likely 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) states that significant effects in the development of the male and female reproductive systems "either early or delayed, should be considered adverse…" and thus supports considering these endpoints for reference value derivation. Delayed eye opening, also found 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). A time-sensitive critical period in the development of the visual system is when 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 (FLR), a clear indicator of postimplantation embryo/fetal mortality, was increased twofold and fourfold in pregnant mice exposed to 175 mg/kg-day or 350 mg/kg-day (respectively) during pregnancy. In the uteri of dams without full resorptions, there was additional evidence of fetal resorptions. In addition, in a separate cohort of gestationally exposed dams that were allowed to deliver litters and were killed after their pups were weaned on lactation day 22,

there was an indication of decreased pre- and postnatal survival of the offspring (as determined by a comparison of the number of maternal implantation sites to the number of pups delivered), the magnitude of which is considered biologically significant (discussed below). Taken together, the potential coherence of decreased pre- or postnatal survival with other effects on early fetal mortality and developmental maturation (i.e., delays in eye opening and reproductive milestones) supports consideration of all these developmental endpoints for deriving PODs.

Individual animal data were obtained from the study authors, which allowed for a thorough consideration of pre- and postnatal mortality data. When the FLR data were combined with data for prenatal mortality from litters without FLR to provide a more complete assessment of embryo/fetal mortality, the response was statistically significant (p = 0.012) using the Cochran-Armitage trend test with a Rao-Scott adjustment (CA/RS) method (Rao and Scott, 1992). Although the embryo/fetal mortality observed as FLR is presumed to have occurred much earlier in pregnancy than fetal mortality in non-FLR litters and could involve different or overlapping contributing mechanisms, combining these endpoints provides information on pregnancy loss and fetal mortality over the entire gestational period, corresponding to the period of PFBA exposure. This was deemed more appropriate than modeling FLR and non-FLR fetal mortality separately. Combining the data in this way has the added benefit of allowing the data to be modeled with the nested dichotomous models and avoids the lower resolution of modeling the FLR data as dam incidence per dose group.

The individual litter data obtained from the study authors also allowed for consideration of modeling postnatal mortality (i.e., number of neonatal deaths compared to the number of implantation sites). Analysis of the individual litter data revealed a nonmonotonic dose-response for postnatal mortality, with response rates of 0.38%, 1.04%, 2.93%, and 1.2% at 0, 35, 175, and 350 mg/kg-day, respectively, and the CA/RS trend test for the dataset was not statistically significant (p = 0.09). Further, the data for postnatal mortality clearly indicates it is a weaker response compared to prenatal mortality. Given that postnatal mortality was a weaker response than prenatal morality, it failed to achieve statistical significance, and prenatal mortality is more closely aligned with the period of exposure, postnatal mortality was not considered further for POD derivation.

The studies (excluding the short-term studies) and outcomes relevant to the identified hazards were selected and advanced for POD derivation as presented in Table 5-1. These selected datasets 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	Exposure duration	Species, sex	POD derivation[a]	Reference[b]
Liver				
Increased relative liver weight	Subchronic	S-D rat, male	Yes	Butenhoff et al. (2012a)
	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	Subchronic	S-D rat, male	Yes	Butenhoff et al. (2012a)
Decreased free T4	Subchronic	S-D rat, male	No	
Increased thyroid follicular hypertrophy	Subchronic	S-D rat, male	No	
Developmental				
Embryo/fetal mortality	Gestational	CD-1 mouse, male and female	Yes	Das et al. (2008)
Postnatal mortality	Gestational	CD-1 mouse, male and female	No	
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] See text for rationale for inclusion/exclusion from POD derivation.
[b] Both the Butenhoff et al. (2012a) and Das et al. (2008) studies were rated as *high* confidence.

Estimation or Selection of Points of Departure (PODs)

Consistent with EPA's *Benchmark Dose Technical Guidance* U.S. EPA (2012), 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 (2012) 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, 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	A 10% extra risk is a commonly used BMR for dichotomous endpoints U.S. EPA (2012) in the absence of information for a biologically based BMR; the endpoint is not considered a frank effect and does not support using a lower BMR.A 10% extra risk is a commonly used BMR for dichotomous endpoints U.S. EPA (2012) in the absence of information for a 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% identified in human and rodent studies (Gilbert et al., 2016; Gilbert, 2011; Haddow et al., 1999).The BMD technical guidance (U.S. EPA, 2012) 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. (2012a) 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		
Embryo/fetal morality	1% extra risk	For quantal endpoints, the BMG Technical Guidance states "[f]from a statistical standpoint, most reproductive and developmental studies with nested study designs support a BMR of 5%" and "[b]iological considerations may warrant the use of a BMR of 5% or lower for some types of effects (e.g., frank effects) …". As increased treatment-related embryo/fetal mortality is clearly a frank effect, BMRs of 5% and 1% were considered. Given that the study employed a nested design with individual animal data available that allow the use of the nested dichotomous models (to account for intra-litter similarity), and the effect of interest was a frank effect (supporting a BMR 5% or lower), a BMR of 1% extra risk was ultimately selected for derivation of the POD to account for the biological severity of these endpoints (i.e., mortality) and the robust statistical power of the study.
Delayed eye opening	5% relative deviations	Biological evidence supports identification of a minimally significant decrease of visual input (1-d delayed eye opening) 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 previously to define biologically relevant responses 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		

When 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. When 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 which causes at least a 10% change, consistent with the rationale for the selecting 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 (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 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 pharmacokinetic 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:

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

where f_{abs} is the fraction absorbed and *dose* is average dose rate expressed at an hourly rate.

If humans are exposed to a regular (daily) dose, D, then use of an estimated human clearance (CL_H) leads to a prediction of an ongoing blood concentration equal to D/CL_H; i.e., that is the steady-state or average blood concentration, C_{AVG}, given the daily dose, D. Hence, this evaluation assumes that the steady-state level increases or decreases in direct proportion to D, with $1/CL_H$ being the proportionality constant.

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:

$$HED = \frac{POD}{CL_A/CL_H} = POD \times \frac{CL_H}{CL_A} \quad (5\text{-}2)$$

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. Note that although this evaluation of relative internal dose (C_{AVG}) assumes that internal dose increases linearly with exposure (as does default allometric scaling), nonlinearity is usually observed only at relative high exposure levels. Further, although clearance of PFBA could be biphasic, it is still linear: A two-compartment classical PK model still uses all linear rate equations, and the predicted C_{AVG} from a two-compartment model still increases linearly with exposure or applied dose.

Clearance values, however, are not reported for humans in the one pharmacokinetic study available for PFBA (Chang et al., 2008). As clearance is a measure of average excretion, to calculate it, 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. Chang et al. (2008) did report V_d for cynomolgus monkeys, however, and as summarized above in Section 3.1.5, the data suggest a difference in V_d between rodents and monkeys. For comparison, the V_d values for PFOA and PFOS estimated from the PBPK parameters of Loccisano et al. (2011) are approximately 0.2 and 0.3 L/kg, respectively, although that obtained from monkeys for PFBA is approximately 0.5 L/kg. This value of V_d for PFBA was obtained from standard analysis of the empirical PK data, which is not influenced by any preliminary chemical-specific assumptions, but as stated by the authors, "Volume of distribution estimates indicated primarily extracellular distribution" (Chang et al., 2008). The difference between V_d for PFBA and those for PFOA and PFOS indicates slightly more intracellular distribution by PFBA. As described in Section 3.1.2 Distribution, V_d for humans is expected to be similar to the value for monkeys, thus the average value for male and female monkeys from Chang et al. (2008) will be used. Human clearance, normalized to body weight, can be calculated as follows:

$$CL_{human}\ (\text{mL/kg-h}) = \ln(2) \times \frac{1}{t_{1/2,\text{human}}(\text{h})} \times V_{\text{d,monkey}}\ (\text{mL/kg}) \quad (5\text{-}3)$$

Note that in equation (5-3), BW normalization is embedded in the fact that V_d is a volume per kg BW. For example, the average blood concentration, C_{AVG} (mg/mL), can then be estimated using equation (5-1) for any given dose (mg/kg/h = (mg/kg/d)/(24 h/d)), independent of specific BW.

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, Minnesota and Cordova, Illinois. Cottage Grove had three subjects, which were not identified by gender. Cordova 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, the available data were assumed insufficient to distinguish male and female humans. The analytic method used replaced concentration measurements below the lower 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), one male subject from Cordova, and one subject from Cottage Grove fell into this category; half-lives for these four subjects were not used. Additionally, the last time point for Subject 2 from Cottage Grove was 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). Other details of the human half-life data are described in Section 3.1.4, Excretion.

As discussed in Section 3.1.4, using the common assumption of $BW^{0.75}$ scaling of clearance and standard species BWs of 0.25 kg in rats and 80 kg in humans, the half-life in humans would be predicted to be 4.2 times greater than rats. Given half-lives of 9.22 and 1.76 hours in male and female rats, one would then predict half-lives of 38.7 hours in men and 7.4 hours in women. Although the value for men is in the range of results for humans, the value for women is much less than that estimated using the human data available from Chang et al. (2008). DAFs based on $BW0^{.75}$ scaling for rats and a standard BW of 0.03 kg for mice are presented in Table 5-3. EPA's guidance on use of $BW0^{.75}$ as the default method for derivation of an oral reference dose states, however, "EPA endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species." It goes on to state that, although use of PBPK models is preferred, "Other approaches may include using chemical-specific information, without a complete physiologically-based pharmacokinetic model" (i.e., the approach described here, using relative clearance) and that use of $BW0^{.75}$ is endorsed, "In lieu of data to support either of these types of approaches" (U.S. EPA, 2011). Thus, because data *are* available to support a chemical-specific approach, it is clearly preferred.

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-hour = 0.12 L/kg-day. 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)	DAF ($BW^{0.75}$)[d]
Male	Rat	21.61[a]	4.95[c]	0.229	0.236
	Mouse	10.10[b]		0.490	0.139
Female	Rat	96.62[a]		0.051	0.236
	Mouse	27.93[b]		0.177	0.139

Data from Tables 2, 3, 5, and 6 of Chang et al. (2008).Data from Tables 2, 3, 5, and 6 of Chang et al. (2008).

[a]Average of CL = dose/AUC (area-under-the-concentration-curve) was calculated using values reported for oral and i.v. exposures reported in Table 2 of Chang et al. (2008) see Table 3-2.

[b]Average of CL = dose/AUC was calculated using values reported for the 10- and 30-mg/kg dose groups reported in Table 3 of Chang et al. (2008) see Table 3-2. CL 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 could be due to saturation of renal absorption or serum binding.

[c]CL value for humans (male and female) as described above.

[d]DAFs based on assumption that elimination scales as $BW^{0.75}$, hence clearance (elimination/BW) scales as $BW^{-0.25}$, using standard BWs of 0.03, 0.25, and 80 kg for mice, rats, and humans, respectively.

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. (2012a) study as an example. Note that the concentration of the ammonium salt first needs to be converted to the concentration of the free acid before HED calculation:

$$HED\ PFBA = POD\ NH_4^+\text{PFB (mg/kg-d)} \frac{MW\ PFBA}{MW\ NH_4^+\text{PFB}} \times \frac{CL\ human\ (mL/kg\text{-}h)}{CL\ animal\ (mL/kg\text{-}h)} \quad (5\text{-}4)$$

$$HED = 9.6\ \text{(mg/kg-d)} \times \frac{214\ g/mol}{231\ g/mol} \times \frac{4.95\ \text{(mL/kg-h)}}{21.61\ \text{(mL/kg-h)}} = 2.04\ \text{(mg/kg-d)}$$

As discussed in Section 3.1.5 (Summary of Pharmacokinetics), the assumed linearity in PK (constant clearance) is likely to be valid for animal POD values of 30 mg/kg-day and below, but these DAFs should not be applied to higher PODs.

Uncertainty of Animal-to-Human Extrapolation of PFBA Dosimetry

There is uncertainty in applying this dosimetric approach given the volume of distribution (V_d) was not measured in humans and the human V_d was assumed equal to that in monkeys to estimate clearance in humans. An 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 use the estimated human clearance values to calculate the HED. This approach, compared to the ratio of the clearance values approach, however, is interpreted to have even greater uncertainty. First, the measured serum concentrations were reported to have been taken 24 hours after the last exposure in the developmental toxicity study

Das et al. (2008) and likely were similarly taken in the subchronic toxicity study (Butenhoff et al., 2012a; van Otterdijk, 2007a). Given the relatively short half-life of PFBA measured in mice and rats, this end-of-exposure measurement of serum concentrations likely did not reflect the average serum concentrations exposed animals experienced. For example, the reported serum levels (see Section 2.1.1) in female mice in the Das et al. (2008). Also, to estimate the HED without 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 be characterized by the same uncertainty as the assumption that human and monkey volumes of distribution are equal and 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. Thus, the approach based on clearance values is the one used here.

That only a single study reported PFBA PK data in rats or mice (or monkeys) introduces qualitative uncertainty, because these results were not validated in independent experiments. Results from different studies cannot be compared quantitatively. In the Chang et al. (2008) study, some results have relatively tight standard errors (SEs), indicating high confidence, but others (especially for mice), indicate high variability/uncertainty. Although the results for AUC in rats have relatively small SEs, they surprisingly show higher AUC (hence lower clearance) following oral doses than following i.v. doses (30 mg/kg). Oral absorption or bioavailability can range between near zero and 100%, but why the blood concentrations after an oral dose are higher than when the same dose is injected directly into the blood is puzzling. The data and plot of the PK model shown in Figures 1 and 2 of Chang et al. (2008) indicate the absorption and clearance phases are well characterized and described by the model, so the uncertainty does not appear to be due to the study design or analysis method. The almost twofold difference in clearance rates estimated from the oral vs. i.v. rat data thus indicate a comparable degree of uncertainty.

Compared to the results for rats, the Chang et al. (2008) clearance estimates at the two lower oral doses in male and female mice are much closer, with only an 8% difference between the two doses for males and a 16% difference for females. The results for both male and female mice show a dose-dependent increase in clearance across all dose levels, consistent with the hypothesis of saturable renal resorption. Although the increase only seems significant with the increase from 30 to 100 mg/kg, the differences between 10 and 30 mg/kg could result from the same mechanism. Thus, those differences might reflect a biological mechanism as much as experimental or analytic variability. The lack of i.v. data in mice at the same dose as any of the oral doses, however, means that one cannot fully compare the apparent self-consistency of the mouse data to the inconsistency noted above for rats.

If the oral vs. i.v. discrepancy in rats is interpreted as indicating an overall factor of 2 uncertainty in the animal clearance values, that can be considered a moderate degree of

uncertainty. ORD's Umbrella quality assurance project plan (QAPP) for dosimetry and mechanism-based models (U.S. EPA, 2020) states that PBPK models are expected to match the corresponding data within a factor of 2 to be considered sufficient for use in risk assessment and similarly IPCS (2010) states that PBPK models can be considered adequate when predictions that are, on average, within a factor of two of experimental data. Hence, this level of uncertainty is considered acceptable in PK analyses. Although the human half-life estimates vary just over fivefold from highest to lowest, this much variability in a human population is not surprising, and with results from just 12 subjects to characterize the mean, uncertainty in that mean can, again, be considered moderate. Given that the physiological fractions of different tissue types is similar in humans and primates and that the blood serum:tissue portioning is reasonably expected to be similar across mammals, the assumption that the volume of distribution in humans is similar to monkeys is considered to have low uncertainty. Considering all these factors, the overall uncertainty in HED calculations using equation (5-4) with the parameters estimated here is considered moderate, that is, within a factor of 3.

Application of Animal-Human Extrapolation of PFBA Dosimetry

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 NH_4^+ PFB (mg/kg-d)	POD PFBA (mg/kg-d)[a]	POD_{HED} PFBA[b] (mg/kg-d)
Increased relative liver weight Butenhoff et al. (2012a)	S-D rat, male	$BMDL_{10RD}$ Exp3 (LN-CV)	9.6	8.89	2.04
Increased relative liver weight Das et al. (2008)	CD-1 mouse, P_0 female	$BMDL_{10RD}$ Exp4 (CV)	15	13.9	2.46
Increased liver hypertrophy Butenhoff et al. (2012a)	S-D rat, male	NOAEL[b] (0% response)	6	5.56	1.27
Decreased total T4 Butenhoff et al. (2012a)	S-D rat, male	NOAEL[c] (15% decrease)	6	5.56	1.27
Embryo/fetal mortality[d] Das et al. (2008)	CD-1 mouse, F_1 male/female	$BMDL_{1ER}$ Nested-Logistic	5.7	5.28	0.93
Delayed eyes opening[d] Das et al. (2008)	CD-1 mouse, F_1 male/female	$BMDL_{5RD}$ Hill (CV)	4.9	4.54	0.80
Delayed vaginal opening[d] Das et al. (2008)	CD-1 mouse, F_1 female	$BMDL_{5RD}$ Hill (CV)	3.8	3.52	0.62
Delayed preputial separation[d] Das et al. (2008)	CD-1 mouse, F_1 male	$BMDL_{5RD}$ Exp3 (CV)	179.1	165.92	n/a[e]

BMDL = 95% lower limit on benchmark dose, RD = relative deviation, LN = log-normal, CV = constant variance, ER = extra risk, NOAEL = no-observed-adverse-effect level.

[a] Both of these studies used the ammonium salt of PFBA as the test article. To calculate a POD for the free acid of PFBA from any PFBA salt, multiply the POD of interest by the ratio of molecular weights of the salt and the free acid. For example, to convert from the ammonium salt of PFBA to the free acid, multiply the ammonium salt POD by 0.926: $\frac{MW\ free\ acid}{MW\ ammonium\ salt} = \frac{214}{231} = 0.926$.

See discussion in Section 5.2.1, Approach for Animal-Human Extrapolation of PFBA Dosimetry, for details on HED.

[b] NOAEL approach used as responses are only seen in the high dose group at levels much higher (90%) than the BMR.

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

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

[e] As noted previously, linearity in clearance values is only valid up to approximately 30 mg/kg-d and the DAF based on a ratio of clearance values should not be applied to PODs greater than 30 mg/kg-d. Therefore, given that the POD for preputial separation is above this limit, and other PODs are below that limit, preputial separation is not considered further for use in estimating a candidate toxicity value for developmental delays. Instead, PODs for delays in vaginal opening and eye opening are advanced for this purpose.

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 UFs 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 pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral NH_4^+PFB/PFBA exposure. Some aspects of the cross-species extrapolation of pharmacokinetic processes have been accounted for by calculating an HED through application of a DAF based on animal and human half-lives; however, some residual pharmacokinetic uncertainty and uncertainty regarding pharmacodynamics 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 pharmacokinetics and pharmacodynamics of NH_4^+PFB/PFBA in humans.
UF_S	10 1	A UF_S of 10 is applied to endpoints observed in the subchronic study Butenhoff et al. (2012a); van Otterdijk (2007a) 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 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. Although these high confidence studies are available for PFBA, the database has some deficiencies, including the lack of information on developmental neurotoxicity and other endpoints; see the text below for further discussion.
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; it accounts for uncertainty regarding the pharmacokinetic and pharmacodynamic differences across species. As is usual in the application of this uncertainty factor, the pharmacokinetic uncertainty is mostly addressed through the application of dosimetric approaches for estimating human equivalent doses (see Section 4.2.2). This leaves some residual uncertainty around the pharmacokinetics and the uncertainty surrounding pharmacodynamics. Typically, a threefold 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, chemical-specific information should be considered further 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 vs. 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, based on statistical significance), and that PPARα null mice generally did not exhibit hepatic effects except for vacuolation. Additionally, in vitro studies suggest that human cells or cells transfected with human PPARα were less sensitive 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 MOA 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 mentioned in the previous paragraph, the pharmacodynamic portion of this UF is typically assigned a value of 3 assuming responses manifest in humans could be more sensitive than those observed in animals). Additional evidence presented in Foreman et al. (2009) and other studies (see Section 2.2.5), however, indicates that non-PPARα MOAs 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 in wild-type mice, although the degree to which null or humanized mice are more susceptible to this effect is difficult 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 that show 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 MOAs 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 pharmacodynamic portion of the UF_A. Therefore, given the remaining uncertainty in additional MOAs that appear active in PFBA-induced liver effects, and the relative contribution of these MOAs to toxicity in humans as compared with rodents, the value of UF_A was set to 3 for the purposes of deriving toxicity values for hepatic effects. No MOA 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 pharmacokinetic and pharmacodynamic uncertainty.

The short-term studies of Butenhoff et al. (2012a), van Otterdijk (2007a), and Foreman et al. (2009) were considered for potential use in informing the selection of the UFS. More specifically, for several 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 pharmacokinetics of PFBA, steady-state levels in potential target tissues might not substantially increase with increasing exposure duration (Butenhoff et al., 2012a; van Otterdijk, 2007a, b). In these studies, the latter conclusion seemed 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 increased slightly or were 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. Preliminarily, this indicates that increased durations of exposure might 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 the 28-day exposure study by Foreman et al. (2009) and the gestational exposure study by Das et al. (2008) basing comparisons on human equivalent external concentrations (see Table 5-6 below for modeling results and application of dosimetric adjustments), liver weight appears affected at similar doses across mice and rats across these three different exposure durations (i.e., gestational, short-term, and subchronic). However, it should be noted that these data indicating no increase of effect when comparing subchronic exposures to short-term exposures (an increase in duration of approximately 3-fold) is not considered sufficient evidence to convincingly argue that effects would not worsen following chronic exposures (an increase of approximately 8-fold increase in duration, compared to subchronic). While it is true that pharmacokinetic data suggests liver concentrations may reach steady-state conditions rapidly (i.e., following 28 days of exposure), it is reasonable to assume that prolonged exposure to those levels of tissue-specific concentrations over the course of multiple years could result in an increased magnitude of effect or effects evident at lower doses. Contributing to this assumption is the consideration of the impact of PFBA exposure duration on related liver effects.

In fact, the lack of increasing effect with increasing duration is not the case for all liver effects. Histopathological evaluations of the liver in male rats exposed to PFBA for 90 days show that hepatocellular hypertrophy occurs at 30 mg/kg-day, whereas hypertrophy occurs only at 150 mg/kg-day in male rats exposed for 28 days (Butenhoff et al., 2012a; van Otterdijk, 2007a, b). Thus, although liver concentrations are equivalent following 28- or 90-day exposures, that prolonged exposure (i.e., 90 days vs. 28 days) elicits adverse effects in the liver is readily apparent. 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 were therefore set to 10 for the purposes of the liver endpoints.

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

Reference	Species/strain/ sex	Duration	POD type/model	POD NH_4^+ PFB (mg/kg-d)	POD PFBA (mg/kg-d)	POD_{HED} PFBA (mg/kg-d)
Relative liver weight Butenhoff et al. (2012a)	S-D rat, male	90 d	$BMDL_{10RD}$ Exp3 (LN-CV)	9.6	8.89	2.04
Relative liver weight Butenhoff et al. (2012a)	S-D rat, male	28 d	$BMDL_{10}$, Exp4 (NCV)	6.34	5.87	1.3
Relative liver weight Foreman et al. (2009)	Sv/129 WT mouse, male	28 d	LOAEL[a]	35	32.42	1.59[b]
Relative liver weight (Foreman et al., 2009)	Sv/129 hPPARα mouse, male	28 d	$BMDL_{10}$, Hill (NCV)	4.41	4.09	2.00
Relative liver weight Das et al. (2008)	CD-1 mouse, P_0 female	Gestational	$BMDL_{10RD}$ Exp4 (CV)	15	13.9	2.46

[a] Data is highly supralinear and BMD modeling guidance recommends against modeling this type of dose-response pattern.
[b] 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.

Regarding thyroid endpoints, effects on total T4 following subchronic exposures were not worse compared to effects following 28-day exposures in the Butenhoff et al. (2012a) study. owever, for thyroid hypertrophy/hyperplasia, although total incidence of hypertrophy/hyperplasia was not observed to worsen with increasing duration (the LOAEL was 30 mg/kg-day for both exposure durations), there is evidence that the severity of the observed lesions worsened after 90-day exposures. As Table 3-4 shows, nine out of ten animals developed thyroid hypertrophy/hyperplasia following 28-day exposures, with all animals displaying minimally severe lesions. However, following 90-day exposures, while the total incidence was the same (9/10), four animals had minimally severe lesions while 5 animals developed mild lesions. While not conclusive, this evidence suggests that damage to the thyroid organ specifically might worsen with increasing duration. Given this potential concern for more severe effects on the thyroid with longer exposures and the small number of studies (e.g., one) available to inform this interpretation for either thyroid histopathology or levels of circulating THs, the default UF_S of 10 was also retained for thyroid endpoints.

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* U.S. EPA (2002), the database uncertainty factor is applied to account for the potential of deriving an underprotective reference value as a result of incomplete characterization of a chemical's toxicity. The PFBA database is relatively small but contains *high* confidence subchronic and developmental toxicity studies investigating effects in multiple organ systems in male and female rats and mice.

For PFBA, given the small number of available studies, both a $UF_D = 10$ or a $UF_D = 3$ were considered due to the limited database (most specifically the lack of a two-generation

developmental/reproductive toxicity study, but also a lack of studies on potential neurodevelopmental or developmental immune effects), and a $UF_D = 3$ ultimately was applied. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. The PFBA database does include a *high* confidence Das et al. (2008) developmental toxicity study in mice. Despite its quality, however, that study fails to cover endpoints related to potential transgenerational impacts of longer-term exposures evaluated in a two-generation study. The 1994 Reference Concentration Guidance U.S. EPA (1994) and 2002 Reference Dose Report U.S. EPA (2002) support applying a UF_D in situations when such a study is missing. The 2002 Reference Dose Report U.S. EPA (2002) states that "[i]f the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproductive study is missing." Consideration of the PFBA, PFBS (a short-chain perfluoroalkane sulfonic acid with a 4-carbon backbone like PFBA), PFHxA (a short-chain perfluoroalkyl carboxylic acid; see public comment draft for PFHxA;),[13] and PFHxS (a long-chain perfluoroalkane sulfonic acid) databases together, however, diminish the concern that the availability of a multigenerational reproductive study would result in reference values lower than those currently derived for PFBA. Although limited in their ability to assess reproductive health or function, measures of possible reproductive toxicity, including reproductive organ weights (i.e., epididymis, testis, and ovary weights) were unaffected when measured after exposure to PFBA for 28 days (Butenhoff et al., 2012a; van Otterdijk, 2007a). Likewise, the available data on reproductive toxicity in the PFBS database is consistent with this general lack of sensitive reproductive effects: No biologically significant changes were observed in male mating and fertility parameters, reproductive organ weights, reproductive hormone levels, or altered sperm parameters (U.S. EPA, 2018b). The female reproductive effects that were observed (e.g., altered estrous cyclicity) occurred at doses equal to or higher than those that resulted in effects in other organ systems (e.g., thyroid, liver), thus indicating they were not more sensitive markers of toxicity. Further, no notable male or female reproductive effects were observed in epidemiological or toxicological studies investigating exposure to PFHxA (see public comment draft for PFHxA, (U.S. EPA, 2021b) and (Luz et al., 2019; NTP, 2019; Klaunig et al., 2015; Chengelis et al., 2009) or PFHxS (MDH, 2019). Therefore, when considering the limited chemical-specific information alongside information gleaned from structurally related compounds, the lack of a multigenerational reproductive study is not considered a major concern relative to UF_D selection.

Another gap in the PFBA database is the lack of measures of thyroid toxicity in gestationally exposed offspring and the lack of a developmental neurotoxicity study. The potential for neurodevelopmental effects, whether thyroid hormone-dependent or independent, remains an

[13]The systematic review protocol for PFBA (see Appendix A) defines perfluoroalkyl carboxylic acids with seven or more perfluorinated carbon groups and perfluoralkane sulfonic acids with six or more perfluorinated carbon groups as "long-chain" PFAS. Thus, PFHxA is considered a short-chain PFAS, whereas PFHxS is considered a long-chain PFAS.

uncertainty for PFBA. Thyroid hormones are critical in myriad physiological processes and must be maintained at sufficient levels during times of brain development in utero and after birth. Although no PFBA-specific data on thyroid hormone levels following gestational exposure are available, total T4 is reduced in both pregnant mice and their offspring following whole-gestation oral exposure to PFBS, with effects evident in offspring at PNDs 1, 30, and 60. Therefore, anticipating that effects due to PFBA exposure also could have been observed had thyroid hormone levels been measured in the Das et al. (2008) developmental study is reasonable. For PFBS, the PODs for effects in dams and offspring on PND 1 were almost identical, indicating that thyroid hormone homeostasis is perturbed at equivalent exposure levels in both pregnant animals and developing offspring. Thus, although some concern remains that thyroid insufficiency during in utero and perinatal development could be a more sensitive effect of PFBA exposure than insufficiency in adults, this concern is mitigated on the basis of data from other PFAS. Likewise, given that neurodevelopmental effects due to thyroid hormone insufficiency would be downstream effects, application of a UF_D (and derivation of reference values) addressing the potential for developmental thyroid insufficiency would presumably be protective of any potential neurodevelopmental endpoints related to that mechanism. The potential for neurodevelopmental effects independent of a thyroid hormone-related mechanism remains an uncertainty for PFBA.

Lastly, the potential for immunotoxicity (including developmental immunotoxicity, in particular) and mammary gland effects represents an area of concern across several constituents of the larger PFAS family (primarily long-chain PFAS). No studies have evaluated these outcomes following PFBA oral exposure or following oral exposure to the structurally related PFBS described above. However, one dermal toxicity study Weatherly et al. (2021) did observe altered cellularity for multiple immune cell types in the draining lymph nodes and ear pinna of exposed animals, raising the concern for immunotoxicity following oral exposures. However, without reported internal serum levels, it is difficult to ascertain whether the exposure levels in the dermal study are equipotent to the oral exposures used in the subchronic and developmental toxicity studies and whether the hepatic, thyroid, or developmental endpoints observed in those studies would be protective of immunotoxicity endpoints. Overall, no chemical-specific information is available to judge the degree to which the existing endpoints in the PFBA Toxicological Review would be protective of mammary gland or immune (including developmental immune) effects after oral exposure.

Given the residual concerns for potentially more sensitive effects outlined above, a database uncertainty factor is considered necessary. Specifically, a value of 3 was selected for the UF_D to account for the uncertainty surrounding the lack of a multigenerational reproductive study, developmental neurotoxicity study (or information on thyroid hormone perturbation in utero and postnatally), immunotoxicity (and developmental immunotoxicity, in particular), or mammary gland effects. A UF_D of 10 was not applied, given that multiple lines of chemical-specific information or data from structural analogs are available to partially mitigate the concern that additional study

would possibly result in reference values one order of magnitude lower than the one currently derived. Thus, a UF_D value of 3 was applied because currently available lines of evidence do not fully eliminate this concern.

The candidate values (see Table 5-7) 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. (2012a), the candidate value is calculated as:

$$\text{Candidate value for PFBA} = \text{BMDL}_{10} \div \text{UF}_c \quad (5\text{-}5)$$

$$\text{Candidate value} = 2.04 \left({}^{\text{mg}}/_{\text{kg-d}}\right) \div 1{,}000$$

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

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

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

Endpoint	POD_{HED} PFBA (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value PFBA (mg/kg-d)	Candidate value NH_4^+ PFB (mg/kg-d)[a]
Increased relative liver weight Butenhoff et al. (2012a)	2.04	3	10	10	1	3	1,000	2.0×10^{-3}	2.2×10^{-3}
Increased relative liver weight Das et al. (2008)	2.46	3	10	10	1	3	1,000	2.5×10^{-3}	2.7×10^{-3}
Increased liver hypertrophy Butenhoff et al. (2012a)	1.27	3	10	10	1	3	1,000	1.3×10^{-3}	1.4×10^{-3}
Decreased total T4 Butenhoff et al. (2012a)	1.27	3	10	10	1	3	1,000	1.3×10^{-3}	1.4×10^{-3}
Embryo/fetal mortality Das et al. (2008)	0.93	3	10	1	1	3	100	9.5×10^{-3}	1.0×10^{-2}
Delayed eyes opening Das et al. (2008)	0.80	3	10	1	1	3	100	8.0×10^{-3}	8.6×10^{-3}
Delayed vaginal opening Das et al. (2008)	0.62	3	10	1	1	3	100	6.2×10^{-3}	6.7×10^{-3}

[a] To calculate candidate values for salts of PFBA, multiply the candidate value of interest by the ratio of molecular weights of the free acid and the salt. For example, for the ammonium salt of PFBA, the RfD would be calculated by multiplying the free acid RfD by 1.079: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{231}{214} = 1.079$. This same conversion can be applied to other salts of PFBA, such as the potassium or sodium salts.

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 PFBA; 1×10^{-3} mg/kg-d NH_4^+ PFB		
Confidence in study[a] used to derive osRfD	High	Confidence in the study Butenhoff et al. (2012a); 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. Although 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 Foreman et al. (2009) 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 a NOAEL (BMD modeling not supported given that responses are only observed in the high dose group at levels (90%) much greater than the BMR) and dosimetric adjustment was based on PFBA-specific pharmacokinetic information, the latter of which introduces some uncertainty. Generally, the use of a NOAEL for the POD would result in a reduced confidence rating. However, in this case, the NOAEL of 6 mg/kg-d is very close to the BMDL (5.4 mg/kg-d) that would be selected had BMD modeling been supported. Therefore, this NOAEL is not interpreted as likely to be substantially more uncertain than a BMD-based POD. This supports a determination that the confidence in the quantification of the POD is *medium*.

Confidence categories	Designation	Discussion
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 quantification of the POD from a *high* confidence study.
Thyroid RfD = 1×10^{-3} mg/kg-d PFBA; 1×10^{-3} mg/kg-d NH_4^+ PFB		
Confidence in study[a] used to derive osRfD	High	Confidence in the study Butenhoff et al. (2012a); 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 a 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 did not provide an adequate fit to the data) and dosimetric adjustment was based on PFBA-specific pharmacokinetic information, the latter of which introduces some uncertainty. Although 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 at a BMR of 1 SD (i.e., the BMD), there is uncertainty regarding how much lower a BMDL would be as compared to the NOAEL[b]. Therefore, while this NOAEL is not likely to be substantially more uncertain than a BMD, it is higher than a BMDL-based POD would be. This introduces some additional uncertainty and 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 by *medium* confidence in the evidence base; however, the *medium-to-low* confidence in the quantification of the POD does warrant decreasing the overall confidence in the osRfD.
Developmental RfD = 6×10^{-3} mg/kg-d PFBA; 7×10^{-3} mg/kg-d NH_4^+ PFB		
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 and reproductive development).

Confidence categories	Designation	Discussion
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 pharmacokinetic information, the latter of which introduces some uncertainty. Other sources of uncertainty are the use of dosimetric adjustments based on the ratio of adult pharmacokinetic parameters, and that the derived BMDL is approximately ninefold 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 quantification 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.
[b] Note that the BMDL would be considerably less than an order of magnitude lower given that the next lower dose tested was only 5-fold lower than the NOAEL and a non-significant *increase* in T4 was observed at that dose.

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 PFBA (mg/kg-d)	osRfD NH_4^+ PFB (mg/kg-d)[b]	Confidence
Hepatic	Increased hepatocellular hypertrophy in adult male S-D rats	$BMDL_{HED}$ from Butenhoff et al. (2012a)	1,000	1×10^{-3}	1×10^{-3}	*Medium*
Thyroid	Decreased total T4 in adult male S-D rats	$NOAEL_{HED}$ from Butenhoff et al. (2012a)	1,000	1×10^{-3}	1×10^{-3}	*Medium-low*
Developmental	Developmental delays after gestational exposure in CD1 mice[a]	$BMDL_{HED}$ from Das et al. (2008)	100	6×10^{-3}	7×10^{-3}	*Medium-low*

[a] POD based on delayed vaginal opening used to represent three developmental delays observed in the study.
[b] See Table 5-7 for details on how to calculate candidate values for salts of PFBA; the osRfDs presented in this table have been rounded to 1 significant digit from the candidate values presented in Table 5-7.

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 PFBA***

based on increased liver hypertrophy and decreased total T4 is selected. The selected RfD for the ammonium salt of PFBA is also 1×10^{-3} mg/kg-day. 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 (for developmental effects) was interpreted with *medium-low* confidence and had a higher osRfD value; thus, it was not selected. Although 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 POD_{HED}s: *medium* for increased liver hypertrophy and *medium-low* for decreased total T4. As noted in Table 5-8, the *medium-low* confidence in the thyroid POD_{HED} reflects that the selected NOAEL would be greater than the BMDL that would be derived if BMD modeling were possible, although this difference would be considerably less than an order of magnitude (see Table 5-8) which reduces the level of concern for this uncertainty. This uncertainty is further mitigated when taken together with the *medium* confidence in the POD_{HED} for the co-critical effect on the liver. Altogether, this supports the determination of *medium* confidence in 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 fivefold lower than the developmental osRfD and thus protective of PFBA-induced effects on the developing organism. If the osRfD for developmental effects were chosen as the overall RfD on the basis of 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. Selection of the RfD based on liver and thyroid effects is presumed to be protective of possible developmental effects in humans, although uncertainty in the database currently available for PFBA remains including a lack of information on the potential for sensitive transgenerational, neurodevelopmental, or developmental immune effects of PFBA exposure (see discussion on UF_D selection above).

Increased liver hypertrophy and decreased total T4 was observed only in male rats exposed to PFBA, thus possibly identifying males as a susceptible population. As discussed in Section 3.3, however, this observation in rats could be driven primarily by the observed sex-dependent differences in pharmacokinetics in rats. No compelling information is available that supports a

similarly strong sex dependence in pharmacokinetics in humans. Therefore, this RfD is presumed equally applicable to both male and female humans.

5.2.2. 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} PFBA (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value PFBA (mg/kg-d)	Candidate value NH_4^+ PFB (mg/kg-d)[a]
Increased relative liver weight Butenhoff et al. (2012a)	2.04	3	10	1	1	3	100	2.0×10^{-2}	2.2×10^{-2}
Increased relative liver weight Das et al. (2008)	2.46	3	10	1	1	3	100	2.5×10^{-2}	2.7×10^{-2}
Increased liver hypertrophy Butenhoff et al. (2012a)	1.15	3	10	1	1	3	100	1.1×10^{-2}	1.2×10^{-2}
Decreased total T4 Butenhoff et al. (2012a)	1.27	3	10	1	1	3	100	1.3×10^{-2}	1.4×10^{-2}
Embryo/fetal mortality Das et al. (2008)	0.93	3	10	1	1	3	100	9.3×10^{-3}	1.0×10^{-2}
Delayed eyes opening Das et al. (2008)	0.80	3	10	1	1	3	100	8.0×10^{-3}	8.6×10^{-3}
Delayed vaginal opening Das et al. (2008)	0.62	3	10	1	1	3	100	6.2×10^{-3}	6.7×10^{-3}

[a] To calculate subchronic candidate values, osRfDs, or the subchronic RfD for salts of PFBA, multiply the value of interest by the ratio of molecular weights of the free acid and the salt. For example, for the ammonium salt of PFBA, the RfD would be calculated by multiplying the free acid RfD by 1.079: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{231}{214} = 1.079$. This same method of conversion can be applied to other salts of PFBA, such as the potassium or sodium salts, using the corresponding molecular weights.

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) (corresponding osRfD of 1×10^{-2} mg/kg-day for the ammonium salt), and an osRfD of 6×10^{-3} mg/kg-day PFBA is selected for developmental effects (developmental delays based on the candidate value for delayed vaginal opening) (corresponding osRfD of 7×10^{-3} mg/kg-day for the ammonium salt). The selection of these candidate values over other candidates and the confidence in these subchronic osRfDs are identical to the confidence in the osRfDs discussed in the previous section and presented in Table 5-8. Note, specifically for developmental delays, the candidate value for delayed eye opening was not selected as the osRfD as it was 33% larger than the candidate value for vaginal opening and thus inadequately protective of human health.

From these subchronic osRfDs, an ***overall subchronic RfD of 6×10^{-3} mg/kg-day PFBA based on developmental delays*** is selected (the corresponding overall subchronic RfD is 7×10^{-3} mg/kg-day for the ammonium salt). 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 need not consider differences in the composite UF, as a value of 100 is applied to all PODs. This is because all the studies considered for the subchronic RfD are subchronic or gestational duration studies. This results in the osRfD for developmental delays being approximately 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 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. Also, although the subchronic RfD is intended to protect health during a less-than-lifetime exposure to PFBA, developmental delays are appropriate endpoints on which to base a subchronic RfD. First, as discussed above (Study Selection subsection), given delayed reproductive milestones occuring during critical periods of development, EPA's Reproductive Toxicity Guidelines U.S. EPA (1996) state that significant effects on puberty (and thus by inference, the development of the male and female reproductive systems more broadly) "either early or delayed, should be considered adverse…". Further, delays in reaching developmental milestones are not phenomena that can be resolved (e.g., after PFBA exposure is removed), and they can result from short (less-than-lifetime) exposures during discrete windows of development. More importantly, the consequences of these delays can have permanent impacts on health (e.g., delays in eye opening leading to permanent decrements in visual acuity). So, although the delay itself might occur only over a short portion of lifetime, the functional consequences are permanent.

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), EPA concluded that there is *inadequate information to assess carcinogenic potential* for PFBA (or salts of PFBA) for any route of exposure. This conclusion precludes the derivation of quantitative estimates for either oral (oral slope factor [OSF]) or inhalation (inhalation unit risk [IUR]) exposure.

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