Human Health Toxicity Values for

Perfluorobutane Sulfonic Acid
(CASRN 375-73-5)
and Related Compound
Potassium Perfluorobutane Sulfonate
(CASRN 29420-49-3)

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Potassium Perfluorobutane Sulfonate (CASRN 29420 49 3)**

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Commonly Used Abbreviations and Acronyms

AEC	absolute eosinophil count	NHANES	National Health and Nutrition Examination Survey
AFFF	Aqueous Film-Forming Foam	NOAEL	no observed adverse effect level
AIC	Akaike's information criterion	NTP	National Toxicology Program
ALT	alanine aminotransferase	NZW	New Zealand White (rabbit breed)
AST	aspartate aminotransferase	OR	odds ratio
AUC	area under the curve	PECO	population, exposure, comparator, outcome
BMD	benchmark dose	PFAA	perfluoroalkyl acid
BMDL	benchmark dose lower confidence limit	PFAS	per- and polyfluoroalkyl substances
BMDS	Benchmark Dose Software	PFOA	perfluorooctanoic acid
BMR	benchmark response	PFOS	perfluorooctane sulfonic acid
BUN	blood urea nitrogen	PFBS	perfluorobutane sulfonic acid
BW	body weight	PFHxA	perfluorohexanoic acid
CA	chromosomal aberration	PND	postnatal day
CASRN	Chemical Abstracts Service Registry Number	POD	point of departure
CHO	Chinese hamster ovary (cell line cells)	RD	relative deviation
CPN	chronic progressive nephropathy	RfC	inhalation reference concentration
D3	deiodinase 3	RfD	oral reference dose
DAF	dosimetric adjustment factor	ROS	reactive oxygen species
DNA	deoxyribonucleic acid	rT3	reverse triiodothyronine
ECP	eosinophilic cationic protein	SD	standard deviation
EPA	U.S. Environmental Protection Agency	T2	3,5-diiodo-L-thyronine
		T3	triiodothyronine
GD	gestation day	T4	thyroxine
HAWC	Health Assessment Workspace Collaborative	TBG	thyroid binding globulin
		TSH	thyroid-stimulating hormone
HED	human equivalent dose	TTR	transthyretin
HPT	hypothalamic-pituitary-thyroid	UF	uncertainty factor
i.v.	intravenous	UF_A	interspecies uncertainty factor
ICR	Institute of Cancer Research	UF_c	composite uncertainty factor
K^+PFBS	potassium perfluorobutane sulfonate	UF_D	database uncertainty factor
LD_{50}	median lethal dose	UF_H	intraspecies uncertainty factor
LOAEL	lowest observed adverse effect level	UF_L	LOAEL-to-NOAEL uncertainty factor
MW	molecular weight	UF_S	subchronic-to-chronic uncertainty factor
NCEA	National Center for Environmental Assessment	VLDL	very low density lipoprotein

Executive Summary

Summary of Occurrence and Health Effects

The U.S. Environmental Protection Agency (EPA) is issuing draft subchronic and chronic oral toxicity values for perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service Registry Number [CASRN] 375-73-5) and its related salt, potassium perfluorobutane sulfonate (K⁺PFBS) (CASRN 29420-49-3) for public comment. The toxicity assessment for PFBS is a scientific and technical report that includes toxicity values associated with potential noncancer health effects following oral exposure (in this case, oral reference doses (RfDs). This assessment evaluates human health hazards. The toxicity assessment and the values contained within is not a risk assessment as it does not include an exposure assessment nor an overall risk characterization. Further, the toxicity assessment does not address the legal, political, social, economic, or technical considerations involved in risk management. When final, the PFBS toxicity assessment can be used by EPA, states, tribes, and local communities, along with specific exposure and other relevant information, to determine, under the appropriate regulations and statutes, if, and when, it is necessary to take action to address potential risk associated with human exposures to PFBS.

PFBS and K⁺PFBS are both four-carbon, fully fluorinated alkane members of a large and diverse class of linear and branched compounds known as "per- and polyfluoroalkyl substances," or PFAS. In the early 2000s, concerns grew over the environmental persistence, long half-lives in humans, and bioaccumulation potential of longer chain PFAS, in particular perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS). As a result, shorter chain PFAS such as PFBS were developed and integrated into various consumer products and applications, as this compound has the desired properties and characteristics associated with this class of compounds with faster elimination from the body than PFOA and PFOS. PFBS has been found in food contact materials, dust, and source and finished drinking water. It is also associated with Aqueous Film-Forming Foams and used during chrome electroplating as a mist suppressant. As such, oral intake of water and food, inhalation, and dermal contact are plausible modes of PFBS exposure, with the oral route being the primary route of exposure. PFBS has been detected in human urine, confirming exposure to this PFAS; however, the magnitude of human exposure likely depends on factors such as occupation (e.g., processing and/or manufacture of PFBS or PFBS-containing products and chrome electroplating) and living conditions (e.g., proximity to locations that make or use PFBS-containing products and well-water use).

Human studies have examined possible associations between PFBS exposure and potential health outcomes such as alteration of menstruation, reproductive hormones or semen parameters, kidney function (uric acid production), lung function (induction of asthma), and lipid profile. The ability to draw conclusions about associations was limited due to the small number of studies per outcome. Of the examined outcomes, only asthma, serum cholesterol, and high-density lipoprotein levels were found to exhibit a statistically significant positive association with PFBS exposure. No studies have been identified that evaluate the association between PFBS exposure and potential cancer outcomes.

Animal studies of repeat-dose PFBS exposure have been exclusively via the oral route, used the potassium salt of PFBS (K^+PFBS) as the source exposure material, and have examined noncancer effects only. The available rat and mouse studies support identification of thyroid, developmental, and kidney endpoints as potential health effects following repeated exposures in utero and/or during adulthood. Animal studies also evaluated other health outcomes such as liver, reproductive parameters, lipid/lipoprotein homeostasis, spleen, and hematology; however, the available evidence does not support a clear association with PFBS exposure.

Noncancer Effects Observed Following Oral Exposure

Oral exposures to PFBS or its K^+ salt in adult and developing rats and mice have been shown to result in thyroid, developmental, and kidney effects. Thyroid effects in adult exposed rats and mice and in developing mice were primarily expressed through significant decreases in circulating levels of hormones such as thyroxine (T4) and triiodothyronine (T3). In early developmental life stages in mice (e.g., newborn), decreases in thyroid hormone were accompanied by other effects indicative of delayed maturation or reproductive development (e.g., vaginal patency and eyes opening). Kidney weight and/or histopathological alterations (e.g., renal tubular and ductal epithelial hyperplasia) were observed in rats following short-term and subchronic oral exposures. Many of the kidney effects, however, occurred at higher doses than did the thyroid and developmental effects. The limited number of human studies examining oral PFBS exposure does not inform the potential for effects in thyroid, developing offspring, or the renal system.

Oral Reference Doses for Noncancer Effects

Subchronic and chronic oral RfDs were derived for PFBS. The hazards of potential concern include thyroid, developmental, and kidney effects. From these identified targets of PFBS toxicity, perturbation of thyroid hormone levels (e.g., thyroxine [T4]) and kidney histopathology (e.g., papillary epithelial tubular/ductal hyperplasia) were used as endpoints for derivation of candidate RfDs. The EPA's *Recommended Use of Body Weight[3/4] as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b) was used to allometrically scale a toxicologically equivalent dose of orally administered PFBS from animals to humans. Following the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), benchmark dose (BMD) modeling of thyroid and kidney effects following exposure to K^+PFBS resulted in a $BMDL_{20}$ human equivalent dose (HED) of 4.2 milligrams per kilogram per day (mg/kg-day) and a $BMDL_{10}$ HED of 11.5 mg/kg-day, respectively. The HEDs associated with the thyroid and kidney effects served as the point of departure (POD) for derivation of the candidate subchronic and chronic RfDs for each effect.

The candidate subchronic RfD for K^+PFBS associated with thyroid effects was calculated by dividing the POD_{HED} for decreased serum total T4 observed in newborn (PND 1) mice, conducted by Feng et al. (2017), by a composite uncertainty factor (UF_C) of 100 to account for extrapolation from mice to humans (an interspecies UF, or UF_A, of 3), for interindividual differences in human susceptibility (intraspecies UF, or UF_H, of 10), and for deficiencies in the toxicity database (database UF, or UF_D, of 3) (a value of 1 was applied for subchronic-to-chronic UF, or UF_S, and LOAEL-to-NOAEL UF, or UF_L) (see Table 9), yielding a candidate subchronic RfD of 4×10^{-2} mg/kg-day. As K^+PFBS is fully dissociated in water at the environmental pH range of 4−9, data for K^+PFBS were used to derive a subchronic RfD for the free acid (PFBS) by

adjusting for differences in molecular weight (MW) between K^+PFBS (338.19) and PFBS (300.10), yielding the same value of 4×10^{-2} mg/kg-day for the candidate subchronic RfD (thyroid effects) for PFBS (free acid).

The candidate subchronic RfD for K^+PFBS associated with kidney effects was calculated by dividing the POD$_{HED}$ for increased papillary epithelial tubular/ductal hyperplasia in P$_0$ female rats, conducted by Lieder et al. (2009b), by a composite uncertainty factor (UF$_C$) of 100 to account for extrapolation from rats to humans (an interspecies UF, or UF$_A$, of 3), for interindividual differences in human susceptibility (intraspecies UF, or UF$_H$, of 10), and for deficiencies in the toxicity database (database UF, or UF$_D$, of 3) (a value of 1 was applied for subchronic-to-chronic UF, or UF$_S$, and LOAEL-to-NOAEL UF, or UF$_L$) (see Table 10), yielding a candidate subchronic RfD of 1×10^{-1} mg/kg-day. As K^+PFBS is fully dissociated in water at the environmental pH range of 4−9, data for K^+PFBS were used to derive a subchronic RfD for the free acid (PFBS) by adjusting for differences in molecular weight (MW) between K^+PFBS (338.19) and PFBS (300.10), yielding the same value of 1×10^{-1} mg/kg-day for the candidate subchronic RfD (kidney effect) for PFBS (free acid).

The candidate chronic RfD for K^+PFBS associated with thyroid effects was calculated by dividing the POD$_{HED}$ for decreased serum total T4 observed in newborn (PND 1) mice, conducted by Feng et al. (2017), by a UF$_C$ of 300 to account for extrapolation from mice to humans (UF$_A$ of 3), for interindividual differences in human susceptibility (UF$_H$ of 10), and deficiencies in the toxicity database (UF$_D$ of 10) (a value of 1 was applied for UF$_S$ and UF$_L$) (see Table 12), yielding a chronic RfD of 1×10^{-2} mg/kg-day. Like the candidate subchronic RfD for thyroid, based on the data for K^+PFBS, a candidate chronic RfD for PFBS (free acid) of 1×10^{-2} mg/kg-day was derived.

The candidate chronic RfD for K^+PFBS associated with kidney effects was calculated by dividing the POD$_{HED}$ for increased papillary epithelial tubular/ductal hyperplasia in P$_0$ female rats, conducted by Lieder et al. (2009b), by a UF$_C$ of 1,000 to account for extrapolation from rats to humans (UF$_A$ of 3), for interindividual differences in human susceptibility (UF$_H$ of 10), to account for less than chronic-duration exposure (UF$_S$ of 10) and deficiencies in the toxicity database (UF$_D$ of 3) (a value of 1 was applied for UF$_L$) (see Table 15), yielding a candidate chronic RfD of 1×10^{-2} mg/kg-day. Like the candidate subchronic RfD for kidney, based on the data for K^+PFBS, a candidate chronic RfD for PFBS (free acid) of 1×10^{-2} mg/kg-day was derived.

Confidence in the Oral RfDs

The overall confidence in the candidate subchronic RfD for thyroid effects is medium. The gestational exposure study conducted by Feng et al. (2017) reports administration of K^+PFBS by gavage in pregnant Institute of Cancer Research (ICR) mice (10/dose) from gestation days (GDs) 1 to 20. This study was of good quality (i.e., high confidence) with adequate reporting and consideration of appropriate study design, methods, and conduct (click to see risk of bias analysis in HAWC). Confidence in the oral toxicity database for derivation of the candidate subchronic RfD is medium because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats, there are no studies available that have specifically evaluated neurodevelopmental effects

following PFBS exposure. Neurodevelopmental effects are of particular concern when perturbations in thyroid hormone occur during a sensitive early life stage, and the absence of a study evaluating neurodevelopmental effects following PFBS exposure is a source of uncertainty in the assessment.

The overall confidence in the candidate subchronic RfD for kidney effects is medium. The subchronic exposure study conducted by Lieder et al. (2009b) reports administration of K^+PFBS by gavage to Sprague-Dawley rats for 90 days. This study was of good quality (i.e., high confidence), was peer-reviewed, applied established approaches, recommendations, and best practices, and employed an appropriate exposure design for the evaluation of systemic toxicity endpoints (click to see risk of bias analysis in HAWC). Confidence in the oral toxicity database for derivation of the candidate subchronic RfD is medium because although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, and one acceptable two-generation reproductive toxicity study in rats, the database lacks studies that have specifically evaluated neurodevelopmental effects.

The overall confidence in the candidate chronic RfD for thyroid effects is low. While the RfD was derived using the same high-confidence principal study conducted by Feng et al. (2017) that was used for the candidate subchronic RfD, there is increased concern pertaining to the potential for identification of hazards following longer (i.e., chronic) duration PFBS exposures. Thus, due to the lack of a study evaluating neurodevelopmental effects and a chronic duration study, confidence in the database specifically for a candidate chronic RfD for thyroid is low.

The overall confidence in the candidate chronic RfD for kidney is low. While the RfD is derived using the same high confidence principal study conducted by Lieder et al. (2009b), as was used for the candidate subchronic RfD, there is increased concern pertaining to the potential for identification of hazards following longer (i.e., chronic) duration PFBS exposures. Thus, due to the lack of a study evaluating neurodevelopmental effects and a chronic duration study, confidence in the database specifically for a candidate chronic RfD for kidney is low.

Effects other than Cancer Observed Following Inhalation Exposure

There are no studies available that examine toxicity in humans or experimental animals following inhalation exposure, precluding the derivation of an inhalation reference concentration (RfC).

Evidence for Carcinogenicity

Under the EPA's *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the Agency concluded that there is "inadequate evidence to assess carcinogenic potential" for PFBS and K^+PFBS by either oral or inhalation routes of exposure. Therefore, the lack of data on the carcinogenicity of PFBS and the related compound K^+PFBS precludes the derivation of quantitative estimates for either oral (oral slope factor) or inhalation (inhalation unit risk) exposure.

1.0   Background

1.1   Physical and Chemical Properties

Perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service Registry Number [CASRN] 375-73-5)[1] and its related salt, potassium perfluorobutane sulfonate (K$^+$PFBS) (CASRN 29420-49-3), are members of the group of per- and polyfluoroalkyl substances (PFAS), more specifically the short-chain perfluoroalkane sulfonates. For purposes of this assessment, "PFBS" will signify the ion, acid, or any salt of PFBS. Concerns about PFBS 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 chemical formula of PFBS is $C_4HF_9O_3S$ and the chemical formula of K$^+$PFBS is $C_4F_9KO_3S$. Their respective chemical structures are presented in Figure 1. K$^+$PFBS differs from PFBS by being associated with a potassium ion. The reported water solubility of each species suggests that in aqueous environments, the sulfonate would be the predominant form. The preferential use of K$^+$PFBS in laboratory studies is related to the optimal dissociation of the salt to the sulfonate (i.e., PFBS) at pH ranging from 4 to 9 (see Table 1). Table 1 provides a table of physicochemical properties for PFBS and K$^+$PFBS.
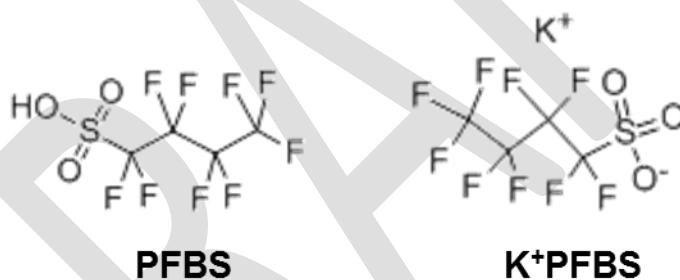
Figure 1. Chemical structures of PFBS and K$^+$PFBS.

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[1] The CASRN given is for linear PFBS; the source PFBS used in toxicity studies was assayed at ≥98% linear, suggesting some minor proportion of other chemicals, such as branched PFBS isomers, are present. Thus, observed health effects may apply to the total linear and branched isomers in a given exposure source.

Table 1. Physicochemical properties of PFBS (CASRN 375-73-5) and related compound K$^+$PFBS (CASRN 29420-49-3)

Property (unit)	Value	
	PFBS (free acid)	K$^+$PFBS (potassium salt)
Boiling point (°C)	80-211 (experimental)[b]	205-447 (predicted)[c]
Density (g/cm^3 at 71 °C)[a]	ND	ND
Vapor pressure (mm Hg at 20 °C)[a]	ND	9.15×10^{-8}
pH (unitless)[a]	ND	ND
Solubility in water (mg/L)[a]	56.6 at 24 °C	46.2 at 20 °C
Molecular weight (g/mol)[a]	300.09	338.19
Dissociation constant[a]	NA	Fully dissociated in water over the pH range of 4−9

Sources:
[a]NICNAS (2005).
[b]U.S. EPA Chemistry Dashboard for CASRN 375-73-5.
[c]U.S. EPA Chemistry Dashboard for CASRN 29420-49-3.
Notes: °C = degrees Celsius; g/cm3 = grams per cubic centimeter; g/mol = grams per mole; mm HG = millimeters of mercury; mg/l = milligrams per liter; NA = not applicable; ND = no data.

1.2   Occurrence

PFBS-based compounds are surfactants used primarily in the manufacture of paints, cleaning agents, and water- and stain-repellent products and coatings. They serve as replacements for perfluorooctane sulfonic acid (PFOS) (3M, 2002b). Various sources report their occurrence in environmental media and consumer products, including drinking water, ambient water, dust, carpeting and carpet cleaners, floor wax, and food packaging.

Oral exposure via drinking water might be expected in areas where contamination has been reported. EPA Unregulated Contaminant Monitoring Reporting data for public drinking water utilities in 2013−2015 showed levels of PFBS above the Minimum Reporting Level (> 0.09 micrograms per liter [µg/L]) in water systems serving Alabama, Colorado, Georgia, the Northern Mariana Islands, and Pennsylvania (U.S. EPA, 2017; Hu et al., 2016). These utilities included both ground and surface drinking water sources, with concentrations ranging from 0.09 to 0.37 µg/L. The estimated combined number of people served by these water systems is more than 340,000 (U.S. EPA, 2018).

Measurements from 37 surface water bodies in the northeastern United States (metropolitan New York area and Rhode Island) collected in 2014 showed an 85% site detection rate (Zhang et al., 2016). PFBS has also been identified in surface waters in Georgia, New Jersey, North Carolina, and the Upper Mississippi River Basin (Post et al., 2013; Lasier et al., 2011; Nakayama et al., 2010; Nakayama et al., 2007). It has been detected in wastewater treatment plant effluent, seawater, soil, and biosolids (Houtz et al., 2016; Zhao et al., 2012; Sepulvado et al., 2011).

PFBS contamination, which has been associated with the use of Aqueous Film-Forming Foams (AFFFs) (ESTCP, 2017; Anderson et al., 2016), was reported at Superfund sites and areas under assessment for Superfund designation. Contaminated sites include the former Wurtsmith Air Force Base, Ellsworth Air Force Base, and Dover Air Force Base (Aerostar SES LLC, 2017;

Anonymous, 2017; ASTSWMO, 2015). At the Wurtsmith site, PFBS was detected at a concentration of 6.4 µg/L in ground water contaminated by a PFAS plume originating from the fire training area (ASTSWMO, 2015). It is also present in some drinking water samples from nearby residential wells at low nanograms per liter concentrations, which were below the screening value cited by the Michigan Department of Community Health (MDCH, 2015). Other sources of PFAS and/or PFBS contamination include chrome plating operations, PFAS manufacture, and sites that use PFAS in product formulations such as textile and electronic industries.

PFBS has also been detected in household dust and consumer products. There was a 92% detection frequency for PFBS among 39 household dust samples (10 from the United States) analyzed with levels ranging from 86 nanograms per gram (ng/g) for the 25th percentile to 782 ng/g for the 75th percentile (Kato et al., 2009). In a separate study, PFBS dust levels were measured in Boston area offices (n = 31), homes (n = 30), and vehicles (n = 13) with detection frequencies being relatively low—10%, 3%, and 0%, respectively—and ranging in the low parts per billion (Fraser et al., 2013). Consumer products could also be an exposure source. Limited quantitative testing showed the presence of PFBS in carpet and upholstery protectors (45.8 and 89.6 ng/g), carpet shampoo (25.7 and 911 ng/g), textiles (2 ng/g), and floor wax (143 ng/g) purchased in the United States (Liu et al., 2014).

PFBS was detected in fast food packaging (7/20 samples) in one U.S. study (Schaider et al., 2017). The European Food Safety Authority reported the presence of PFBS in various food and drink items, including fruits, vegetables, cheese, and bottled water. For average adult consumers, the estimated exposure ranges for PFBS were 0.03−1.89 nanograms per kilogram per day (ng/kg-day) (minimum) to 0.10−3.72 ng/kg-day (maximum) (EFSA, 2012).

PFBS has been reported in serum of humans in the general population. In American Red Cross samples collected in 2015, 8.4% had a quantifiable serum PFBS concentration; the majority of samples were below the lower limit of quantitation (4.2 nanograms per milliliter [ng/mL]) (Olsen et al., 2017). The National Health and Nutrition Examination Survey (NHANES) 2013–2014 data reported the 95th percentile for PFBS at or below the level of detection (0.1 ng/mL). Another study with a lower limit of detection (0.013 ng/g) reported increasing levels of PFBS in serum from primiparous nursing women in Sweden from 1996 to 2010 (Glynn et al., 2012).

1.3   Toxicokinetics

1.3.1   Overview

Animal evidence has shown that PFBS, like other PFAS, is well absorbed following oral administration. PFBS distributes to all tissues of the body (Bogdanska et al., 2014), but a study evaluating the volume of distribution concluded that distribution is predominantly extracellular (Olsen et al., 2009). Because of its chemical resistance to metabolic degradation, PFBS is primarily eliminated unchanged in urine and feces.

Two sets of investigators have conducted toxicokinetic studies in rats and monkeys (Chengelis et al., 2009; Olsen et al., 2009). Olsen et al. (2009) also measured the half-life of PFBS in humans. More recently, Bogdanska et al. (2014) and Rumpler et al. (2016) have reported limited toxicokinetic information in mice.

Results of all studies discussed in this section are summarized in Table 2.

Table 2. Summary of toxicokinetics of serum PFBS (mean ± standard error)

Species/Sex	Study design	Elimination half-life (hr)	AUC (µg-hr/mL)	Clearance	Volume of distribution (L/kg)	Reference
Mice						
Mice/male	Single i.v. dose (30 or 300 mg/kg)	4.6[a]			0.4	Rumpler et al. (2016)
Mice/female	Single i.v. dose (30 or 300 mg/kg)	2.5[a]			0.4	Rumpler et al. (2016)
Rats						
Rats/male	Single i.v. dose (10 mg/kg)	2.1	254	0.0394 (L/hr-kg)	0.118	Chengelis et al. (2009)
	Single i.v. dose (30 mg/kg)	4.51 ± 2.22	294 ± 77	119 ± 34 (L/hr)[b]	0.330 ± 0.032	Olsen et al. (2009)
	Single oral dose (30 mg/kg)	4.68 ± 0.43	163 ± 10	NA	0.676 ± 0.055	Olsen et al. (2009)
Rats/female	Single i.v. dose (10 mg/kg)	0.64	32	0.311 (L/hr-kg)	0.288	Chengelis et al. (2009)
	Single i.v. dose (30 mg/kg)	3.96 ± 0.21	65 ± 5	469 ± 40 (L/hr)[b]	0.351± 0.034	Olsen et al. (2009)
	Single oral dose (30 mg/kg)	7.42 ± 0.79	85 ± 12	NA	0.391± 0.105	Olsen et al. (2009)
Monkeys[c]						
Cynomolgus macaque/male	Single i.v. dose (10 mg/kg)	15 (9.65)[d]	1,115 ± 859	0.016 (L/hr-kg)	0.209 ± 0.028	Chengelis et al. (2009)
	Single i.v. dose (10 mg/kg)	95.2 ± 27.1	24.3 ± 8.6	511 ± 141 (mL/hr)	0.254 ± 0.031	Olsen et al. (2009)
Cynomolgus macaque/female	Single i.v. dose (10 mg/kg)	8.1	489 ±180	0.0229 ± 0.0099 (L/hr-kg)	0.248 ± 0.045	Chengelis et al. (2009)
	Single i.v. dose (10 mg/kg)	83.2 ± 41.9	35.4 ± 13.3	368 ± 120 (mL/hr)	0.255 ± 0.017	Olsen et al. (2009)
Humans						
Males and females	NA	27.7 ± 4.53 days	NA	NA	NA	Olsen et al. (2009)

Notes: AUC = area under the curve; hr = hour; i.v. = intravenous; L/hr-kg = liters per hour per kilogram; L/kg = liter per kilogram; mL/hr = milliliters per hour; µg-hr/mL = micrograms per hour per milliliter; NA = not available.

[a]Rumpler et al. (2016) is a published abstract only.
[b]Body weights were reported to be 0.200−0.250 kg (approximately 476 L/kg-hour).
[c]The data were monitored 48 hours and 31 days postdosing for Chengelis et al. (2009) and Olsen et al. (2009), respectively.
[d]One male monkey had a serum concentration more than tenfold higher than the others at 48 hours postdosing with an estimated half-life of 26 hours.

1.3.2   Absorption

Olsen et al. (2009) conducted intravenous (i.v.) and oral uptake studies in rats (three male and female) that were given a single oral dose (30 milligrams per kilogram [mg/kg]) of potassium PFBS (K^+PFBS). The serum area under the concentration curve (AUC) after i.v. was 294 ± 77 and 65 ± 5 (µg-h/mL) in male and female rats, respectively, and 163 ± 10 and 85 ± 12 in males and females, respectively, after oral dosing. The large variance in AUC for male rats after i.v. dosing and greater AUC after oral dosing compared to i.v. dosing in females makes it difficult to interpret these results with certainty. Peak concentrations occurred at $0.3-0.4$ hours after oral dosing, showing that absorption was fairly rapid.

1.3.3   Distribution

In a published abstract, Rumpler et al. (2016) evaluated the pharmacokinetic properties of PFBS in CD-1 mice at 8 weeks of age. Male and female mice were given a single dose of 0, 30, or 300 mg/kg PFBS via gavage. Liver and kidney were harvested 24 hours postdosing. PFBS did not accumulate in either liver or kidney, and the volume of distribution was 0.4 liter per kilogram [L/kg].

Olsen et al. (2009) estimated volumes of distribution for K^+PFBS as 0.7 and 0.4 L/kg in male and female rats, respectively, and 0.25 L/kg in cynomolgus macaques, and concluded that K^+PFBS is primarily distributed in the extracellular space.

Bogdanska et al. (2014) characterized the tissue distribution of ^{35}S-labeled PFBS in male C57BL/6 mice. Animals (3/group) were exposed for either 1, 3, or 5 days to an average of 16 mg of PFBS/kg/day in the diet. Following 1, 3, and 5 days of exposure, total estimated recovery of PFBS from all tissues evaluated was 10%, 5%, and 3.4% of the ingested dose, respectively. The declining recovery with time reflects the lack of accumulation in tissues after the first few days, with continued elimination in the urine. The study authors suggest that these low recovery rates most likely reflect rapid excretion of PFBS and/or potentially limited uptake of the compound, but the results of Rumpler et al. (2016) and Olsen et al. (2009) suggest that limited tissue distribution is also a factor.

Bogdanska et al. (2014) found that blood levels of PFBS did not change when comparing values observed after 1 and 5 days of exposure. As with PFOS, PFBS was found to distribute to most of the 20 tissues examined at all exposure durations, but the levels of PFBS were significantly lower (fivefold to fortyfold lower) than those of PFOS in tissues after similar exposure to PFOS, especially in liver and lungs (Bogdanska et al., 2014). These differences might be attributed to chain length-dependent active transport of perfluorinated chemicals (Weaver et al., 2010). Excluding stomach and fat tissue, PFBS tissue levels increased between 1 and 3 days of exposure, but there were no significant changes in tissue levels between 3 and 5 days of exposure in any tissue examined. Similar to PFOS, whole bone, liver, blood, skin, and muscle accounted for approximately 90% of the recovered PFBS at all time points. The highest tissue concentrations outside of blood, however, were found in liver, GI tissues, kidney, and cartilage. The significant total mass found in muscle and skin was due to the total volume of these tissues as much as the concentration in them. The liver contained the highest tissue concentration of PFBS at all time points, while the brain contained the lowest.

Human studies were not available on lactational or transplacental transfer of PFBS, but developmental studies in animals indicate the potential for effects in offspring following gestational exposure (Feng et al., 2017; York, 2003a, 2002).

1.3.4 Metabolism

There is no evidence of biotransformation of PFBS. It is expected that PFBS, a short-chain (C4) of perfluoroalkyl acids (PFAAs), is metabolically inert because of the chemical stability that also exists in the longer chain PFAA chemicals, including perfluorohexane sulfonic acid (PFHxS) (C6), PFOS (C8), and perfluorooctanoic acid (PFOA) (C8).

1.3.5 Elimination

To facilitate comparison of differing studies for a given species, results for elimination are organized by species.

1.3.5.1 Mice

Rumpler et al. (2016) dosed male and female CD-1 mice with 0, 30, or 300 mg/kg PFBS via gavage. Trunk blood was collected at 0.5, 1, 2, 4, 8, 16, 24, and 48 hours and urine at 24 hours after dosing. The half-life of PFBS was estimated to be 4.6 hours in the male mice and 2.5 hours in the females. Within 24 hours, more than 95% of the serum PFBS was excreted into urine.

1.3.5.2 Rats

Chengelis et al. (2009) conducted a single-dose pharmacokinetic study in Sprague-Dawley (S-D) rats, designed to compare the toxicokinetic behavior of PFBS to that of perfluorohexanoic acid (PFHxA), another PFAA. In this study, 12 male and 12 female rats were each administered a bolus dose of PFBS (10 mg/kg) via i.v. injection. Blood samples were collected from three animals per sex at 0.5, 1, 1.5, 2, 4, 8, and 24 hours after dose administration. Additionally, to determine urinary excretion, three animals per sex were housed in metabolic cages following dose administration and urine was collected over the following time intervals: 0−6, 6−12, and 12−24 hours postdosing. Female rats had an approximately threefold shorter mean elimination half-life of PFBS in serum (0.64 hours) than male rats (2.1 hours). This could be in part due to the difference in clearance and volume of distribution; the mean apparent clearance of PFBS from the serum was approximately eightfold higher for female rats (0.311 L/h/kg) than for male rats (0.0394 L/h/kg) and the mean apparent volume of distribution for PFBS in the serum was approximately 2.4-fold higher for female rats (0.288 L/kg) than for male rats (0.118 L/kg). Approximately 70% of the administered dose of PFBS was recovered in the urine during 24 hours postdosing regardless of sex. Using the urine data, the mean half-life values for male rats and female rats were determined to be 3.1 and 2.4 hours, respectively; the finding of longer urinary half-lives in males is consistent with those observed for serum half-lives.

Olsen et al. (2009) evaluated the elimination of PFBS in S-D rats after i.v. and oral exposure to K^+PFBS. The mean terminal serum elimination half-lives following i.v. administration of 30 mg/kg K^+PFBS were 4.51 ± 2.22 hours for males and 3.96 ± 0.21 hours for females. Although there was not a statistically significant difference between the terminal serum half-lives in male and female rats, there was a statistically significant difference in the urinary clearance rates ($p \leq 0.01$), with female rats (469 ± 40 mL/hour) having faster clearance rates than male rats (119 ± 34 mL/h). (Since clearance [CL] is calculated from the ratio of the volume of distribution

[Vd] to the half-life [t1/2], CL = 0.693*Vd/t1/2, differences in Vd can lead to differences in CL, even when t1/2 is similar between comparison groups.) For rats receiving an oral dose, terminal serum K⁺PFBS elimination half-lives were significantly different ($p \leq 0.05$) for males (t1/2 = 4.68 ± 0.43 hours) versus females (t1/2 = 7.42 ± 0.79 hours). Thus, the half-life estimates of Olsen et al. (2009) (4−7.5 hours) are roughly twice those estimated by Chengelis et al. (2009) based on urine data (2.4 and 3.1 hours); there is more of a difference with the values estimated by Chengelis et al. (2009) from serum data (0.64 and 2.1 hours).

1.3.5.3   Monkeys

Similar to their study in rats, Chengelis et al. (2009) investigated the toxicokinetic profile of PFBS through a series of experiments in the cynomolgus macaque (*Macaca fascicularis*). Monkeys (three males and three females) were each administered a bolus i.v. dose of 10 mg/kg PFBS. The controlled exposure to PFBS occurred 7 days after the same animals were each administered a bolus dose of PFHxA (10 mg/kg). Blood samples were collected at 0 hours (immediately prior to dosing) and at 1, 2, 4, 8, 24, and 48 hours after dose administration and were analyzed to determine PFBS concentration in serum. Only a single clearance half-life was estimated. The estimated half-life of PFBS in serum ranged from 5.8 to 26.0 hours in this experiment, and the median half-life was 9.55 hours for the six animals.

The study of Chengelis et al. (2009) indicated that, under conditions of equivalent exposure, the areas under the serum concentration-time curves (AUCs) were lower and the elimination half-lives were shorter for PFHxA than those for PFBS in both S-D rats and cynomolgus macaques. In the monkeys, for instance, PFHxA was cleared more rapidly and resulted in a lower AUC value (approximately an order of magnitude lower) with a shorter terminal half-life (2.4−5.3 hours, data not shown in the study) than PFBS at an equivalent dose (i.v. dose at 10 mg/kg).

Olsen et al. (2009) also evaluated the elimination of PFBS (specifically, K⁺PFBS) in cynomolgus macaques after i.v. dosing. A significant difference in design from the study of Chengelis et al. (2009) is that Olsen et al. (2009) followed PFBS elimination for 31 days in monkeys (versus 48 hours), allowing Olsen and colleagues to identify both an initial clearance half-life and a terminal phase-half-life. Olsen et al. (2009) did not observe statistically significant sex-related differences in half-life or clearance between male and female monkeys, unlike those observed in rats. In monkeys, the mean terminal serum elimination half-lives, after i.v. administration of 10 mg/kg K⁺PFBS, were 95 ± 27 hours in males and 83 ± 42 hours in females.

The serum half-life data in Olsen et al. (2009) clearly show a slow elimination phase in monkeys that does not begin until 4−10 days after dosing. Chengelis et al. (2009) followed elimination for only 48 hours, hence could not have observed this terminal clearance phase. The initial elimination half-life (t0.5β) estimated by Olsen et al. (2009) in monkeys—13 hours for males, 11 hours for females—is essentially identical to the values estimated by Chengelis et al. (2009)—10 or 15 hours for males (without/with outlier) and 8 hours in females. Hence the two studies appear consistent in identifying an initial elimination half-life, but the difference in design precluded Chengelis and colleagues from identifying the longer (terminal) half-life of PFBS.

1.3.5.4   Humans

In addition to their experimental studies in rats and monkeys, Olsen et al. (2009) evaluated the elimination of human serum K$^+$PFBS in a group of workers with occupational exposure, with serum concentrations measured up to 180 days after cessation of further K$^+$PFBS work-related activity. Given that the workers had been occupationally exposed, distribution into the tissues is expected to have been complete before the observations began. Among the six subjects (five male, one female), the geometric mean serum elimination half-life for K$^+$PFBS was 25.8 days (95% confidence interval = 16.6−40.2 days). Urine appeared to be a major route of elimination based on observed levels of PFBS in urine in the human study.

1.3.6   Summary

Collectively, elimination half-lives appear to be similar for mice and rats, with potential sex-specific toxicokinetic differences being reported (i.e., females appearing to have a faster elimination rate). Humans have a longer serum elimination half-life (~weeks) than both rodents (~hours) and monkeys (~days).

2.0 Problem Formulation

2.1 Conceptual Model

A conceptual model was developed to summarize the availability of data to understand potential health hazards related to exposure to PFBS and/or K$^+$PFBS. The potential sources of these chemicals, the routes of exposure for biological receptors of concern (e.g., various human activities related to ingested drinking water, and food preparation and consumption), the potential assessment endpoints (e.g., effects such as developmental toxicity), and adverse health effects in the populations at risk due to exposure to PFBS and/or potassium salt are depicted in the conceptual diagram in Figure 2.
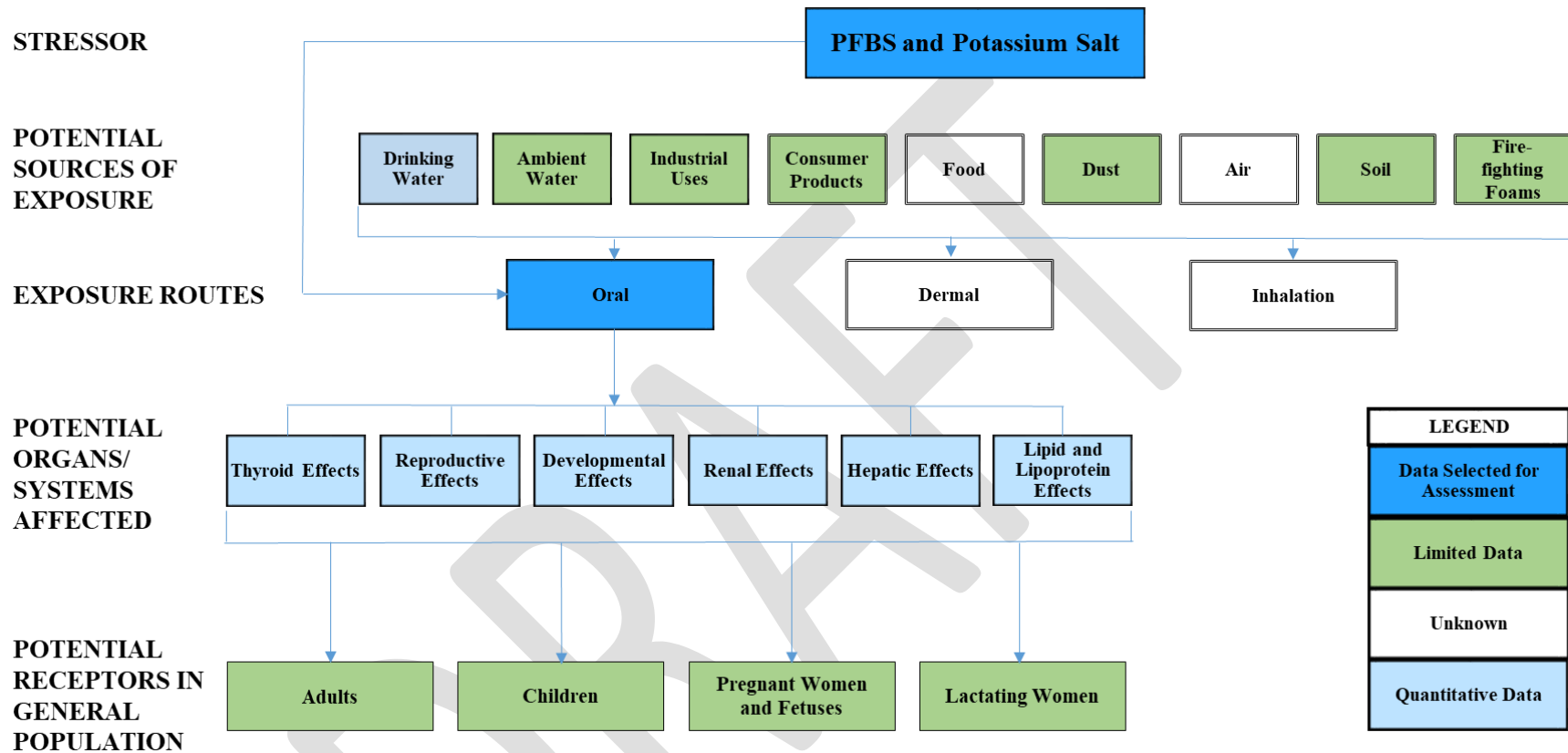
Figure 2. Conceptual model for PFBS and/or potassium salt.

2.2   Objective

The overall objective of this assessment is to provide the health effects basis for the development of oral reference doses (RfDs) for PFBS (CASRN 375-73-5) and a related compound, K⁺PFBS (CASRN 29420-49-3), including the science-based decisions providing the basis for identification of potential human health effects and estimating PODs. Based on the needs of the EPA partner Program Offices, Regions, States, and/or Tribes as they pertain to diverse exposure scenarios and human populations, subchronic and chronic RfDs have been derived. The assessment includes studies and information previously provided in the 2014 Provisional Peer-Reviewed Toxicity Value assessment (U.S. EPA, 2014f) and builds upon the amount of literature containing studies published since that review.

2.3   Methods

2.3.1   Literature Search

Four online scientific databases (PubMed, Web of Science, Toxline, and TSCATS via Toxline) were searched by the EPA's Health and Environmental Research Online (HERO) staff and stored in the HERO database.[2] The literature search focused on chemical name and synonyms with no limitations on publication type, evidence stream (i.e., human, animal, *in vitro*, and *in silico*), or health outcomes. Full details of the search strategy for each database are presented in appendix A. The initial database searches were conducted on July 18, 2017 and last updated on February 28, 2018. Studies were also identified from other sources relevant to PFBS, including studies submitted to the EPA by the manufacturer of PFBS (i.e., 3M) as part of TSCA premanufacture notices for other PFAS chemicals or as required under Toxic Substances Control Act (TSCA) reporting requirements and studies referenced in prior evaluations of PFBS toxicity (MDH, 2017; ATSDR, 2015). In addition, on March 29, 2018, the National Toxicology Program (NTP) published study tables and individual animal data from a 28-day toxicity study of PFBS (http://doi.org/10.22427/NTP-DATA-002-01134-0003-0000-4). Although a peer-reviewed NTP Technical Report for the PFBS study is not yet available, this information was included in the assessment because these data have undergone standard NTP quality assurance/control processing and are publicly available. A protocol outlining the NTP study methods is available in HERO (https://hero.epa.gov/hero/index.cfm/reference/details/reference_id/4309741) (NTP, 2011). During the process of deriving toxicity values, the EPA conducted further quantitative analyses (e.g., BMD modeling) beyond what was reported by the NTP.

2.3.2   Screening Process

Two screeners independently conducted a title and abstract screening of the search results using DistillerSR[3] to identify study records that met the Population, Exposure, Comparator, Outcome (PECO) eligibility criteria (see appendix B for a more detailed summary):

- Population: Human and nonhuman mammalian animal species (whole organism) of any life stage and *in vitro* models of genotoxicity.

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[2]The EPA's Health and Environmental Research Online (HERO) database provides access to the scientific literature behind EPA science assessments. The database includes more than 2,500,000 scientific references and data from the peer-reviewed literature used by the EPA to develop its regulations.

[3]DistillerSR is a web-based systematic review software used to screen studies available at https://www.evidencepartners.com/products/distillersr-systematic-review-software.

- Exposure: Any qualitative or quantitative estimates of exposure of PFBS or K$^+$PFBS, via oral or inhalation routes of exposure.
- Comparator: A comparison or reference population exposed to lower levels or for shorter periods of time for humans. Exposure to vehicle-only or untreated control in animals.
- Outcome: Any examination of cancer or noncancer health outcomes.

In addition to the PECO criteria, the following additional exclusion criteria were applied, although these study types were tracked as supplemental material as described following the exclusion criteria:

- Records that do not contain original data such as other agency assessments, scientific literature reviews, editorials, and commentaries;
- Abstract only (e.g., conference abstracts); and
- Retracted studies.

Records that were not excluded based on title and abstract screening advanced to full-text review using the same PECO eligibility criteria. Studies that have not undergone peer review were included if the information could be made public and sufficient details of study methods and findings were included in the reports. Full-text copies of potentially relevant records identified from title and abstract screening were retrieved, stored in the HERO database, and independently assessed by the screeners using DistillerSR to confirm eligibility. At both title/abstract and full-text review levels, screening conflicts were resolved by discussion between the primary screeners in consultation with a third reviewer to resolve any remaining disagreements. During title/abstract or full-text level screening, studies that were not directly relevant to the PECO, but could provide supplemental information, were categorized (or "tagged") by the type of supplemental information they provided (e.g., review, commentary, or letter with no original data; conference abstract; toxicokinetics; mechanistic information aside from *in vitro* genotoxicity studies; other routes of exposure; exposure only). Conflict resolution was not required during the screening process to identify supplemental information (i.e., tagging by a single screener was sufficient to identify the study as potential supplemental information).

2.3.3 Study Evaluation

Study evaluation was conducted by one reviewer for epidemiological studies and by two independent reviewers for animal studies using the EPA's version of Health Assessment Workspace Collaborative (HAWC), a free and open source web-based software application designed to manage and facilitate the process of conducting literature assessments.[4] For pragmatic purposes, only one reviewer was considered necessary for epidemiological studies because it was apparent during literature screening that the animal evidence would be most informative for deriving toxicity values. The available outcomes in the epidemiological studies were heterogeneous and unrelated to each other, and only a single study was available for each outcome. This approach is consistent with recommendations from the National Academies of Science encouraging the EPA to explore ways to make systematic review more feasible, including a "rapid review in which components of the systematic review process are simplified

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[4]HAWC: A Modular Web-Based Interface to Facilitate Development of Human Health Assessments of Chemicals. https://hawcproject.org/.

or omitted (e.g., the need for two independent reviewers)" ([NASEM, 2017](#)). Study evaluation was not conducted for studies tagged as supplemental information during screening.

The general approach for evaluating epidemiology and animal toxicology was the same (see Figure 3), but the specifics of applying the approach differed. These evaluations were focused on the methodological approaches and completeness of reporting in the individual studies, rather than on the direction or magnitude of the study results. Evaluation of epidemiology studies was conducted for the following domains: exposure measures, outcome measures, participant selection, confounding, analysis, sensitivity, and selective reporting. For animal studies, the evaluation process focused on assessing aspects of the study design and conduct through three broad types of evaluations: reporting quality, risk of bias, and study sensitivity. A set of domains with accompanying core questions fall under each evaluation type and directed individual reviewers to evaluate specific study characteristics. For each domain evaluated for experimental animal studies—reporting quality, selection or performance bias, confounding/variable control, reporting or attrition bias, exposure methods sensitivity, and outcome measures and results display—basic considerations provided additional guidance on how a reviewer might evaluate and judge a study for that domain. Core and prompting questions used to guide the criteria and judgment for each domain are presented in appendix C. Key concerns for the review of epidemiology and animal toxicology studies are potential sources of bias (factors that could systematically 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).

For each study in each evaluation domain, reviewers reached a consensus rating regarding the utility of the study for hazard identification, with categories of *good*, *adequate*, *deficient*, *not reported,* or *critically deficient*. These ratings were then combined across domains to reach an overall classification of *high*, *medium*, or *low confidence* or *uninformative* (definitions of these classifications are available in appendix C). The rationale for the classification, including a brief description of any identified strengths and/or limitations from the domains and their potential impact on the overall confidence determination, is documented and retrievable in HAWC. Uninformative studies were not used in evidence synthesis or dose-response analysis. Studies were evaluated for their suitability for each health outcome investigated and could receive different ratings for each outcome.

For epidemiological studies, exposure-specific criteria were developed prior to evaluation and are described in detail in appendix C. In brief, standard analytical methods of measurement of PFBS in serum or whole-blood using quantitative techniques such as liquid chromatograph-triple quadrupole mass spectrometry and high-pressure liquid chromatography with tandem mass spectrometry were preferred. In addition, exposure must have been assessed in a relevant time-window for development of the outcome.

Individual study level domains

Animal	Epidemiological
Reporting Quality	Exposure measurement
Selection or Performance Bias	Outcome ascertainment
Confounding/Variable Control	Population Selection
Reporting or Attrition Bias	Confounding
Exposure Methods Sensitivity	Analysis
Outcome Measures and Results Display	Sensitivity
	Selective reporting

Domain judgements

	Judgement	Interpretation
🟢	Good	Appropriate study conduct relating to the domain & minor deficiencies not expected to influence results.
🟢	Adequate	A study that may have some limitations relating to the domain, but they are not likely to be severe or to have a notable impact on results.
🟡	Deficient	Identified biases or deficiencies interpreted as likely to have had a notable impact on the results or prevent reliable interpretation of study findings.
🔴	Critically Deficient	A serious flaw identified that is interpreted to be the primary driver of any observed effect or makes the study uninterpretable. Study is not used without exceptional justification.

Overall study rating

Rating	Interpretation
High	No notable deficiencies or concerns identified; potential for bias unlikely or minimal; sensitive methodology.
Medium	Possible deficiencies or concerns noted, but resulting bias or lack of sensitivity would be unlikely to be of a notable degree.
Low	Deficiencies or concerns were noted, and the potential for substantive bias or inadequate sensitivity could have a significant impact on the study results or their interpretation.
Uninformative	Serious flaw(s) makes study results unusable for hazard identification

Figure 3. Approach for evaluating epidemiological and animal toxicology studies.

2.3.4   Data Extraction

Information on study design, methods, results, and data from animal toxicology studies were extracted into the HAWC and are available at https://hawcprd.epa.gov/assessment/100000037/. Visual graphics prepared from HAWC are embedded as hyperlinks and are fully interactive when viewed online by way of a "click to see more" capability. Clicking on content allows access to study evaluation ratings, methodological details, and underlying study data. The action of clicking on content contained in those visual graphics (e.g., data points, endpoint, and study design) will yield the underlying data supporting the visual content. *NOTE. The following browsers are fully supported for accessing HAWC: Google Chrome (preferred), Mozilla Firefox, and Apple Safari. There are errors in functionality when viewed with Internet Explorer.* Study methods and findings from epidemiological studies were described in narratives given the small size and heterogeneity of the evidence base. Data extraction was performed by one member of the evaluation team and checked by one to two other members. Any discrepancies in data extraction were resolved by discussion or consultation with a third member of the evaluation team. Digital rulers such as WebPlotDigitizer and Grab It (https://automeris.io/WebPlotDigitizer/ and https://grab-it.soft112.com/, respectively) were used to extract numerical information from figures. Use of digital rulers was documented during extraction. Dose levels were extracted as reported in the study and converted to milligrams per kilogram per day(mg/kg-day) human equivalent dose (HED) for endpoints that were considered for use in the dose-response and derivation of toxicity values.

2.3.5 Evidence Synthesis

For the purposes of this assessment, after study evaluation, the informative evidence for each outcome was summarized from the available human studies and, separately, the available animal studies. This synthesis provides a short synopsis of the breadth of data available to inform each outcome and summarizes information on the general study design, doses tested, outcomes evaluated, and results for the endpoints of interest within each study. While the evidence synthesis describes inferences about the methodological rigor and sensitivity of the individual studies (i.e., study confidence) and discusses the pattern and magnitude of the experimental findings within studies, it does not include conclusions drawn across the sets of studies (see "Evidence Integration and Hazard Characterization," next).

2.3.6 Evidence Integration and Hazard Characterization

In this assessment, the evaluation of the available evidence from informative human and animal studies was described in an evidence integration narrative for each outcome, including overall evidence integration judgments as to whether the data provide evidence sufficient to support a hazard. These integrated judgments serve to characterize the extent of the available evidence for each outcome, including information on potential susceptible populations and life stages, as well as important uncertainties in the interpretation of the data.

The evidence integration for each health effect considered aspects of an association that might suggest causation first introduced by Austin Bradford Hill (Hill, 1965), including the consistency, exposure-response relationship, strength of association, biological plausibility, and coherence of the evidence. This involved weighing the PFBS-specific human and animal evidence relating to each of these considerations within or across studies, including both evidence that supported causation as well as evidence that indicated lack of support. For example, the evaluation of consistency examined the similarity of results across studies (e.g., direction and magnitude). When inconsistencies across studies were identified, the evaluation considered whether results were "conflicting" (i.e., unexplained positive and negative results in similarly exposed human populations or in similar animal models) or "differing" (i.e., mixed results explained by differences between human populations, animal models, exposure conditions, or study methods), based on analyses of potentially important explanatory factors such as confidence in studies' results (the results of higher confidence studies were emphasized), exposure levels or duration, or differences in populations or species (including potential susceptible groups) across studies (U.S. EPA, 2005). While consistent evidence across studies increases support for hazard, unexplained inconsistency or conflicting evidence decreases support for hazard. The evaluations of these considerations were informed by EPA guidelines, including *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a) and *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996b).

The overall evidence integration judgments were developed using a structured framework based on evaluation of the considerations above (see Table 3). Using this framework, the human and animal evidence for each health effect was judged separately as *supports a hazard*, *equivocal*, or *supports no hazard*. Evidence integration judgments of *supports a hazard* span a range of supportive evidence bases that can be further differentiated by the quantity and quality of information available to rule out alternative explanations for the results. *Equivocal* evidence is limited in terms of the quantity, consistency, or confidence level of the available studies and

serves to encourage additional research. *Supports no hazard* requires several high-confidence studies across potentially susceptible populations with consistent null results; this judgment was not reached in this assessment. Overall evidence integration judgments were drawn across the human and animal conclusions, considering the available information on the human relevance of findings in animals. Thus, for example, evidence in animals that *supports a hazard* alongside *equivocal* human evidence in the absence of information indicating that the responses in animals are unlikely to be relevant to humans would result in an overall judgment of *supports a hazard* for that outcome.

Table 3. Criteria for overall evidence integration judgments

	Animal	**Human**
Supports a hazard	The evidence for effects is consistent or largely consistent in at least one high- or medium-confidence experiment.[a] Although notable uncertainties across studies might remain, any inconsistent evidence or remaining uncertainties are insufficient to discount the cause for concern from the positive experiments. In the strongest scenarios, the set of experiments provide evidence supporting a causal association across independent laboratories or species. In other scenarios, including evidence for an effect in a single study, the experiment(s) demonstrate additional support for causality such as coherent effects across multiple related endpoints; an unusual magnitude of effect, rarity, age at onset, or severity; a strong dose-response relationship; and/or consistent observations across exposure scenarios (e.g., route, timing, or duration), sexes, or animal strains.	One or more high- or medium-confidence independent studies reporting an association between the exposure and the health outcome. In general, the study results are largely consistent or any inconsistent results are not sufficient to discount the cause for concern from the higher confidence study or studies, and there is reasonable confidence that alternative explanations, including chance, bias, and confounding, have been ruled out. In situations in which only a single study is available, the results of multiple studies are heterogeneous, or alternative explanations, including chance, bias and confounding, have not been ruled out, there is additional supporting evidence such as associations with biologically related endpoints in other human studies (coherence), large estimates of risk, or strong evidence of an exposure-response within or across studies.
Equivocal	The evidence is generally inadequate to determine hazard. This includes a lack of relevant studies available or a set of low-confidence experiments. It also includes scenarios with a set of high- or medium-confidence experiments that are not reasonably consistent or not considered informative to the hazard question under evaluation. This category would also include a single high- or medium-confidence experiment with weak evidence of an effect (e.g., changes in one endpoint among several related endpoints, and without additional evidence supporting causality).	The evidence is considered inadequate to describe an association between exposure and the health outcome with confidence. This includes a lack of studies available in humans, only low-confidence studies, or considerable heterogeneity across medium- or high-confidence studies. This also includes scenarios in which there are serious residual uncertainties across studies (these uncertainties typically relate to exposure characterization or outcome ascertainment, including temporality) in a set of largely consistent medium- or high-confidence studies.

	Animal	**Human**
Supports no hazard	A set of high-confidence experiments examining the full spectrum of related endpoints within a type of toxicity, with multiple species, and testing a reasonable range of exposure levels and adequate sample size in both sexes, with none showing any indication of effects. The data are compelling in that the experiments have examined the range of scenarios across which health effects in animals could be observed, and an alternative explanation (e.g., inadequately controlled features of the studies' experimental designs) for the observed lack of effects is not available. The experiments were designed to specifically test for effects of interest, including suitable exposure timing and duration, post-exposure latency, and endpoint evaluation procedures, and to address potentially susceptible populations and life stages.	Several high-confidence studies, showing consistently null results (e.g., an odds ratio of 1.0) ruling out alternative explanations including chance, bias, and confounding with reasonable confidence. Each of the studies should have used an optimal outcome and exposure assessment and adequate sample size (specifically for higher exposure groups and for sensitive populations). The set as a whole should include the full range of levels of exposures that human beings are known to encounter, an evaluation of an exposure response gradient, and at-risk populations and life stages and should be mutually consistent in not showing any indication of effect at any level of exposure.

Note:
ᵃ "Experiment" refers to measurements in a single population of exposed animals (e.g., a study that included separate evaluations of rats and of mice, or separate cohorts exposed at different life stages, would be considered as multiple experiments). Conversely, two papers or studies that report on the same cohort of exposed animals (e.g., examining different endpoints) would not be considered separate experiments.

The primary evidence and rationale supporting these decisions were summarized in a single evidence profile table to transparently convey the aspects of the evidence that were considered to increase or decrease the hazard support for each health effect. For the purposes of this assessment, only the integrated evidence that *supports a hazard* was considered for use in the dose-response and derivation of toxicity values.

2.3.7 *Derivation of Values*

Development of the dose-response assessment for PFBS and/or the potassium salt has followed the general guidelines for risk assessment put forth by the National Research Council (NRC, 1983) and the EPA's *Framework for Human Health Risk Assessment to Inform Decision Making* (U.S. EPA, 2014b). Other EPA guidelines and reviews considered in the development of this assessment include the following:

- *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002).
- *A Framework for Assessing Health Risks of Environmental Exposures to Children* (U.S. EPA, 2006).
- *Exposure Factors Handbook* (U.S. EPA, 2011a).
- *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b).
- *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012).
- *Child-Specific Exposure Scenarios Examples* (U.S. EPA, 2014a).

The EPA's *A Review of the Reference Dose and Reference Concentration Processes* document describes a multistep approach to dose–response assessment, including analysis in the range of observation followed by extrapolation to lower levels (U.S. EPA, 2002). As described above, prior to deriving toxicity values, the EPA conducted a comprehensive evaluation of available human epidemiological and animal toxicity studies to identify potential health hazards and associated dose-response information through the literature search and screening, study evaluation, evidence synthesis, and evidence integration steps. This evaluation informed the selection of candidate key studies and critical effects for dose-response analysis, from which the EPA identified a critical effect and point of departure (POD) for subchronic and chronic reference value derivation and extrapolated a selected POD to a corresponding RfD (e.g., subchronic RfD). For dose-response analysis of PFBS and/or the potassium salt, the EPA used the BMD approach to identify a POD. The steps for deriving an RfD using the BMD approach are summarized below.

- **Step 1: Evaluate the data to identify and characterize endpoints related to exposure to PFBS chemicals.** This step involved determining the relevant studies and adverse effects to be considered for BMD modeling. Once the appropriate data were collected, evaluated for study quality, and characterized for adverse outcomes, endpoints were selected that were judged to be relevant (i.e., for the purposes of this assessment, effects that were sufficient to *support a hazard*) and sensitive as a function of dose (typically defined by the no observed adverse effect level [NOAEL] value). In this assessment, these decisions were directly informed by the evidence integration judgments arrived at for each assessed health outcome. Some of the most important considerations that influenced selection of endpoints for BMD modeling include data with dose-response, percent change from controls, adversity of effect, and consistency across studies. For PFBS, kidney, thyroid, and developmental endpoints were considered for toxicity value derivations.

- **Step 2: Convert the adjusted daily doses to an HED.** The adjusted daily doses were converted to HEDs using the EPA's *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b). Study-reported body weight (BW) was used when available; otherwise, default animal BW information was retrieved from U.S. EPA (1988).

- **Step 3: Select the benchmark response (BMR) level.** Using the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), the endpoints selected were modeled. The BMR is a predetermined change in the response rate of an adverse effect. It serves as the basis for obtaining the benchmark dose lower confidence limit (BMDL), which is the 95% lower bound of the BMD. BMRs were identified and applied consistent with quantal and continuous data and, when possible, informed by understanding of biological significance.

- **Step 4: BMD Model the data.** This step involved fitting a statistical model to the dose-response data that describes the data set of the identified adverse effect. Typically, this involved selecting a family or families of models (e.g., polynomial continuous, hill continuous, or exponential continuous) for further consideration based on the data and experimental design. In this step, a BMDL was derived by placing confidence limits (one- or two-sided) and a confidence level (typically 95%) on a BMD to obtain the dose that ensures with high confidence that the BMR is not exceeded.

- **Step 5: Determine a POD$_{HED}$.** If modeling was feasible, the estimated BMDL(HED)s were used as PODs (i.e., POD$_{HED}$). If dose-response modeling was not feasible, NOAEL (HED)s or lowest observed adverse effect level (LOAEL) (HED)s were identified.
- **Step 6: Provide rationale for selecting Uncertainty Factors (UFs).** UFs were selected in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the key study compared to a lifetime of the species studied, and the potential limitations of the toxicology database.
- **Step 7: Calculate the subchronic and chronic RfDs.** The RfDs were calculated by dividing a POD$_{HED}$ by the selected UFs.

$$\text{RfD} \quad = \quad \frac{\text{POD}_{HED}}{\text{UF}_C}$$

where:

POD$_{HED}$ = The POD$_{HED}$ is calculated from the BMDL or NOAEL using a BW$^{3/4}$ allometric scaling approach consistent with EPA guidance ([U.S. EPA, 2011b](#))

UF$_C$ = Composite UF established in accordance with EPA guidelines considering variations in sensitivity among humans, differences between animals and humans, the duration of exposure in the key study compared to a lifetime of the species studied, and the potential limitations of the toxicology database.

3.0 Overview of Evidence Identification for Synthesis and Dose-Response Analysis

3.1 Literature Search and Screening Results

The database searches yielded 373 unique records, with 50 records identified from additional sources such as TSCA submissions, posted NTP study tables, peer-review recommendations, and review of reference lists from other authoritative sources. Of the 373 studies identified, 261 were excluded during title and abstract screening, 112 were reviewed at the full-text level, and 37 were considered relevant to the PECO eligibility criteria (see Figure 4). This included 14 epidemiologic studies (described in 17 publications), 10 *in vivo* animal studies (described in 15 peer-reviewed and nonpeer-reviewed publications), and five *in vitro* genotoxicity studies. The detailed search approach, including the query strings and PECO criteria, is provided in appendix A and appendix B, respectively.
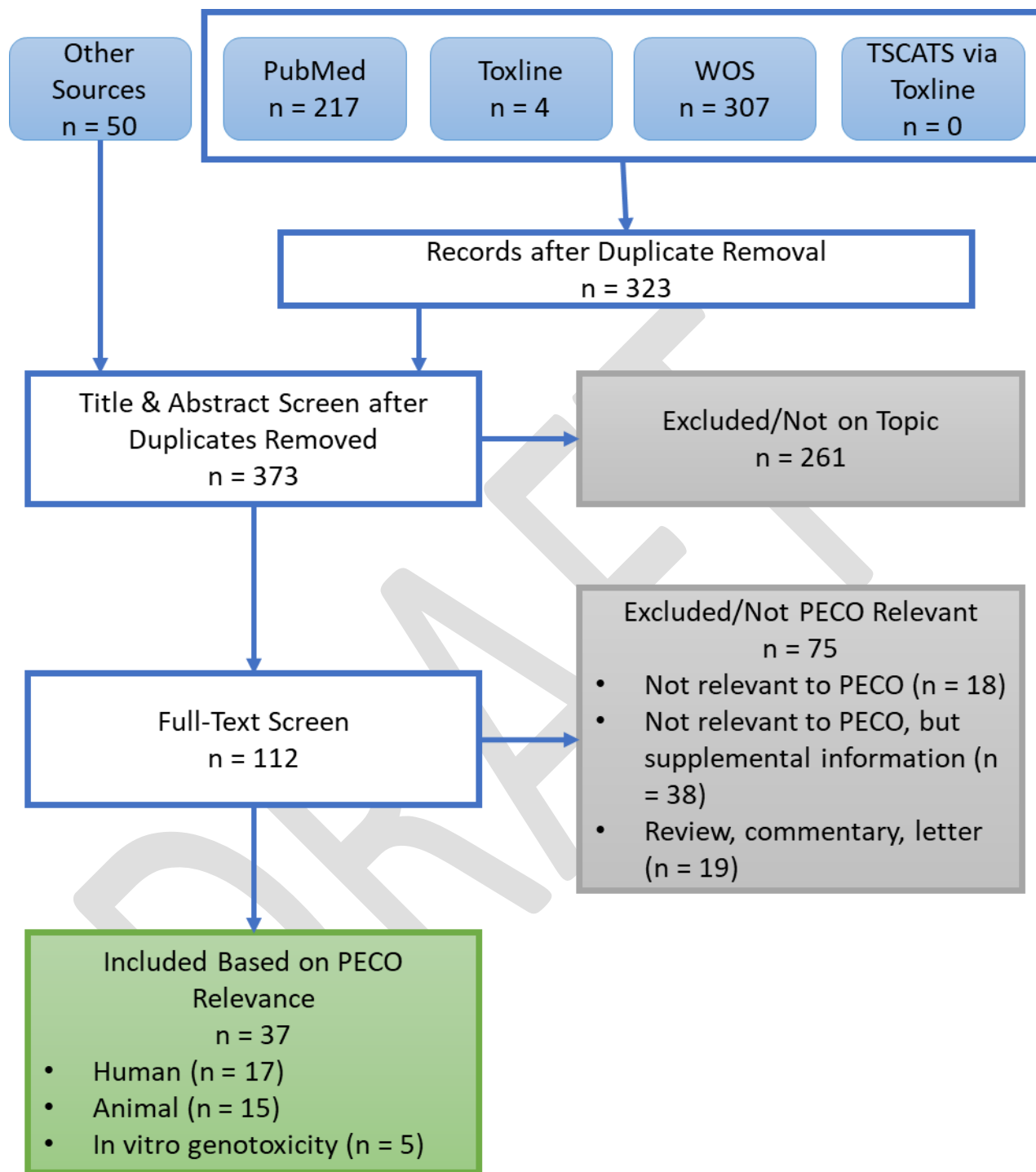
Figure 4. Literature search and screening flow diagram for PFBS (CASRN 375-73-5).

3.2   Study Evaluation Results

Based on the study evaluations, seven human epidemiology studies were considered uninformative and are not discussed any further in this assessment (see Table 4). No animal studies were considered uninformative and, thus, all animal studies identified as relevant during literature screening were included in the evidence synthesis and dose-response analysis. Overall, seven epidemiologic studies (described in 10 publications) and 10 *in vivo* animal studies (described in 15 peer-reviewed and nonpeer-reviewed publications) were included in the evidence synthesis and further evaluated for use in the development of toxicity values for PFBS. As shown in Figures 5 and 6, while the database of studies on PFBS is not large, a number of high- and medium-confidence oral exposure studies in animals were identified, as were several medium-confidence studies in humans. No studies were identified evaluating the toxicity of PFBS or K$^+$PFBS following inhalation exposure or on the carcinogenicity of PFBS or K$^+$PFBS in humans or animals.

Table 4. Epidemiological studies excluded based on study evaluation

Reference	Outcome	Reason for exclusion
Bao et al. (2017)	Blood pressure	Extremely poor sensitivity (96% of participants below the LOD) with no observed association.
Berk et al. (2014)	Depression	Serious concerns with temporality between exposure and outcome, confounding, and analysis.
Gyllenhammar et al. (2018)	Birth size, weight gain	Extremely poor sensitivity (median exposure = 0.01 ng/g) with no observed association.
Kim et al. (2016)	Congenital hypothyroidism	Excluded from full statistical analysis by study authors because of high percent below the LOD (72%).
Seo et al. (2018)	Cholesterol, uric acid, diabetes, BMI, thyroid hormones	No consideration of potential confounding.
Shiue (2016)	Sleep disturbances	Not evaluated due to nonspecific effect.
Wang et al. (2017)	Endometriosis-related infertility	Exposure measured concurrent with outcome for chronic outcome; serious concerns for exposure and outcome misclassification.

Note: LOD = limit of detection.

	Bao, 2017, 3860099	Berk, 2014, 2713574	Chen, 2018, 4238372	Dong, 2013, 1937230	Gyllenhammar, 2018, 4238300	Kim, 2016, 3351917	Qin, 2016, 3981721	Seo, 2018, 4238334	Song, 2018, 4220306	Wang, 2017, 3856459	Zeng, 2015, 2851005	Zhou, 2016, 3856472	Zhou, 2017, 3859799
Participant selection	+	+	++	+	N/A	--	+	N/A	-	-	+	+	++
Exposure measurement	--	-	++	+	--	-	-	--	++	--	-	-	-
Outcome ascertainment	++	+	+	+	N/A	+	++	N/A	-	-	+	-	+
Confounding	+	-	++	+	N/A	--	+	--	-	-	+	-	+
Analysis	+	-	+	+	N/A	-	++	N/A	-	+	+	-	++
Sensitivity	+	-	+	+	--	--	+	N/A	-	+	+	-	-
Selective Reporting	+	-	+	+	N/A	--	+	N/A	+	+	+	+	+
Overall confidence	--	--	+	+	--	--	-	--	-	--	-	-	-

++	Good (metric) or High (overall)	+	Adequate (metric) or Medium (overall)	-	Deficient (metric) or Low (overall)	N/A	Not assessed due to critical deficiency in other domain	--	Critically deficient (metric) or Uninformative (overall)

Figure 5. Evaluation results for epidemiological studies assessing effects of PFBS (click to see interactive data graphic **for** rating rationales).

Figure 6. Evaluation results for animal studies assessing effects of PFBS exposure (click to see interactive data graphic for rating rationales).

4.0 Evidence Synthesis: Overview of Included Studies

The database of all repeated-dose oral toxicity studies for PFBS and the related compound K$^+$PFBS that are potentially relevant to the derivation of RfD values includes a short-term range finding study in rats (3M, 2000d), two 28-day studies in rats (NTP, 2018, 2011; 3M, 2001), one subchronic-duration study in rats (Lieder et al., 2009a; York, 2003b), one subchronic-duration lipoprotein metabolism study in mice (Bijland et al., 2011; 3M, 2010), three gestational exposure studies in mice and rats (Feng et al., 2017; York, 2003a, 2002), and one two-generation reproductive toxicity study in rats (Lieder et al., 2009b; York, 2003c, d, e). In addition, seven epidemiologic studies (described in 10 publications) were identified that report on the association between PFBS and human health effects. Specific study limitations identified during evaluation (see HAWC) are discussed only for studies interpreted as low confidence or if a limitation impacted a specific inference for drawing conclusions.

Human and animal studies have evaluated potential effects on the thyroid, reproductive systems, developing organism, kidneys, liver, and lipid and lipoprotein homeostasis following exposure to PFBS. The evidence base for these outcomes is presented in this section. For each potential health effect, the synthesis describes the database of human and animal studies, as well as an array of the animal results across studies. NOAELs and LOAELs in presented in figures and text are based on statistical significance and/or biological significance (e.g., directionality of effect [statistically significantly decreased cholesterol/triglycerides is of unclear toxicological relevance], abnormal or irregular dose-response [nonmonotonicity], tissue-specific considerations for magnitude of effect [nonstatistically significant increase of ≥10% in liver weight interpreted as biologically significant]). For this section, evidence to inform organ-/system-specific effects of PFBS in animals following developmental exposure is discussed in the individual organ-/system-specific sections (e.g., reproductive cycling endpoints after developmental exposure are discussed in "Reproductive Effects"). Other effects informing potential developmental effects (e.g., pup BW) are discussed in the "Offspring Growth and Early Development" section.

Evidence integration analyses and overall judgments on the hazard support for each outcome domain provided by the available human and animal studies are discussed in "Evidence Integration and Hazard Characterization." Notably, in that section, the evidence informing organ-/system-specific endpoints after developmental exposure was considered potentially informative to both the developmental effects outcome domain and the organ-/system-specific outcome domain.

4.1 Thyroid Effects

4.1.1 Human Studies

No human studies were available to inform the potential for PFBS exposure to cause effects on the thyroid.

4.1.2 Animal Studies

Two high-confidence studies evaluated the effects of PFBS exposure on thyroid, specifically thyroid hormone levels, thyroid histopathology, and thyroid weight (NTP, 2018; Feng et al., 2017; NTP, 2011) (see Figure 7). Dams exposed to K$^+$PFBS through gestation (gestation days [GDs] 1−20) exhibited statistically significantly decreased total triiodothyronine (T3),

total thyroxine (T4), and free T4 (reduced 17%, 21%, and 12%, respectively, relative to control at 200 mg/kg-day and reduced 16%, 20%, and 11%, respectively, relative to control at 500 mg/kg-day) on GD 20 at doses of 200 and 500 mg/kg-day, but not at 50 mg/kg-day (Feng et al., 2017). Decreased total T3 and total T4 were also reported at postnatal day (PND) 1, PND 30, and PND 60 in offspring gestationally exposed to K+PFBS at the same doses (up to 37% reduction in T3 and 52% reduction in T4). Increased thyroid-stimulating hormone (TSH) was reported in dams and pubertal (PND 30) offspring (21% and 14% relative control at 200 mg/kg-day, respectively) exposed gestationally to K+PFBS. Statistically significant dose-dependent decreases in total T3, total T4, and free T4 were also reported after exposure in male and female rats to K+PFBS for 28 days at all doses tested ($\geq$ 62.6 mg/kg-day) (NTP, 2018, 2011). The reported reductions in total T3 were up to −57% and −43% in male and female rats, −86% and −77% in free T4, and −97% and −71% in total T4, respectively. Dose-response graphics for T4, T3, and TSH, including effect size and variability, are included in appendix E, Figures E-1, E-2, and E-3, respectively. Thyroid gland weight, thyroid histopathology, and TSH levels were not changed after 28 days of PFBS exposure in male or female rats at up to 1,000 mg/kg-day (NTP, 2018, 2011).

Figure 7. Thyroid effects from K+PFBS exposure (click to see interactive data graphic and rationale for study evaluations for effects on the thyroid in HAWC).

4.2   Reproductive Effects

4.2.1   Human Studies

Three studies that evaluate populations in China and Taiwan examined different reproductive outcomes in women and men (Song et al., 2018; Zhou et al., 2017a; Zhou et al., 2016).

Although not statistically significant, one low-confidence cross-sectional study (Zhou et al., 2017a) reported adjusted odds ratios (OR) of 1.30 (95% CI: 0.54−3.12) for menorrhagia and 1.48 (95% CI: 0.54−4.03) for hypomenorrhea in preconception women in China for each one unit change in PFBS. They also reported inverse statistically nonsignificant associations for these two outcomes, however, based on exposure quartiles (OR range: 0.61−0.84 for the highest quartiles relative to the referent) with no evidence of an exposure-response relationship. The potential for reverse causation from uncertain temporality from these cross-sectional data temper any conclusions that might be drawn from this one study.

One low-confidence study (Zhou et al., 2016) in Taiwanese adolescents reported no clear associations between PFBS levels and reproductive hormones among the entire population or stratified by sex.

One low-confidence cross-sectional study (Song et al., 2018) examined the association between PFBS exposure and semen parameters. There was no indication of decreased semen quality in this study (correlation coefficients of −0.022 for semen concentration and 0.195 [$p < 0.05$] for progressive motility), although issues were noted regarding the ability of this study to detect an effect and important methodological details were missing.

4.2.2   Animal Studies

Reproductive outcomes were evaluated in a high-confidence study of prenatal exposure to PFBS in mice (Feng et al., 2017), in two high-confidence gestational exposure studies in rats (York, 2003c, 2002), in high-confidence short-term and subchronic-duration studies in rats (NTP, 2018, 2011; Lieder et al., 2009a), and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include fertility and pregnancy outcomes, hormone levels, markers of reproductive development, and reproductive organ weights.

4.2.2.1   Female fertility and pregnancy outcomes

Female fertility parameters were evaluated in both Feng et al. (2017) and Lieder et al. (2009b), which reported generally no effects in exposed parents, but some effects after gestational exposure in the F1 offspring (click to see interactive graphic for Female Fertility Effects in HAWC). Female fertility (e.g., fertility index and days in cohabitation) and delivery parameters (e.g., length of gestation, % deliveries, stillborn pups, and implantation sites) evaluated in Lieder et al. (2009b) generally unaffected by K$^+$PFBS treatment for P0- and F1-generation dams up to 1,000 mg/kg-day. The mean number of live born F1 pups was statistically significantly decreased in the 30-mg/kg-day group, but this change was not dose-dependent. The viability index in F1 pups and the lactation index in F1 and F2 pups showed statistically significant changes at various doses but were not dose-dependent (Lieder et al., 2009b). Similarly, no effects were observed in delivery and litter parameters (e.g., implantations, litter sizes, live fetuses, corpora lutea, and early resorptions) following prenatal exposure from GDs 6 to 20 (York, 2003c, 2002). Adult (PND 60) F1 females gestationally exposed to PFBS at doses greater than 200 mg/kg-day, however, exhibited fewer primordial follicles, primary follicles, secondary follicles, early antral follicles, antral follicles, and preovulatory follicles, as well as fewer corpora lutea compared to control (Feng et al., 2017). Importantly, no effects on the health (e.g., weight gain) of the exposed dams were observed at any dose (Feng et al., 2017). Lieder et al. (2009b) evaluated ovarian follicles in F1 females after they were mated and their pups had been weaned

(i.e., lactation day [LD] 22), and observed no effects compared to controls at 1,000 mg/kg-day; however, the data were not reported. These parameters were not evaluated in York (2002).

4.2.2.2 Male fertility

Two studies using S-D rats evaluated several potential responses in the male reproductive system (NTP, 2018; Lieder et al., 2009b). Male fertility parameters and reproductive effects (e.g., sperm parameters) were generally unaffected by K$^+$PFBS treatment in P0- and F1-generation males observed by Lieder et al. (2009b). At the highest dose, there were statistically significant increases in the percentage of abnormal sperm in F1 animals and decreases in testicular sperm count in P0-generation males. In addition, the study authors report the number of spermatids per gram testis was within the historical control of the testing facility. These effects were not dose-dependent. Alterations in parameters such as sperm count/number and morphology are considered indicative of adverse responses in the male reproductive system (Foster and Gray, 2013; Mangelsdorf et al., 2003; U.S. EPA, 1996a). A 28-day exposure study reported a decreased trend in testicular spermatid count per mg testis evaluated at the time of necropsy; however, no significant effects on other sperm measures were reported, including caudal epididymal sperm count and sperm motility (NTP, 2018).

The differences in responses observed in the two available studies might have been due to experimental design differences as Lieder et al. (2009b) exposed P0 animals for 70 days and F1 animals during the entire period of gestation plus lactation, whereas NTP (2018) exposed animals for 28 days. Future studies should be developed to ascertain whether long-term and/or gestational exposure to PFBS significantly affects sperm measures in sexually mature and developing animals.

4.2.2.3 Reproductive hormones (female and male)

Reproductive hormones were evaluated in mice (Feng et al., 2017) and, to a limited extent, in rats (NTP, 2018, 2011) (see Figure 8). Exposure to K$^+$PFBS for 28 days resulted in a significant trend for increased testosterone levels in females, but not in males (NTP, 2018, 2011). The increase in testosterone was not statistically significant when compared to control at any dose by pairwise analysis. Other reproductive hormone levels were not measured. Prenatal exposure to PFBS at and above 200 mg/kg-day resulted in statistically significant reduced serum estradiol levels and increased serum luteinizing hormone levels in pubertal offspring (i.e., PND 30) (Feng et al., 2017). The change in serum estradiol levels, but not luteinizing hormone, continued into adulthood in the K$^+$PFBS-exposed offspring (i.e., PND 60). Adult PFBS-exposed offspring also exhibited decreased serum progesterone levels at doses of 200 mg/kg-day and greater. PFBS exposure did not alter maternal estradiol-, progesterone-, or gonadotropin-releasing hormone. Reproductive hormone levels in males and females were not evaluated by Lieder et al. (2009b). The changes in follicle and corpora lutea development reported in the same study, however, may be associated with alterations in hormone production/levels, as ovarian follicles and corpora lutea produce estrogen and progesterone, respectively (Foster and Gray, 2013; U.S. EPA, 1996a).

The hormonal effects observed in the NTP (2018) and Feng et al. (2017) studies might be associated with adverse reproductive effects reported in these studies. Androgens, luteinizing hormone, estradiol, and progesterone play an important role in normal development and functions of the female reproductive system (Woldemeskel, 2017; Foster and Gray, 2013). Alterations in the levels and production of these reproductive hormones can disrupt endocrine

signals at the hypothalamic-pituitary level and lead to delayed reproductive development and changes in functions (Rudmann and Foley, 2018; Woldemeskel, 2017; Foster and Gray, 2013).
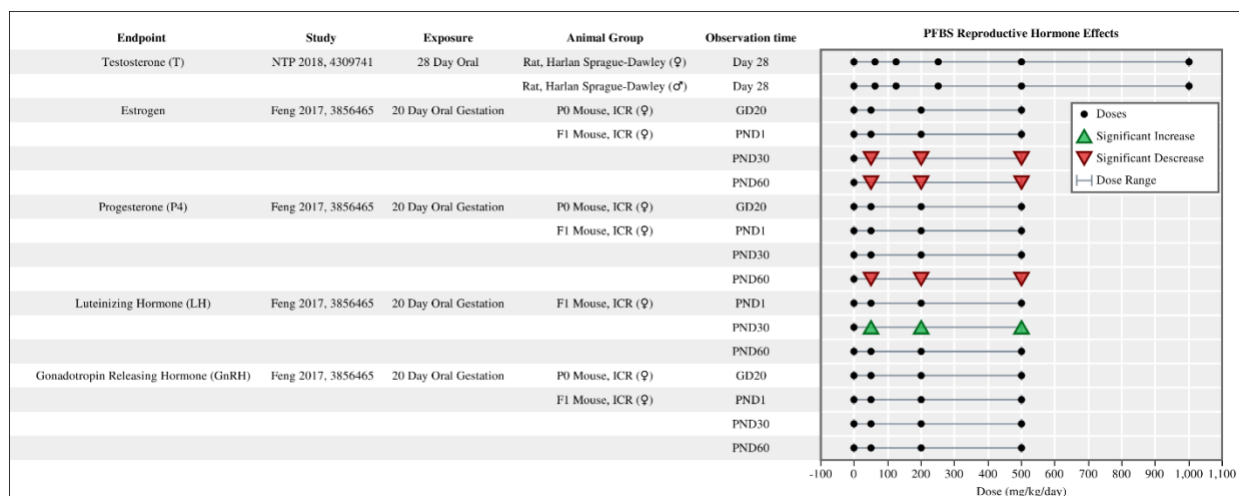
Figure 8. Reproductive hormone response to K$^+$PFBS exposure (click to see interactive data graphic and rationale for study evaluations for reproductive hormone levels in HAWC).

4.2.2.4   Reproductive system development, including markers of sexual differentiation and maturation (female and male)

Several measures of female reproductive development were affected by gestational K$^+$PFBS exposure in mice (see Figure 9). Feng et al. (2017) reported a delayed first estrous in female PFBS-exposed offspring ($\geq$ 200 mg/kg-day) compared to control. Estrous cyclicity was also affected in K$^+$PFBS-exposed PNDs 40−60 offspring as exhibited by a prolongation of the diestrus stage compared to control. Estrous cycling was generally not statistically significantly altered in P0- or F1-generation females treated with K$^+$PFBS in the two-generation study by Lieder et al. (2009b). An increase in the number of rats with $\geq$ 6 consecutive days of diestrus was observed in the F1 females exposed to 100 mg/kg/day; however, the increase was not present at higher doses (Lieder et al., 2009b). Estrous cyclicity was affected after adult exposure to K$^+$PFBS for 28 days exhibited by a dose-dependent prolongation of diestrus at doses of 250 mg/kg-day and greater with marginal significance at the lowest dose tested (125 mg/kg-day) (p = 0.063) (NTP, 2018, 2011). Lieder et al. (2009b) reported a delay in the days to preputial separation in F1 males of the 30- and 1,000-mg/kg-day groups;[5] however, the measure was no longer statistically significant when adjusted for BW. There was similarly no change in the days to vaginal patency in F1 female rats (Lieder et al., 2009b). Unlike Lieder et al. (2009b), Feng et al. (2017) reported a delay in vaginal patency in F1 females after gestational exposure of 200 mg/kg-day and greater.

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[5]A marker of delayed reproductive development (_ENREF_37; _ENREF_110).

Figure 9. Effects to reproductive development and estrous cycling following PFBS exposure (click to see interactive data graphic).

4.2.2.5   Reproductive organ weights and histopathology (female and male)

Studies have not consistently reported changes in reproductive organ weights (click to see interactive graphic for Reproductive Organ Effects in HAWC). Reproductive organ weights, including testes, ovaries, and uterus, were unchanged in the two-generation reproductive study in P0 and F1 males and females (Lieder et al., 2009b) and following short-term and subchronic exposure to K+PFBS (NTP, 2018, 2011; Lieder et al., 2009a; 3M, 2001, 2000d). F1 females gestationally exposed to PFBS, however, exhibited decreased size and weight of the ovaries and uterus (Feng et al., 2017). In addition, the total uterine section diameter and endometrial and myometrial thickness were significantly reduced. There were no significant histopathological alterations in the male or female reproductive organs evaluated following exposure to K+PFBS for 28 days (NTP, 2018, 2011) or in parental or offspring from the two-generation reproductive study (Lieder et al., 2009b).

4.3   Offspring Growth and Early Development

4.3.1   Human Studies

No human studies were available to inform the potential for PFBS exposure to cause effects on offspring growth or early development.

4.3.2   Animal Studies

Evidence to inform organ-/system-specific effects of PFBS in animals following developmental exposure are discussed in the individual hazard sections (e.g., reproductive cycling after developmental exposure is discussed in "Reproductive Effects"). This section is limited to discussion of other, specific developmental effects commonly evaluated in guideline developmental toxicity studies, including pup BW, developmental markers, and bone measures. Four high- or medium-confidence studies examined potential alterations in offspring growth and

early development following PFBS exposure, including two gestational exposure studies in rats (York, 2003a, 2002) and one gestational exposure study in mice (Feng et al., 2017), as well as a two-generation study in rats (Lieder et al., 2009b; York, 2003c) (click to see interactive graphic for Developmental Effects in HAWC).

None of the studies identified significant effects in either rats or mice on measures of fetal alterations (i.e., malformations and variations). BW of female offspring of PFBS-exposed mice at doses greater than 200 mg/kg-day was statistically significantly lower than control at PND 1, and the pups remained underweight through weaning, pubertal, and adult periods, with decreases of approximately 25% observable in pups nearing weaning (Feng et al., 2017). At these ages (i.e., around PND 16), Feng et al. (2017) also reported an ~1.5-day developmental delay in eye opening in pups gestationally exposed to 200 mg/kg-day PFBS and greater. Importantly, no effects on the health of the exposed dams (e.g., weight gain) were observed at any dose (Feng et al., 2017). Dose response graphics for eye opening, including effect size and variability, are included in appendix E, Figure E-4. Fetal BWs (male and female) were also reduced (approximately 10%) compared to controls following gestational exposure from GDs 6 to 20 at the highest tested dose (1,000 mg/kg-day in York (2002)] and 2,000 mg/kg-day in York (2003a)]). Parental BWs and organ weights, however, were also affected to a similar degree at those doses (Lieder et al., 2009b; York, 2003c, 2002), limiting the interpretation of the results. No statistically significant changes in F1- and F2-generation pups mean pup weight at birth and mean pup weight at weaning were reported by Lieder et al. (2009b) or York (2003c).

Several measures of thyroid hormone development and female reproductive development were affected by gestational PFBS exposure in mice and are described in more detail in "Thyroid Effects" and "Reproductive Effects," respectively.

4.4 Renal Effects

4.4.1 Human Studies

One medium-confidence study (Qin et al. (2016), with additional details in Bao et al. (2014), selected 225 subjects ages 12−15 years old from a prior cohort study population in seven public schools in northern Taiwan (Tsai et al., 2010) and examined the association between PFBS exposure and uric acid concentrations. There was no association between ln(PFBS) concentration and uric acid concentrations in the total population ($\beta = 0.0064$ mg/dL increase in uric acid per 1 ln-µg/L increase in PFBS, 95% CI = −0.22, 0.23). A nonsignificant positive association in boys was offset by a nonsignificant negative association in girls, and there is not enough information to determine whether there are sex-specific susceptibility differences. When PFBS exposure was analyzed for high uric acid (> 6 mg/dL), the risk was somewhat elevated in boys (OR = 1.53; 95% CI: 0.92, 2.54), but not in girls (OR = 0.99; 95% CI: 0.58, 1.73). The potential for reverse causation tempers any conclusions that might be able to be drawn.

4.4.2 Animal Studies

Renal effects were evaluated in high-confidence short-term and subchronic-duration exposure studies in rats (NTP, 2018, 2011; Lieder et al., 2009a; 3M, 2001, 2000d) and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include kidney weights, histopathological changes, and serum biomarkers of

effect (see Figure E-8 and Figure E-9). Dose-response graphics for histopathological effects, including effect size and variability, are included in appendix E, Figure E-7.

Absolute and relative kidney weights of male and female S-D rats were unchanged in rats exposed daily for 90 days to K^+PFBS at doses up to 600 mg/kg-day compared to control rats (Lieder et al., 2009a). This lack of effect on kidney weight was also observed in parental and F1 male and female rats of the same strain exposed to K^+PFBS at doses up to 1,000 mg/kg-day during a two-generation reproductive study (Lieder et al., 2009b). Although none of the findings reached statistical significance, however, an approximate 9% increase in absolute kidney weight was observed in female S-D rats exposed to 1,000 mg/kg-day K^+PFBS for 10 days (3M, 2000d); relative-to-body kidney weights were also increased approximately 6%−9%. This organ-weight effect was not observed in corresponding males of the study. In a follow-on 28-day study by the same lab, a 9%−11% increase in absolute and relative-to-body kidney weight was observed in female S-D rats exposed to 900 mg/kg-day K^+PFBS (3M, 2001), although these changes were not statistically significant. Smaller, nonsignificant increases in kidney weight were also observed in male rats. In another 28-day study, K^+PFBS exposure significantly increased absolute and relative right kidney weights in high-dose male (500 mg/kg-day) S-D rats (NTP, 2018, 2011). Only relative-to-BW kidney weights were altered in female rats; but this effect was significant at all tested K^+PFBS doses ($\geq$ 62.6 mg/kg-day). Click to see interactive graphic for Kidney Weight Effects in HAWC.

After 90 days of exposure, Lieder et al. (2009a) observed increased incidences of histopathological alterations of the kidneys of male and female rats of the high-dose group (600 mg/kg-day). Increased incidence of hyperplasia of the epithelium of renal papillary tubules and ducts was observed in rats of both sexes (see Figures E-7 and E-8). A single incidence of papillary necrosis in both kidneys was observed in one male in the high-dose group. Further, focal papillary edema was observed in 3/10 rats of both sexes of the high-dose groups compared to no evidence of this effect in control rats. Similar histopathological alterations were observed in parental and F1 male and female rats in the two-generation reproduction study (Lieder et al., 2009b). Compared to control rats, increased incidences of hyperplasia of the renal tubular and ductal papillary epithelium, and focal papillary edema were observed in parental male and female rats at PFBS doses $\geq$ 300 mg/kg-day. Hyperplastic foci in the same locations of the kidney were also observed in male and female F1 rats exposed to $\geq$ 300 mg/kg-day PFBS across life stages from gestation to adulthood (Lieder et al., 2009b). Focal papillary edema was observed in male ($\geq$ 1,000 mg/kg-day) and female ($\geq$300 mg/kg-day) F1 rats, although this specific alteration did not appear to be dose-dependent in females. Although kidney alterations such as hydronephrosis, mineralization, and tubular degeneration were observed in male or female S-D rats after just 10 days of oral K^+PFBS exposure, these effects were not significant compared to control and/or did not appear to be dose-dependent (3M, 2000d). The same histopathological lesions were noted in the 28-day rat study albeit with lack of significance compared to control or dose-dependence (3M, 2001). In another 28-day oral gavage study in S-D rats, chronic progressive nephropathy (CPN) was observed in all male and female PFBS treatment groups and control rats, with no evidence of dose-dependence for this effect (NTP, 2018, 2011). Renal papillary necrosis was also observed in these rats but only at the highest exposure dose (1,000 mg/kg-day).

Serum levels of biomarkers indicative of kidney injury and/or function, including blood urea nitrogen (BUN) and creatinine, have been examined across multiple studies of varying exposure durations, and were found to be unchanged in male and female rats treated with K$^+$PFBS at doses up to 1,000 mg/kg-day (Lieder et al., 2009a; 3M, 2001, 2000d). After 28 days of oral gavage exposure in S-D rats, however, NTP (2018, 2011) observed significantly increased levels of BUN in males (≥ 250 mg/kg-day). This increased circulating BUN was not observed in female rats at doses up to 1,000 mg/kg-day. Click to see interactive graphic for other Kidney Effects in HAWC.

4.5   Hepatic Effects

4.5.1   Human Studies

No human studies were available to inform the potential for PFBS exposure to cause hepatic effects.

4.5.2   Animal Studies

Hepatic effects were evaluated in high-confidence short-term and subchronic-duration studies in rats (NTP, 2018, 2011; Lieder et al., 2009a; 3M, 2001, 2000d) and in a high-confidence two-generation reproductive study in rats (Lieder et al., 2009b). Endpoints evaluated in these studies include liver weights, histopathological changes, and serum biomarkers of effect (see Figure E-10).

Ten days of daily oral gavage exposure to K$^+$PFBS significantly increased absolute, relative-to-body, and relative-to-brain weights of liver in adult male and female S-D rats exposed to 1,000 mg/kg-day (3M, 2000d). The absolute liver mass of male rats was increased by 36% compared to females (22%). A similar profile of liver weight alteration in S-D rats was observed following 28 days of exposure where absolute and relative liver weights of high-dose (900 mg/kg-day) male rats were increased 25%−30% (3M, 2001). Female rats of the same treatment dose did not experience a similar magnitude increase in absolute or relative liver weights (4%−6%). In another 28-day study in S-D rats, K$^+$PFBS exposure significantly increased absolute and relative liver weights in males (≥ 125 and ≥ 62.6 mg/kg-day, respectively) and females (≥ 250 and ≥ 125 mg/kg-day, respectively) (NTP, 2018, 2011). In contrast, the livers of male and female S-D rats exposed to K$^+$PFBS at doses up to 600 mg/kg-day for 90 days were not significantly changed compared to respective controls (Lieder et al., 2009a). In a two-generation reproduction study using the same strain of rat, however, increased absolute and relative liver weights were observed in male parental rats exposed to doses of K$^+$PFBS ≥ 300 mg/kg-day for approximately 70 days (Lieder et al., 2009b). In the F1 adult males, only relative liver weight was significantly increased at the high dose (1,000 mg/kg-day), although terminal BW was significantly decreased in this group compared to control.

Histopathological examination of the livers of S-D rats across three separate oral gavage studies of increasing K$^+$PFBS exposure duration (10-day (3M, 2000d)]; 28-day (3M, 2001)]; 90-day (Lieder et al., 2009a)]) did not reveal any significant dose-dependent alterations or lesions. For example, focal/multifocal hepatic inflammation was observed in 3/10 male and 4/10 female rats of the high-dose group (no incidence at the low- or mid-dose) compared to 6/10 male and female rats in the control treatment groups (Lieder et al., 2009a). The Lieder et al. (2009b) two-generation reproduction oral gavage study did identify increased incidences of

hepatocellular hypertrophy in parental and F1 adult male rats at ≥ 300 mg/kg-day; however, this effect was absent in female rats at doses of K⁺PFBS up to 1,000 mg/kg-day. NTP (2018) and NTP (2011) identified significantly increased incidence of hepatocellular hypertrophy in male (≥ 125 mg/kg-day) and female (≥ 500 mg/kg-day) S-D rats after 28 days of K⁺PFBS exposure. Further, significantly increased cytoplasmic alteration of hepatocytes was observed in these rats (male and female at ≥ 500 mg/kg-day). Hepatic necrosis was also observed but was not significant compared to control and only occurred at the high dose (1,000 mg/kg-day) in both sexes (NTP, 2018, 2011).

In general, serum biomarkers associated with altered liver function or injury, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were not significantly changed in male and female S-D rats across multiple oral gavage studies of varying exposure durations up to 90 days, at K⁺PFBS doses up to 1,000 mg/kg-day (Lieder et al., 2009a; 3M, 2001, 2000d). NTP (2018) and NTP (2011), however, reported increased serum ALT and AST in male (500 mg/kg-day only) and female (≥ 250 mg/kg-day for ALT; ≥ 500 mg/kg-day for AST) rats exposed to K⁺PFBS for 28 days. Click to see interactive graphic for Liver Effects in HAWC.

4.6   Lipids and Lipoproteins

4.6.1   Human Studies

One low-confidence study (Zeng et al., 2015) used the controls from the case-control study of asthma described below (Dong et al., 2013a) and examined the association between PFBS exposure and serum lipids. There was a statistically significant increase in total cholesterol (β = 19.3 mg/dL increase per 1 µg/l increase in PFBS, 95% CI = 0.6−38.0) but when PFBS exposure was analyzed in quartiles, no exposure-response gradient was observed.

4.6.2   Animal Studies

PFBS studies have not particularly focused on perturbations in lipids or lipoproteins as a potential health outcome, as studies have typically focused only on measures of serum cholesterol and triglyceride as part of a broader panel of clinical chemistry measures in high- or medium-confidence rat studies of 10, 28, and 90 days (see Figure E-11) (3M (2000d)]; 3M (2001)]; and Lieder et al. (2009a)], respectively). Circulating levels of cholesterol and triglycerides were unchanged in male and female S-D rats following daily oral gavage exposure to K⁺PFBS for 10 days at doses up 1,000 mg/kg-day (3M, 2000d). In a similarly designed study from the same laboratory, serum cholesterol and triglyceride levels were decreased in male rats but at the high dose only, and, this effect was not statistically significant compared to control nor was this effect observed in female rats of the same dose group (3M, 2001). Following exposure for up to 90 days, cholesterol and triglycerides were unchanged in male and female rats at doses up to 600 mg/kg-day (Lieder et al., 2009a). PFBS was included in a multi-PFAS study specifically designed to interrogate the mechanism of effect on lipid and lipoprotein metabolism in a transgenic mouse line (APOE*3-Leiden CETP) that is highly responsive to fat and cholesterol intake, consistent with human populations exposed to a western-type diet (Bijland et al., 2011; 3M, 2010). Adult male mice were fed a western-type, high-fat diet for 4 weeks prior to initiation of PFBS exposure and throughout the 4−6 weeks PFBS exposure period (at approximately 30 mg/kg-day). This study included several measures of lipid and lipoprotein synthesis, modification, and transport or clearance such as circulating plasma levels, *in vivo* clearance of very low density lipoprotein (VLDL)-like particles, fecal bile acid and sterol

excretion, hepatic lipid levels, lipase activity, VLDL-triglyceride and VLDL-apoB production, and gene expression profiles. After 4 weeks of PFBS exposure, fasting plasma triglycerides, cholesteryl ester transfer protein, and glycerol were significantly decreased compared to mice on the control diet. Further, the half-life of VLDL-like particles and hepatic lipase activity, and hepatic cholesteryl ester and free cholesterol levels were decreased (Bijland et al., 2011; 3M, 2010). Hepatic uptake of VLDL-like particles (represents fatty acid/lipid transport into hepatic tissue) was modestly, but significantly increased compared to control mice. This increased hepatic lipid uptake in the liver was accompanied by increased expression of genes associated with lipid binding, activation, and metabolism (e.g., β-oxidation).

4.7   Other Effects

4.7.1   Human Studies

Two studies in China examined different immune outcomes in children (Chen et al., 2018; Dong et al., 2013a).

One medium-confidence study reported in five publications (Qin et al., 2017; Zhou et al., 2017b; Zhou et al., 2017a; Zhu et al., 2016; Dong et al., 2013b) examined the association between PFBS exposure and asthma, asthma symptoms, pulmonary function, and related immune markers (IgE, absolute eosinophil count [AEC], eosinophilic cationic protein [ECP], T-helper cell-specific cytokines, and 16-kDa club cell secretory protein). The primary finding was a statistically significant positive association between incident asthma (i.e., diagnosis in the previous year) and PFBS exposure (OR [95% CI] for Q2: 1.3 [0.7, 2.3], Q3: 1.2 [0.7, 2.2], Q4: 1.9 [1.1, 3.4]). There were also increases in AEC and ECP with increased exposure (not statistically significant with the exception of AEC in children with asthma). There was no clear association with IgE or T-helper cell-specific cytokines. There was also no clear association with asthma severity or control of asthma symptoms (Dong et al., 2013a), or pulmonary function measured with spirometry among children with asthma (Qin et al., 2017). While pulmonary function could be considered an outcome separate from asthma, the authors noted no associations in pulmonary function (i.e., in nonasthmatics across the PFAS they studied), so for these purposes, it was considered an indicator of asthma severity.

One medium-confidence study (Chen et al., 2018) examined the association between PFBS exposure and atopic dermatitis and reported a nonstatistically significant increase in atopic dermatitis with increased exposure (OR: 1.23, 95% CI: 0.74−2.04).

4.7.2   Animal Studies

Other effects were evaluated following exposure to PFBS, including outcomes related to the spleen, hematological system, BW, neurotoxicity, and nonspecific clinical chemistry. These groups of outcomes were not synthesized due to inadequate available information, uncertain biological relevance, and/or inconsistencies across studies and sexes.

4.8   Other Data

Other studies that used PFBS or K⁺PFBS are described in this section. These studies are not adequate for the determination of RfD values and were considered supportive data. These data might include acute duration exposures, genotoxicity, mechanistic, and other studies (see Table 5).

Table 5. Other studies

Test	Materials and methods	Results	Conclusions	References
Genotoxicity				
Mutagenicity test	*Salmonella typhimurium* (strains TA98 and TA100) and *Escherichia coli* (*E. coli*) (strain pKM101) in the presence or absence of S9. Concentrations of PFBS were between 0−5,000 μg/plate.	Test was negative for TA100 and pKM101 strains and equivocal for TA98 strain.	There is no *in vitro* evidence of PFBS mutagenicity.	NTP (2005)
Ames	*S. typhimurium* (strains TA98, TA100, TA1535, and TA1537) and *E. coli* (strain WP2uvrA) were tested in the presence or absence of S9 and with or without a preincubation treatment. Concentrations of K^+PFBS were between 0−5,000 μg/plate.	The results of both mutation assays indicate that PFBS did not induce any significant increase in the number of revertant colonies for any of the tester strains in the presence or absence of induced rat liver S9.	There is no *in vitro* evidence of PFBS mutagenicity.	Pant (2001)
Genotoxicity test	Human hepatoma (HepG2) cells were treated with 0.4 μM to 2 mM PFBS. Intracellular ROS production was measured by use of 2′,7′-dichlorofluorescein diacetate and DNA damage was measured with the comet assay.	The amount of ROS and DNA strand breaks remained unaffected by PFBS treatment.	PFBS did not generate ROS or DNA damage in human liver cells.	Eriksen et al. (2010)
CHO chromosomal aberration	Cultures of CHO cells were treated with K^+PFBS at concentrations ranging from 0 to 5,000 μg/mL with or without exogenous metabolic activation. The *in vitro* exposure duration was 3 hr.	PFBS did not induce a statistically significant increase in the percentage of cells with aberrations at any of the concentrations tested, either with or without metabolic activation, in either assay when compared to the solvent controls.	Based on the negative results in the *in vitro* CA assay in CHO cells, PFBS is not considered to be a clastogenic agent.	Xu (2001)
Micronucleus assay	Male and female S-D rats (5/group) were exposed twice daily to K^+PFBS by oral gavage at doses of 31.3, 62.5, 125, or 250 mg/kg for 28 d.	PFBS did not induce a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes.	PFBS was negative for micronuclei in the blood of male and female rats, indicating a lack of genotoxic potential.	NTP (2012)
Acute duration and other routes of exposure				
Acute	10 rats/group, young adult male rat (strain not specified), administered PFBS by gavage, single dose, 50, 100, 300, 600, or 800 μL/kg and observed for 14-d postexposure.	Mortality: 0%, 20%, 60%, 80%, and 100% at 50, 100, 300, 600, and 800 μL/kg PFBS, respectively.	Acute oral PFBS rat LD_{50} in male rats is 236 μL/kg (corresponding to 430 mg/kg).	Bomhard and Löser (1996) Low confidence

Test	Materials and methods	Results	Conclusions	References
Acute dermal	Adult (8 wk of age) male and female S-D rats (5/group) were exposed dermally (10% of body surface area) to 500, 1,000, or 2,000 mg/kg K$^+$PFBS for 24 hr and then observed for 15-d postexposure for signs of clinical toxicity, mortality, BW changes, or gross pathology (terminus of study).	No treatment-related observations were noted.	PFBS is not acutely toxic via the dermal route of exposure in rats.	3M (2000b)
Dermal irritation	Adult (14-wk of age) female NZW rabbits (3 rabbits total for study) were exposed dermally (6 cm^2 of skin) to 500 mg K$^+$PFBS for approximately 4 hr and then observed for 9-d postexposure for signs of clinical toxicity, mortality, or BW changes.	Draize scoring was performed on the patch site immediately following the exposure period and 24, 48, and 72 hr postexposure. No signs of dermal irritation were observed. No signs of clinical toxicity or mortality occurred. No treatment-related alterations in BW were noted.	PFBS did not induce erythema, edema, or other possible dermal findings during the scoring periods, indicating a lack of dermal irritant properties in rabbits.	3M (2000a)
Ocular sensitivity	Adult (16-wk of age) female NZW rabbits (3 rabbits total for study) were exposed to approximately 80 mg K$^+$PFBS via ocular installation in the left eye for 2 sec. Eyes were flushed with 0.9% saline after 24 hr and then observed and scored for up to 21-d postexposure. The rabbits were also followed for clinical signs of toxicity or mortality/moribundity.	Excessive lacrimation of the left eyes noted throughout study postexposure. Based on the laboratory scoring system, PFBS was "moderately" irritating at 24 and 72 hr postexposure.	PFBS is a moderate ocular irritant in rabbits.	3M (2000c)
Contact hypersensitivity	Adult male (10−12 wk old) and female (9 wk old) CRL:(HA)BR Hartley guinea pigs were injected intradermally with sterile water, Freund's adjuvant, or adjuvant containing 125 mg/mL K$^+$PFBS (induction phase). Day 7 after induction, a petrolatum paste containing 0.5 g K$^+$PFBS was applied to the previous injection site of the guinea pigs for 48 hr (topical induction phase). Day 22, a challenge dose of 0.5 g K$^+$PFBS (petrolatum paste) was applied to the shaved left cranial flank (right flanks were treated with petrolatum paste only) (challenge phase). This challenge procedure was repeated on Day 29. Challenge sites were observed and scored following each challenge period (days 24−25 males and females and days 31−32 males only). Guinea pigs were also followed for signs of clinical toxicity, mortality/moribundity, or alterations in BW.	No mortalities, clinical signs of toxicity, or changes in BW associated with PFBS exposure. Dermal scores were zero (no response) in females and did not exceed 1 in males (discreet or patchy edema), which was not considered significant compared to control guinea pigs exposed to Freund's adjuvant alone.	PFBS is not considered an allergen in the guinea pig maximization test.	3M (2002a)

CA = chromosomal aberration; CHO = Chinese hamster ovary; cm^2 = square centimeters; d = day(s); DNA = deoxyribonucleic acid; LD$_{50}$ = median lethal dose; μg/plate = microgram per plate; μM = micromol; mM = millimol; NZW = New Zealand White; ROS = reactive oxygen species; wk = weeks(s).

4.8.1 Tests Evaluating Genotoxicity and Mutagenicity

Genotoxic, mutagenic, and clastogenic effects of PFBS have been tested in mammalian and prokaryotic cells *in vitro* (Eriksen et al., 2010; NTP, 2005; Pant, 2001; Xu, 2001), and in rats *in vivo* (NTP, 2018). PFBS was negative for mutagenicity in *Escherichia coli* (*E. coli*) strain pKM101 and *Salmonella typhimurium* strain TA100 (NTP, 2005). Mutagenicity test results were equivocal in *S. typhimurium* strain TA98. Pant (2001) tested PFBS at concentrations up to 5,000 µg/plate in *E. coli* strain WP2uvrA and *S. typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence or absence of exogenous metabolic activation and found no evidence of mutagenic activity. In mammalian cells *in vitro*, PFBS did not generate reactive oxygen species (ROS) or oxidative deoxyribonucleic acid damage in HepG2 cells (Eriksen et al., 2010). PFBS also failed to induce chromosomal aberrations in Chinese hamster ovary cells, suggesting a lack of clastogenic activity (Xu, 2001). Adult male and female S-D rats exposed twice daily to oral PFBS at doses up to 250 mg/kg for 28 days did not experience any significant increases in micronucleated polychromatic erythrocytes, indicating a lack of genotoxic activity (see Table 5) (NTP, 2012).

5.0 Evidence Integration and Hazard Characterization

The epidemiology database of studies of PFBS exposure and health effects consists of seven epidemiologic studies (described in 10 publications), illustrated and summarized in the previous section. The experimental animal database of all repeated-dose oral toxicity studies for PFBS and the related compound K⁺PFBS includes a short-term range finding study in rats (3M, 2000d), two 28-day studies in rats (NTP, 2018, 2011; 3M, 2001), one subchronic-duration study in rats (Lieder et al., 2009a), one subchronic-duration lipoprotein metabolism study in mice (Bijland et al., 2011; 3M, 2010), three gestational exposure studies in mice and rats (Feng et al., 2017; York, 2003a, 2002), and one two-generation reproductive toxicity study in rats (Lieder et al., 2009b). Health outcomes evaluated across available studies included effects on the thyroid, reproductive organs and tissues, developing offspring, kidneys, liver, and lipids/lipoproteins following oral exposure to PFBS. Table 6 provides an overview of this database of potentially relevant studies and effects. This table includes only the high- and medium-confidence animal studies (a single, low-confidence animal study was not considered informative to drawing judgments on potential health hazard[s]); the available epidemiology studies are not included as their ability to inform conclusions about associations was limited due to the small number of studies (typically one) per outcome and poor sensitivity resulting from low exposure levels.

Following the summary of the available database in Table 6, narrative summaries describe the evidence integration judgments and the primary rationales supporting these decisions for each health effect. These narratives are supported by an evidence profile table that succinctly lays out the various factors that were judged to increase or decrease the support for hazard. While the epidemiology studies were not influential to drawing evidence integration judgments (i.e., they were judged as equivocal for all outcomes) or the derivation of toxicity values (i.e., these studies are not discussed in the next section), the general findings are summarized below to provide context to the animal study findings and identify potential areas of future research.

Table 6. Summary of noncancer data for oral exposure to PFBS (CASRN 375-73-5) and the related compound K$^+$PFBS (CASRN 29420-49-3)

Exposure duration[a]	Reference	Study confidence	Number of male/female, strain, species, study type, study duration	Doses tested (mg/kg-d)	Effects observed at LOAEL	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Short-term	3M (2000d)	Medium confidence	5/5, S-D rat, K$^+$PFBS administered by gavage, 10 d	0, 100, 300, 1,000	Increased absolute and relative liver weight.	300	1,000
Short-term	3M (2001)	High confidence	10/10, S-D rat, K$^+$PFBS administered by gavage, 28 d	0, 100, 300, 900	Increased liver weight (male) and kidney weight (female).	300	900
Short-term	NTP (2018); NTP (2011)	High confidence	10/10, S-D rat, PFBS administered by gavage, twice/d, 28 d	0, 62.6, 125, 250, 500, 1,000[b]	Decreased T3, free T4, total T4 in males and females. Increased relative liver weight in females, and increased relative right kidney weight in males.	ND	62.6
Subchronic	Lieder et al. (2009a); York (2003b)	High confidence	10/10, S-D rat, K$^+$PFBS administered by gavage, 7 d/wk, 90 d	0, 60, 200, 600	Increased incidence of renal hyperplasia in males and females.	200	600
Subchronic	Bijland et al. (2011); 3M (2010)	Medium confidence	6−8/0, Apoe*3-Leiden CETP mice, K$^+$PFBS in diet, 4−6 wk	0, 30	Alterations in lipid homeostasis (e.g., decreased hepatic lipase, triglycerides) is of uncertain biological significance.	ND	ND
Developmental	Feng et al. (2017)	High confidence	0/10, ICR mice, K$^+$PFBS administered by gavage, GDs 1−20	0, 50, 200, 500	Decreased T3, free T4, and total T4 and increased TSH in maternal and offspring (PND 30 only). Delayed eyes opening, vaginal opening, and final estrous and decreased BW in pups.	50	200
Developmental	York (2003a)	High confidence	0/8, S-D rat, K$^+$PFBS administered by gavage, GDs 6−20	0, 100, 300, 1,000, 2,000	Decreased maternal feed consumption, BW gain, and gravid uterine weight. Decreased pup BW at doses where maternal health was affected limiting the interpretation of the results; thus developmental effect levels were not determined. (Limited endpoints evaluated—pilot study).	P0: 1,000 F1: ND	P0: 2,000 F1: ND

Exposure duration[a]	Reference	Study confidence	Number of male/female, strain, species, study type, study duration	Doses tested (mg/kg-d)	Effects observed at LOAEL	NOAEL (mg/kg-d)	LOAEL (mg/kg-d)
Developmental	York (2002)	High confidence	0/25, S-D rat, K$^+$PFBS administered by gavage, GDs 6−20	0, 100, 300, 1,000	Decreased maternal feed consumption and BW gain. Decreased pup BW at doses where maternal health was affected limiting the interpretation of the results; thus developmental effect levels were not determined.	P0: 300 F1: ND	P0: 1,000 F1: ND
Reproductive	Lieder et al. (2009b); York (2003c); York (2003d); York (2003e)	High confidence	30/30, S-D rat, K$^+$PFBS administered by gavage, two-generation reproductive study	P0 adults: 0, 30, 100, 300, 1,000 F1 adults: 0, 30, 100, 300, 1,000	P0 and F1 adults: increased incidence of hyperplasia and focal papillary edema in the kidneys of males and females. F2 pups: no dose-related effects at the highest dose tested (1,000 mg/kg-d).	P0, F1: 100 F2: 1,000	P0, F1: 300 F2: ND

Notes: ND = no data; ICR = Institute of Cancer Research
[a] Duration categories are defined as follows: Acute = exposure for ≤ 24 hours; short term = repeated exposure for 24 hours to ≤ 30 days; long term (subchronic) = repeated exposure for > 30 days ≤ 10% lifespan for humans (> 30 days up to approximately 90 days in typically used laboratory animal species); chronic = repeated exposure for > 10% lifespan for humans (>~ 90 days to 2 years in typically used laboratory animal species) (U.S. EPA, 2002).
[b] Rats were gavaged twice daily at administered doses of 0, 31.3, 62.6, 125, 250, and 500 mg/kg in NTP (2018) and NTP (2011).

5.1   Thyroid Effects

PFBS-induced perturbation of the thyroid was consistently observed across two species, sexes, life stages, and exposure durations in two independent, high-confidence studies. These perturbations involved a coherent pattern of hormonal changes. Significant changes in tissue weight or histopathology were not observed.

Similar patterns of decreases in total T3, total T4, and free T4 were observed in PFBS-exposed pregnant mice, nonpregnant adult female and adult male rats from a 28-day study, and gestationally exposed female mouse offspring (NTP, 2018; Feng et al., 2017; NTP, 2011). These decreases were of a concerning magnitude (~20% in dams and ~50% in offspring), they were shown to persist at least 60 days after gestational exposure in offspring, and they exhibited a clear dose-dependence in both studies.

Development of numerous organ systems, including neuronal, reproductive, hepatic, and immune systems, are affected by altered thyroid homeostasis since adequate levels of thyroid hormones are necessary for normal growth and development in early life stages (Forhead and Fowden, 2014; Gilbert and Zoeller, 2010; Hulbert, 2000). Thus, the observed effects of PFBS exposure on thyroid hormone economy are biologically consistent with the reported delays and abnormalities in organ/system development discussed below. It is well-established that the presence of sufficient thyroid hormones during the gestational and neonatal period is essential for brain development and maturation. Studies specifically evaluating the effect of PFBS on neurodevelopment were not identified, leaving uncertainty as to the potential for more sensitive developmental effects. Nonetheless, the coherence of these PFBS findings, in addition to the large number of xenobiotic exposure studies demonstrating associations between thyroid hormone economy and decrements in early life stage growth, development, and survival, provides support for thyroid hazard.

Taken together, the evidence in animals for thyroid effects *supports a hazard*. No studies in humans were available. Although there are some differences in hypothalamic-pituitary-thyroid (HPT) regulation across species (e.g., serum hormone-binding proteins, hormone turnover rates, and timing of *in utero* thyroid development), rodents are generally considered to be a good model for evaluating the potential for thyroid effects of chemicals in humans (Zoeller et al., 2007). Overall, based on findings in animal models considered to be informative for evaluating the potential for thyroid effects in humans, the available evidence *supports a hazard* and the thyroid is considered a potential target organ for PFBS toxicity in humans.

5.2   Developmental Effects

Overt effects on birth parameters and early development have generally not been observed in either rats or mice after PFBS exposure. Specifically, the available studies do not provide evidence of effects on endpoints relating to pregnancy loss, fetal survival, or fetal alterations (Feng et al., 2017; Lieder et al., 2009a; York, 2003a, c, 2002). While one mouse study indicated pronounced decreases in female offspring BW at several ages after gestational exposure (Feng et al., 2017), several other studies either did not observe decreases in offspring BW or only detected these changes when parental BWs were similarly affected (Feng et al., 2017; Lieder et al., 2009a; York, 2003a, c, 2002).

Delays in development have been reported following gestational PFBS exposure in mice, including delayed development of the female reproductive organs (i.e., ovaries, uterus, and vaginal patency), delayed and abnormal estrous cycling (i.e., first estrous and prolongation of diestrus), and delayed eye opening ([Feng et al., 2017](#)). Age at vaginal patency and ovarian follicle counts (i.e., in F1 rat offspring after delivery of the F2 generation) were unaffected at 1,000 mg/kg-day in a two-generation reproductive toxicity study ([Lieder et al., 2009a](#)). This observed lack of effects (i.e., on vaginal patency) is inconsistent with the findings in mice and not easily explained by the current understanding of PFBS toxicokinetics. However, [Feng et al. (2017)](#) also noted changes in reproductive hormones that might be relevant to the delays in female sexual development, including a decrease in serum estradiol and increased luteinizing hormone in pubertal offspring (i.e., PND 30 [Note: progesterone was decreased at a later age, PND 60, but not PND 30]. As the changes reported in mice by [Feng et al. (2017)](#) were observed in parallel with effects on thyroid hormone levels (discussed above), it is plausible that these developmental delays and hormonal changes could represent sequalae of reduced thyroid function, although that was not directly tested.

For the most part, developmental effects have been reported in a single study and species (mouse); however, the findings are coherent with one another as well as with the consequences of decreased thyroid hormone levels. Due to the coherence across effects on the thyroid and several interrelated developmental effects in mice (i.e., delays and hormonal changes), the evidence in animals for developmental effects *supports a hazard*. There is no reason to expect that the specific developmental delays observed in mice would not be directly relevant to similar processes in humans. Thus, based on findings in animals that are presumed to be relevant to humans, the available evidence *supports a hazard* and the developing offspring is considered a potential target for PFBS toxicity in humans. As no studies in humans were available, this represents an area deserving of additional research.

5.3   Reproductive Effects

Reproductive outcomes, including male and female fertility, pregnancy outcomes, hormone levels, markers of reproductive development, and reproductive organ weights and histopathology, have been evaluated in a number of high-confidence studies in mice ([Feng et al., 2017](#)) and rats ([NTP, 2018](#), [2011](#); [Lieder et al., 2009a](#); [Lieder et al., 2009b](#)). In addition, three low-confidence human studies evaluated potential associations between PFBS exposure and reproductive effects ([Song et al., 2018](#); [Zhou et al., 2017a](#); [Zhou et al., 2016](#)).

PFBS exposure has resulted in no significant changes in male mating and fertility parameters, reproductive organ weights, or reproductive hormones. While there were some slight, statistically significant effects on male reproductive endpoints in two rat studies (specifically, altered sperm parameters such as percentage of abnormal sperm or testicular sperm count ([NTP, 2018](#); [Lieder et al., 2009a](#)) and delayed preputial separation at 1,000 mg/kg-day ([Lieder et al., 2009a](#))), these findings were not clearly dose-dependent and the levels of change were of questionable biological significance. No significant reproductive effects in men were noted across two human studies ([Song et al., 2018](#); [Zhou et al., 2016](#)).

In general, PFBS exposure in adults has also resulted in no significant alterations in female fertility or pregnancy outcomes in rats or mice ([NTP, 2018](#); [Feng et al., 2017](#); [NTP, 2011](#); [Lieder et al., 2009a](#); [Lieder et al., 2009b](#)) or in two human studies ([Zhou et al., 2017a](#); [Zhou et al., 2016](#)), and

inconsistent changes in rodent reproductive organ weights were reported across studies regardless of duration and timing of exposure. However, changes in normal estrous cyclicity, specifically prolongation of the diestrus stage, have been reported in both nonpregnant adult rats exposed to PFBS (NTP, 2018) and adult mouse offspring exposed gestationally from GDs 1 to 20 (Feng et al., 2017). PFBS exposures in NTP (2018) began between 8 and 10 weeks of age; although the exposures might overlap with some aspects of reproductive development or changes in function during adolescence, these rats were sexually mature and thus the endpoints are considered in the context of reproductive, rather than developmental, effects. The mouse offspring in the study by Feng et al. (2017) also displayed delayed vaginal patency and histopathological markers of decreased fertility (i.e., decreased follicles and corpora lutea); however, the reproductive function of those offspring was not tested. While adult rat offspring (F1) in a two-generation toxicity study also exhibited variable changes in estrous cyclicity (Lieder et al., 2009b), including prolonged diestrus at 100 mg/kg-day, this effect was not observed at higher doses, limiting interpretation, and no effects on vaginal patency were observed. Female reproductive hormones can inform the potential for effects on reproductive organ development, estrous cyclicity, and fertility. Changes in serum hormones included increased testosterone after exposure of female rats as adults (NTP, 2018), increased luteinizing hormone and decreased estradiol in pubertal mice after gestational exposure (Feng et al., 2017), and decreased estradiol and progesterone when these gestationally exposed mice were assessed as adults. Overall, the pattern and timing of hormonal changes after PFBS exposure is difficult to interpret and likely incomplete. However, the hormonal alterations after gestational PFBS exposure in mice are most relevant to conclusions about female reproductive health.

Taken together, the evidence indicates that the developing reproductive system, particularly in females, might be a target for PFBS toxicity. However, the potential for reproductive effects in adults was less clear, and significant impacts on mating or fertility parameters were not observed across the available studies. Therefore, the evidence in developing animals is considered most informative to conclusions relating to potential developmental effects (see above) and the evidence for reproductive effects (i.e., in adults) is *equivocal*. In the three studies of potential reproductive effects in humans, no clear associations were observed, and so the evidence in human studies is *equivocal*. Overall, based on *equivocal* human and animal evidence, the available evidence for reproductive effects is *equivocal*.

5.4   Renal Effects

Renal effects associated with oral exposure to PFBS have been observed in adult or developing rats across high- or medium-confidence gavage studies of various duration (NTP, 2018, 2011; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d).

Statistically significant increases in kidney weights have been observed in male and female rats after short-term exposure in one study (NTP, 2018), with strong dose-dependence for changes in relative weights in female rats at doses as low as 62.5 mg/kg-day. This study was likewise the only study to observe changes in serum markers of renal injury, specifically increased BUN in males at ≥ 250 mg/kg-day. However, while several other studies noted slight increases in weights, typically at higher PFBS doses (≥ 500 mg/kg-day), these nonsignificant changes were not consistently observed across the set of available studies and no other studies reported changes in serum markers of renal injury (Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d).

Kidney histopathology for some effects (i.e., CPN, hydronephrosis, tubular degeneration, and tubular dilation) was unaffected by PFBS exposure in rats, although each of these endpoints was not assessed across several studies (NTP, 2018, 2011; Lieder et al., 2009a; 3M, 2000d). Mixed results were reported for mineralization and necrosis. Both of these endpoints were noted in females, but not males, after subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a), whereas mineralization was unaffected in male or female rats after short-term exposure (3M, 2000d) and necrosis was unaffected in male or female rats in short-term and 2-generation (in both generations) studies (NTP, 2018; Lieder et al., 2009b). Multiple markers of inflammatory changes were consistently noted in the two longest exposure duration studies, which were the only studies to report on these endpoints. Specifically, increases in chronic pyelonephritis, tubular basophilia, and mononuclear cell infiltration were observed in female, but not male, rats following subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a). Similarly, increases in papillary edema and hyperplasia were observed in male and female rats after subchronic exposure to 600 mg/kg-day (Lieder et al., 2009a), and in both generations of rats in the two-generation study at ≥ 300 mg/kg-day (Lieder et al., 2009b), with female rats being more sensitive than males.

Overall, the evidence in animals suggests an increased sensitivity of female rats (i.e., based on histopathology and organ weight changes). Due primarily to the consistency and coherence in renal effects observed in the subchronic-duration study by Lieder et al. (2009a) and the reproductive toxicity study by Lieder et al. (2009b) in male and female rats, the evidence in animals *supports a hazard*. No information from PFBS studies informs the human relevance of these findings. Taken together, the renal histopathology evidence in rodents identifies a toxicologically significant spectrum of effects that is presumed to be relevant to similar changes known to occur in humans. Renal effects (i.e., uric acid) were evaluated in one low-confidence human study and no clear association was observed, and so the evidence in human studies is *equivocal*. Overall, based on findings in animals that are presumed to be relevant to humans, the available evidence *supports a hazard* and indicates the kidney as a target organ of PFBS toxicity.

5.5   Hepatic Effects

Hepatic effects, including organ-weight changes and histopathology associated with oral exposures to PFBS, have been observed in high- or medium-confidence studies in adult or developing rats following short-term and subchronic durations (NTP, 2018, 2011; Lieder et al., 2009a; 3M, 2001, 2000d) and in a two-generation reproductive study in rats (Lieder et al., 2009b). Increased absolute and/or relative liver weights were consistently observed in male and female rats after short-term and multigenerational exposure (NTP, 2018, 2011; Lieder et al., 2009b; 3M, 2001, 2000d). In some studies, the magnitude of the liver weight changes and the doses at which effects occurred differed across sexes of rat, although the pattern across studies was unclear and did not consistently indicate one sex as more sensitive. Liver histopathology, including necrosis and inflammation, was not consistently observed across PFBS studies. One possible exception is increases in hepatocellular hypertrophy in male rats observed across two studies (NTP, 2018; Lieder et al., 2009b), although female rats were unaffected in the multigenerational study and this lesion was not observed at up to 600 mg/kg-day in the subchronic study by Lieder et al. (2009a). The only study to observe changes in serum markers of liver injury was NTP (2018), at ≥ 250 mg/kg-day in females and ≥ 500 mg/kg-day in males. The biological relevance or significance of the observed liver effects is not clear. In particular,

the adversity of the variable changes in liver weight and observations of cellular hypertrophy is unclear. Further, the observed lesions either occurred in only one sex of rat, were not dose-dependent compared to control, and/or occurred only at the highest PFBS dose tested. Thus, the evidence in animals is *equivocal*. Overall, based on *equivocal* animal evidence and a lack of human studies, the available evidence for hepatic effects is *equivocal*.

5.6 Effects on Lipid or Lipoprotein Homeostasis

Few studies have examined the effects of PFBS on circulating or hepatic lipid or lipoprotein homeostasis. It is recognized that increased circulating levels of lipids and lipoprotein products and/or increased hepatic lipid load are clinical observations of concern in humans. However, the lack of effect on lipid dynamics in most studies of rats exposed to high oral K^+PFBS doses for up to 90 days and the generally modest effects in transgenic mice, designed to interrogate mechanisms of lipid transport and metabolism, fed a high-fat, western-type diet renders this potential health outcome of unclear toxicological significance at this time. Thus, given the inconsistent, modest effects and the unclear biological relevance of these changes in isolation (i.e., lipids/lipoproteins were decreased, not increased) the evidence in animals is *equivocal*. Effects on serum lipids were evaluated in one low-confidence human study and, although an association was observed between increased PFBS exposure and increased total cholesterol, this evidence in humans is *equivocal*. Overall, based on *equivocal* evidence in both animal and human studies, the available evidence for effects on lipid or lipoprotein homeostasis is *equivocal*.

5.7 Immune Effects

Immune effects were observed in two human studies, including associations with asthma (Dong et al., 2013a) and atopic dermatitis (Chen et al., 2018). Because of the lack of additional evidence and some concerns about risk of bias, the evidence in human studies is *equivocal*. Overall, based on *equivocal* evidence in human studies and a lack of animal studies, the available evidence for immune effects is *equivocal*.

5.8 Evidence Integration and Hazard Characterization Summary

Based on the evidence integration judgments regarding the potential for PFBS exposure to cause health effects (the narrative above is summarized in Table 7), the animal studies informing the potential effects of PFBS exposure on thyroid function, renal function, and development were concluded to support hazard. Thus, for the purposes of this assessment, the animal data supporting these outcomes were considered for use in dose-response analysis, and other data were considered no further.

Table 7. Summary of hazard characterization and evidence integration judgments

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Thyroid effects				
Human studies				*Supports a hazard (animal evidence supports a hazard; human evidence is equivocal).*
No studies available to evaluate	--	--	--	
Animal studies (all oral gavage)				
Mouse Studies: • High-confidence gestational (GDs 1–20) exposure study (Feng et al., 2017) Rat Studies: • High-confidence short-term (28-d) toxicity study (NTP, 2018, 2011)	• Consistent thyroid hormone decreases (i.e., for total T3, total T4, and free T4) across two high-confidence studies of varied design. The findings were consistent across two species, sexes, life stages, and exposure durations. • Dose-response gradients were observed for those thyroid hormones. • Large magnitudes of effect (e.g., up to ~50% reductions in offspring serum hormones) were reported for those thyroid hormones.	• No factors noted.	Similar patterns of decreases in thyroid hormones (i.e., for total T3, total T4, and free T4) were observed in PFBS-exposed pregnant mice and gestationally exposed female mouse offspring at ≥ 200 mg/kg-d (Feng et al., 2017) and in adult female and male rats at ≥ 62.6 mg/kg-d (NTP, 2018, 2011). Increased TSH was reported in mouse dams and in pubertal (PND 30) offspring following gestational exposure (Feng et al., 2017), but no changes were noted in rats exposed as adults (NTP, 2011). Thyroid weight and histopathology were not changed after short-term exposure in adult male or female rats (NTP, 2018, 2011).	The primary basis for this judgment is thyroid hormone decreases in mice and rats at ≥ 62.6 mg/kg-d.
Developmental effects				
Human studies				
No studies available to evaluate	--	--	--	

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Animal studies (all oral gavage)				
Mouse Studies: • High-confidence gestational (GDs 1−20) exposure study (Feng et al., 2017) Rat Studies: • Two high-confidence gestational exposure (GDs 6−20) studies: a range finding study and a follow-up study (York, 2003c, 2002) • High-confidence 2-generation study (Lieder et al., 2009b)	• Biologically consistent spectrum of developmental effects in female offspring in a high-confidence mouse study at doses not causing maternal toxicity, including pronounced and persistent effects on BW, delays in developmental milestones and sexual maturation, concordant effects on reproductive organs, and altered serum hormones. • Concerning magnitude of effect (e.g., ~25% change in pup weight) and dose-dependence for several parameters. • Coherence of effects with thyroid hormone insufficiency (see above). Note: these effects were also coherent with effects on estrous cyclicity observed after short-term exposure in adult rats (NTP, 2018, 2011), but this was categorized as a reproductive effect (see below).	• Developmental effects were limited to changes in one study, sex, and species. • A high-confidence rat study reported some inconsistent evidence, including lack of a delay in vaginal patency and lack of clear effects on estrous cyclicity or ovarian morphology, although the latter endpoint was assessed in much older animals. These potential differences across species are not explainable based on toxicokinetics alone.	In the only mouse study (Feng et al., 2017), developmental effects and altered markers of female reproductive development or function were observed in female offspring after gestational PFBS exposure, including decreased BW, delayed eye opening, delayed vaginal opening, altered estrous cyclicity (including prolonged diestrus), altered reproductive hormones (e.g., decreased estradiol and progesterone), and effects on reproductive organs (e.g., weight and ovarian morphology). Most effects were observed at $\geq$ 200 mg/kg-d, with several changes noted at PND 60. Endpoints relating to fertility, pregnancy, survival, and fetal alterations were unchanged in both rats and mice across the four available studies, although this was not tested in mouse offspring (Feng et al., 2017). Developmental BW changes in rat offspring were either unchanged (Lieder et al., 2009b) or observed only at doses causing parental toxicity (York, 2003c, 2002). In a rat two-generation study, while some statistically significant findings were noted for markers of female reproductive development or function, they were not dose-dependent or were of questionable biological relevance; thus, no clear changes in F1 offspring were noted at doses up to 1,000 mg/kg-d regarding vaginal patency or estrous cycling at comparable ages to (Feng et al., 2017), or in ovarian morphology after the F1 females gave birth to the F2 pups.	*Supports a hazard* (animal evidence *supports a hazard*; human evidence is *equivocal*). The primary basis for this judgment is a set of persistent developmental delays and alterations in reproductive system maturation in female mice, generally at $\geq$ 200 mg/kg-d.

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Reproductive effects				
Human studies				*Equivocal (equivocal* human and animal evidence).

Note: As the strongest evidence for female reproductive effects was in offspring that were gestationally exposed, these findings were considered most relevant to developmental, not reproductive, effects. |
Male reproductive effects				
• Low-confidence cohort study (Zhou et al., 2016) • Low-confidence cross-sectional study (Song et al., 2018)	• No factors noted.	• Lack of clear association in studies of low confidence with poor sensitivity (i.e., due to low exposure levels, range).	No clear association between PFBS exposure and male reproductive hormones (Zhou et al., 2016) or semen parameters (Song et al., 2018).	
Female reproductive effects				
• Low-confidence cross-sectional study (Zhou et al., 2017a) • Low-confidence cohort study (Zhou et al., 2016)	• No factors noted.	• Lack of clear association in studies of low confidence with poor sensitivity (i.e., due to low exposure levels, range). • Potential for reverse causation for menstrual cycle characteristics.	No clear association between PFBS exposure and female reproductive hormones (Zhou et al., 2016) or menstrual cycle characteristics (Song et al., 2018).	
Animal studies (all oral gavage)				
Male reproductive effects				
Rat Studies: • High-confidence short-term (28-d) toxicity study (NTP, 2018, 2011) • High-confidence 2-generation study (Lieder et al., 2009b) • High-confidence subchronic study (Lieder et al., 2009a)	• No factors noted.	• A few small, statistically significant changes were not dose-dependent or were of questionable biological relevance. • Lack of effects on male mating and fertility, hormones, or reproductive organs in rats.	Statistically significant effects on sperm health (NTP, 2018; Lieder et al., 2009a) and delayed preputial separation at 1,000 mg/kg-d (Lieder et al., 2009b) were not dose-dependent, were within the normal range of historical controls for the laboratory, and/or were no longer significantly changed after correcting for other variables (e.g., BW). Other relevant parameters (e.g., organ weights, mating success, and so forth) were unchanged in the three studies.	

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Female reproductive effects				
<u>Mouse Studies:</u> • High-confidence gestational (GDs 1−20) exposure study (Feng et al., 2017) <u>Rat Studies:</u> • High-confidence short-term (28-d) toxicity study (NTP, 2018, 2011) • High-confidence subchronic study (Lieder et al., 2009a) • High-confidence 2-generation study (Lieder et al., 2009b)	• Effects on markers of female reproductive function (i.e., estrous cyclicity) were observed in high-confidence studies in rats and mice. • Changes in reproductive serum hormones were observed in female rats (i.e., increased testosterone) and mice (e.g., decreased estradiol and progesterone). Although the pattern of change is difficult to interpret and likely incomplete, there were no conflicting data.	• Lack of similar effects on reproductive function (i.e., estrous cyclicity) in a second high-confidence rat study. • Lack of effects on female fertility or pregnancy measures, although this was untested in prenatally exposed female mouse offspring. • Lack of organ weight changes in three rat studies. Note: The lack of effects on ovarian follicles in rats did not decrease the support for hazard provided by findings in mice, as the age at endpoint assessment was not comparable.	See "Developmental effects" (above) for findings from (Feng et al., 2017) and (Lieder et al., 2009b). Altered estrous cyclicity (including prolonged diestrus) and increased serum testosterone were observed in female rats after short-term exposure, primarily at ≥ 250 mg/kg-d (NTP, 2018, 2011). Female reproductive organ weights were reduced in gestationally exposed mouse offspring (Feng et al., 2017), but were unchanged after short-term, subchronic, or 2-generational exposure (NTP, 2018, 2011; Lieder et al., 2009a; Lieder et al., 2009b).	

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Renal effects				
Human studies				*Supports a hazard.* (animal evidence *supports a hazard*; human evidence is *equivocal*).
• Low-confidence cross-sectional study (Qin et al., 2016)	• No factors noted.	• Inconsistency across subpopulations in single study. • Single study of low confidence with concern for potential reverse causality.	Overall, there was no clear association for PFBS and uric acid. No association observed between PFBS and uric acid in the total population. Increase in uric acid with increased exposure in boys, but decrease for girls (neither was statistically significant).	
Animal studies (all oral gavage)				The primary basis for this judgment is kidney histopathology in rats, primarily females, at ≥300 mg/kg-d.
Rat Studies: • One high-confidence subchronic study (Lieder et al., 2009a) • Two high-confidence study (NTP, 2018, 2011; 3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study • One high-confidence 2-generation study (Lieder et al., 2009b)	• Two high-confidence studies with the longest exposure durations reported consistent effects on kidney histopathology in male and female rats (females were more sensitive). • The histopathological effects related to inflammation were largely dose-dependent and of a concerning magnitude, although primarily at high doses (300 or 600 mg/kg-d).	• Inconsistency in kidney weight changes across studies. • Findings are from a single laboratory and species. Note: The general lack of effects on other pathology endpoints in the shorter term studies was not considered to decrease support for hazard, as this was not interpreted as inconsistent.	Increases in kidney weight in male and female rats were observed in one short-term study at ≥ 62.5 mg/kg-d, but clear changes were not observed in the other short-term, subchronic, or two-generation rat studies. Kidney histopathology for some effects (i.e., CPN, hydronephrosis, tubular degeneration, and tubular dilation) was unchanged in single-study evaluations, and mixed results across studies were reported for mineralization and necrosis (NTP, 2018; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2000d). Multiple markers potentially related to inflammation and most notably papillary edema and hyperplasia were increased in the two longest duration studies (Lieder et al., 2009a; Lieder et al., 2009b), without contrary evidence. Other markers of renal injury, including BUN and creatinine, were mostly unaffected across studies (NTP, 2018, 2011; Lieder et al., 2009a; Lieder et al., 2009b; 3M, 2001, 2000d), although the NTP study did observe effects on BUN in males at ≥ 250 mg/kg-d.	

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Hepatic effects				
Human studies				*Equivocal* (*equivocal* human and animal evidence).
No studies available to evaluate	--	--	--	
Animal studies (all oral gavage)				
Rat Studies: • One high-confidence subchronic study (Lieder et al., 2009a) • Two high-confidence study (NTP, 2018, 2011; 3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study • One high-confidence 2-generation study (Lieder et al., 2009b)	• Consistent changes in liver weights in rats of both sexes across four studies. Although the pattern (e.g., by sex and dose) and magnitude of changes varied across studies, weights were consistently increased.	• Other than liver-weight changes, there were notable unexplained inconsistencies in the findings across studies. • One high-confidence study was entirely inconsistent.[a]	Absolute or relative liver weights were increased in all studies except the 90-d exposure component of the study by Lieder et al. (2009a), which tested doses up to 600 mg/kg-d. Note: 70 d of exposure in this study did elicit effects. Effects generally occurred at ≥ 300 mg/kg-d, although one study reported effects at lower doses (NTP, 2011; 3M, 2001), and two others (3M, 2001, 2000d) observed changes at ≥ 900 mg/kg-d. Serum markers of liver injury were unchanged in three studies (Lieder et al., 2009a; 3M, 2001, 2000d) and increased in one short-term study at ≥ 250 mg/kg-d (NTP, 2018, 2011). Liver histopathology, specifically hepatocellular hypertrophy and cytoplasmic alterations in males and females (NTP, 2018, 2011) or hypertrophy in females only (Lieder et al., 2009a), were noted in two studies, but not in the others.	
Lipid or lipoprotein homeostasis				
Human studies				*Equivocal* (*equivocal* human and animal evidence).
• Low-confidence cross-sectional study (Zeng et al., 2015)	• No factors noted.	• Association in a single study with concern for potential reverse causality.	Increase in total cholesterol (statistically significant, $\beta = 19.3$ mg/DL increase per unit increase in PFBS).	
Animal studies				

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Mouse Studies (diet): • Medium-confidence short-term (4−6 wk) study (Bijland et al., 2011); transgenic mice (human-like lipid metabolism) were fed a high-fat diet Rat Studies (all oral gavage): • One high-confidence subchronic study (Lieder et al., 2009a) • One high-confidence study (3M, 2001) and one medium-confidence (3M, 2000d) short-term (10−28 d) study	• Decreases in serum cholesterol and triglycerides were observed in male rats and mice.	• Inconsistent evidence in other rat studies and across sexes. • Small effect magnitudes and unclear direction (decreases) of changes are of questionable biological relevance and could not be informed by evaluating dose-dependency (i.e., only single-dose or high-dose effects were observed).	Serum lipids, specifically cholesterol and triglyceride levels, were slightly decreased (~20%) at 900 mg/kg-d in males, but not females, in one rat study (3M, 2001), but not in two other rat studies at up to 1,000 mg/kg-d. Serum and hepatic lipids and lipoproteins were also decreased in male mice exposed to ~30 mg/kg-d in diet.	
Immune effects				
Human studies				*Equivocal (equivocal human and animal evidence).*
Asthma				
• Medium-confidence case-control study (Zhou et al., 2016; Zhu et al., 2016; Dong et al., 2013b)	• Statistically significant association in a medium-confidence study. Note: Increases in eosinophil markers were not interpreted to increase support for hazard, as they were not statistically significant and other markers important to asthma etiology (e.g., IgE) were unchanged.	• Association was observed in a single study with concern regarding the potential for residual confounding (e.g., with other PFAS chemicals).	Statistically significant increase in odds of asthma diagnosis in the previous year (OR = 1.2−1.9) with increased PFBS exposure. Eosinophil markers (i.e., AEC and ECP) were increased with increased PFBS exposure in asthmatics and nonasthmatics; however, this did not reach statistical significance. IgE and T-helper cell-specific cytokines were unchanged (Zhu et al., 2016).	

Studies and confidence	Factors that increase support for hazard	Factors that decrease support for hazard	Summary of findings	Overall evidence integration judgment and basis
Atopic dermatitis				
• Medium-confidence cohort study ([Chen et al., 2018](#))	• No factors noted.	• Slight associations were not statistically significant in a single study with concern regarding the potential for residual confounding (e.g., with other PFAS chemicals).	Nonstatistically significant increase in odds of atopic dermatitis (OR = 1.2) with increased PFBS exposure.	
Animal studies				
No studies available to evaluate	--	--	--	

Notes:

a The lack of liver effects in the subchronic study was not interpreted to significantly reduce support for hazard, as the maximum tolerated dose was 600 mg/kg-d, and other studies reported only liver effects at ≥ 900 mg/kg-d.

T3 = triiodothyronine; T4 = thyroxine.

6.0   Derivation of Values

The hazard and dose-response database for PFBS and the potassium salt is primarily associated with the oral route of exposure. There are a limited number of dermal studies (see Table 5) and no known inhalation studies. There are no known studies evaluating potential cancer effects of PFBS. As such, only noncancer reference values are derived in this assessment for the oral route.

6.1   Derivation of Oral Reference Doses

6.1.1   Derivation of Candidate Subchronic RfDs

As described above, the hazards of potential concern for oral PFBS exposure include thyroid, developmental, and kidney effects. Thus, available studies that evaluated these effects are considered in the derivation of oral RfDs, and selected data sets from studies with multiple exposure levels for thyroid, developmental, and kidney effects were modeled using the EPA's Benchmark Dose Software (BMDS) Version 2.7.

Consistent with the EPA's *Benchmark Dose Technical Guidance Document* (U.S. EPA, 2012), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a benchmark response (BMR) to represent a minimal, biologically significant level of change. Based on BMD guidance, in the absence of information regarding the level of change that is considered biologically significant, a BMR of 1 SD from the control mean for continuous data or a BMR of 10% extra risk for dichotomous data is used to estimate the BMD and BMDL, and to facilitate a consistent basis of comparison across endpoints, studies, and assessments.

For thyroid hormone effects in pregnant females and offspring, a 20% RD was applied based on a biological level of concern. Multiple lines of evidence regarding the degree of thyroid hormone disruption and developmental outcomes in pregnant dams or offspring were considered in the identification of this BMR. During developmental life stages 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). 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; Patel et al., 2011). Indeed, human epidemiological studies have demonstrated key relationships between decreased levels of thyroid hormones such as T4 and *in utero* and early postnatal life neurodevelopmental status. For example, children born euthyroid but who were exposed to thyroid hormone insufficiency *in utero*, present with cognitive impairments (e.g., decreased intelligence quotient) and/or concomitant abnormalities in brain imaging (Korevaar et al., 2016; Lavado-Autric et al., 2003; Mirabella et al., 2000). With regard to what level of decrease in thyroid hormone is sufficient for anatomical and/or functional alterations, particularly in neurodevelopment in developing fetuses or newborns, several studies have identified a fairly stable range across humans and experimental rodents. 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). In other studies, mild-to-moderate thyroid insufficiency in pregnant women was defined as having serum T4 levels below the 10th percentile for the study population, which was associated with a 15%−30% decrease relative to the corresponding median (Finken et al., 2013; Julvez et al., 2013; Román et al., 2013; Henrichs et al., 2010). Similarly,

decreases in mean maternal T4 levels of ~10%−17% during pregnancy and lactation have been found to elicit neurodevelopmental toxicity in rat offspring (Gilbert et al., 2016; Gilbert, 2011). As the lower end of the range of T4 changes associated with untoward developmental health outcomes (e.g., 10%) commonly falls within normal experiment-to-experiment variation in control values, a BMR of 20% RD from control mean was determined to be a minimally biologically significant degree of change when performing BMD modeling on thyroid hormone alterations in pregnant females and associated offspring. For comparison purposes only, a BMR of 1 SD is presented as well. While significantly decreased thyroid hormone (e.g., T4 and T3) was also observed in adult rats exposed twice daily to oral K^+PFBS (NTP, 2018), a biologically significant level of change was not determined for the BMR as it is unclear what magnitude of hormone perturbation would be considered adverse in adult animals. As such, for thyroid hormone effects in nonpregnant adult rats, a BMR of 1 SD from control mean was applied.

For effects in the developing offspring, other than the thyroid, a BMR of 1 SD change from the control mean is used for continuous data to account for effects occurring in a sensitive life stage. For comparison purposes only, a 0.5 SD is presented as well.

For kidney hyperplasia data from the subchronic-duration study by Lieder et al. (2009a) and two-generation reproductive toxicity study by Lieder et al. (2009b), a BMR of 10% extra risk was used because it is the recommended approach for dichotomous data in the absence of information on the minimally significant level of change.

Where modeling was feasible, the estimated BMDLs were identified as PODs (summarized in Table 8). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in HAWC and are discussed in Appendix F. Where dose-response modeling was not feasible, NOAELs or LOAELs were identified (summarized in Table 8).

In *Recommended Use of Body Weight$^{3/4}$ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b), the EPA endorses a hierarchy of approaches to derive human equivalent oral exposures from data from laboratory animal species, with the preferred approach being physiologically based toxicokinetic modeling. Other approaches might include using some chemical-specific information, without a complete physiologically based toxicokinetic model. In the absence of chemical-specific models or data to inform the derivation of human equivalent oral exposures, the EPA endorses $BW^{3/4}$ as a default to extrapolate toxicologically equivalent doses of orally administered agents from all laboratory animals to humans for the purpose of deriving an RfD under certain exposure conditions. More specifically, the use of $BW^{3/4}$ scaling for deriving an RfD is recommended when the observed effects are associated with the parent compound or a stable metabolite, but not for portal-of-entry effects. Although the pharmacokinetic study by Olsen et al. (2009) suggests a longer half-life for K^+PFBS in humans than in rats, these results were obtained by single-dose administration and it is uncertain whether this reflects the compound's half-life after repeated dosing. The EPA considered the 2014 *Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation* in determining interspecies and intraspecies UFs (UF_{AS} and UF_{HS}, respectively) (U.S. EPA, 2014c). Using the decision process described in Figure 2 of that guidance (U.S. EPA, 2014c), the EPA concluded that data are inadequate to support derivation of data-derived extrapolation factors. Specifically, given the lack of available models and data to address external dose and clearance in humans with any certainty or the magnitude of difference in half-life across species as a function of dose or time, the default approach of the use of $BW^{3/4}$ scaling to obtain a HED is considered appropriate in this case.

Table 8. PODs considered for the derivation of the subchronic RfD for K⁺PFBS (CASRN 29420-49-3)

Endpoint/reference	Species/life stage— sex	POD_{HED}[a] (mg/kg-d)	Comments
Thyroid effects†			
Total T4—Feng et al. (2017)[b]	Mouse/P_0—Female	$BMDL_{20}$ = 7.8 ($BMDL_{1SD}$ = 3.4)	Adequate model fit
Free T4—Feng et al. (2017)[b]	Mouse/P_0—Female	NOAEL = 7.5	No models provided adequate statistical or visual fit to mean responses
TSH—Feng et al. (2017)[b]	Mouse/P_0—Female	NOAEL = 7.5	No models provided adequate statistical or visual fit to mean responses
Total T4 PND 1 (fetal n)[c]—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5	No models provided adequate fit to the data, specifically variance
Total T4 PND 1 (litter n)[c]—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{20}$ = 4.2 ($BMDL_{1SD}$ = 8.9)	Adequate model fit
Total T4 PND 30—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{20}$ = 7.8 ($BMDL_{1SD}$ = 7.0)	Adequate model fit
Total T4 PND 60—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5	No models provided adequate fit to the data, specifically variance
TSH PND 30—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5	No models provided adequate statistical or visual fit to mean responses
Total T4—NTP (2018)[d]	Rat—Male	LOAEL = 15.5	No models provided adequate statistical or visual fit to mean responses
	Rat—Female	$BMDL_{1SD}$ = 1.6	Adequate model fit
Free T4—NTP (2018)[d]	Rat—Male	LOAEL = 15.5	No models provided adequate statistical or visual fit to mean responses
	Rat—Female	LOAEL = 14.3	No models provided adequate statistical or visual fit to mean responses
Developmental effects			
Eyes opening (fetal n)[c]—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5	No models provided adequate fit to the data, specifically variance
Eyes opening (litter n)[c]—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 14.8 ($BMDL_{0.5SD}$ = 7.4)	Adequate model fit
Vaginal opening (fetal n)[c]—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 12.4 ($BMDL_{0.5SD}$ = 5.1)	Adequate model fit
Vaginal opening (litter n)[c]—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 7.9 ($BMDL_{0.5SD}$ = 3.3)	Adequate model fit
First estrous (fetal n)[c]—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5	No models provided adequate statistical or visual fit to mean responses

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Endpoint/reference	Species/life stage—sex	POD$_{HED}$[a] (mg/kg-d)	Comments
First estrous (litter *n*)[c]—Feng et al. (2017)	Mouse/F$_1$—Female	NOAEL = 7.5	No models provided adequate statistical or visual fit to mean responses
Kidney effects			
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009a)[e]	Rat—Male	BMDL$_{10}$ = 47.0	Adequate model fit
	Rat—Female	BMDL$_{10}$ = 12.6	Adequate model fit
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)[f]	Rat/P$_0$—Male	BMDL$_{10}$ = 20.0	Adequate model fit
	Rat/P$_0$—Female	BMDL$_{10}$ = 11.5	Adequate model fit
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)[f]	Rat/F$_1$—Male	BMDL$_{10}$ = 42.8	Adequate model fit
	Rat/F$_1$—Female	BMDL$_{10}$ = 20.2	Adequate model fit

Notes:

BMDL$_{10}$ = 10% benchmark dose lower confidence limit; BMDL$_{1SD}$ = benchmark dose lower confidence limit for 1 SD change from the control.

[a] Following U.S. EPA (2011b) guidance, animal doses from candidate principal studies were converted to HEDs through the application of a dosimetric adjustment factor (DAF), where HED = dose × DAF. DAFs for each dose are calculated as follows: DAF = (BW$_a^{1/4}$ ÷ BW$_h^{1/4}$), where BW$_a$ = animal BW and BW$_h$ = human BW. For all DAF calculations, a reference BW$_h$ of 80 kg (U.S. EPA, 2011a) was used. The default BW$_h$ assumption was updated from 70 kg to 80 kg based on NHANES data from 1999 to 2006 and represents the mean weight for adults ages 21 and older (U.S. EPA, 2011a). The BW$_h$ also includes estimates of BW of pregnant women reported in the EPA's *Exposure Factors Handbook* (U.S. EPA, 2011a).

[b] DAFs were calculated using dam terminal BWs (BW$_a$) as reported by the study authors for ICR mice. For example, dams in the 200 mg/kg-day group had a terminal BW of 0.0399 kg. The DAF was calculated DAF = (BW$_a^{1/4}$ ÷ BW$_h^{1/4}$) = (0.0399$^{1/4}$ ÷ 80$^{1/4}$) = 0.149. The HED was calculated as dose × DAF = 200 × 0.149 = 29.9 mg/kg-day.

[c] Fetal endpoints from Feng et al. (2017) were modeled alternatively using dose group sizes based either on total number of fetuses or dams. Given that it appears that Feng et al. (2017) did not use the litter as the statistical unit of analysis, it is unclear if the study-reported standard errors pertain to litters or fetuses. Alternatively, modeling fetal endpoints using litter *n* or fetal *n* provides two modeling results that bracket the "true" variance among all fetuses in a dose group. Individual animal data were requested from study authors but were unable to be obtained.

[d] DAFs were calculated using study-specific time-weighted average BW (BW$_a$) data for female and male S-D rats using study-specific weekly BW data.

[e] DAFs were calculated using study-specific time-weighted average BW$_a$ data for female and male S-D rats using study-specific initial and terminal BW data. For example, female rats in the 600 mg/kg-day group had a time-weighted average BW of 211.35 g. The DAF was calculated as follows: DAF = (BW$_a^{1/4}$ ÷ BW$_h^{1/4}$) = (0.21135$^{1/4}$ ÷ 80$^{1/4}$) = 0.2344. The HED was calculated as dose × DAF = 600 × 0.2344 = 140.65.

[f] For the P$_0$-male rats (S-D), DAFs were calculated using BWs (BW$_a$) estimated from study-specific initial and terminal BWs. For the P$_0$-female rats, DAFs were calculated using BWs (BW$_a$) estimated from study-specific initial, day 70, LD 1, and LD 22 BWs. For the F$_1$-male rats, DAFs were calculated using BWs (BW$_a$) estimated from study-specific postweaning day 1 and terminal BWs. For the F$_1$-female rats, DAFs were calculated using BWs (BW$_a$) estimated from study-specific postweaning day 1, day of cohabitation, LD 1, and LD 22 BWs.

[‡] BMD modeling methods and links to modeling inputs and results in HAWC are found in appendix F.

HAWC visualization: Candidate PODs for Subchronic and Chronic RfD

As presented in Table 8, effects in the thyroid were considered when determining potential PODs for derivation of a subchronic RfD. Similar patterns of decreases in total T3, total T4, and free T4 were observed in PFBS-exposed pregnant mice, nonpregnant adult female rats, adult male rats, and gestationally exposed female mouse offspring (NTP, 2018; Feng et al., 2017). Reflex increases in TSH in response to decreased T4 or T3 were not observed in male or female rats following 28 days of exposure. Such an increase in TSH was observed in pregnant mice and their corresponding female offspring, at PND 30 only, with an irregular dose-response or time course. This pattern of decreased thyroid hormone without a concomitant increase in TSH is consistent with a human clinical status referred to as "hypothyroxinemia." Importantly, it has been noted that milder forms of thyroid perturbation are up to 10 times more prevalent in human populations than overt gestational hypothyroidism (Korevaar et al., 2016; Stagnaro-Green et al., 2011). Hypothyroxinemia has been associated with impairments in neurodevelopment and/or cognition later in life (Thompson et al., 2018; Min et al., 2016). As discussed in the opening of the "Derivation of a Subchronic RfD" section above (i.e., identification of a biologically informed BMR for thyroid hormone effects), several studies have identified a fairly stable range across humans and experimental rodents at which decrements in thyroid hormone levels are associated with developmental health outcomes. This hypothyroxinemia, rather than overt hypothyroidism, is further supported by the lack of effect on thyroid weight or tissue architecture in rats after 28 days of PFBS exposure, which might be expected in a condition that lacks a coordinated feedback activation of TSH (NTP, 2018). While both the Feng et al. (2017) and NTP (2018) studies are considered high confidence, it should be noted that the NTP thyroid data is nonpeer-reviewed and has not yet been published in a completed report. However, NTP studies are conducted with the highest level of standards and laboratory practices and employ study designs highly relevant for the systematic evaluation of chemical toxicities.

Developmental effects, as presented in Table 8, were considered in the determination of the potential PODs for derivation of a subchronic RfD. Specifically, in Feng et al. (2017), developmental delays or abnormalities in growth (i.e., BW and eye opening), reproductive organs (i.e., ovaries, uterus, and vaginal opening), and reproductive cycling (i.e., first estrous and prolongation of diestrus) were observed in mouse offspring. These effects were observed in mice from litters in which thyroid hormone deficiency occurred at PND 1 and was sustained through pubertal and adult periods (i.e., PND 30 and PND 60, respectively).

As presented in Table 8, effects in the kidney were considered when determining the potential PODs for derivation of a subchronic RfD. Mild-to-moderate hyperplasia was reported in the kidneys of male and female rats following subchronic-duration exposure to PFBS by Lieder et al. (2009a) and in the P0- and F1-generation animals of the reproductive toxicity study by Lieder et al. (2009b). Other studies evaluating effects in the kidney were of shorter duration and thus less suitable as a candidate principal study. Additional histopathological alterations accompanied the hyperplasia observed in the kidney, including papillary edema and inflammatory changes, specifically increases in chronic pyelonephritis, tubular basophilia, and mononuclear cell infiltration (Lieder et al., 2009a; Lieder et al., 2009b). Across kidney histopathological effects reported following PFBS exposure, in general, female rats were more sensitive than males. Although histopathological effects in the kidney have not been reported in human populations exposed to PFBS, the spectrum of renal effects observed in rodents is presumed to be relevant to similar changes known to occur in humans.

Across the body of evidence supporting hazards via the oral exposure route, candidate RfDs were derived for the most sensitive effects in the thyroid and kidney. Across all life stages evaluated, the thyroid (specifically, decreased thyroid hormone [T4]) was identified as the most sensitive target of PFBS toxicity. It should be noted that developmental effects (e.g., delayed eyes opening, vaginal opening, or first estrous) were often observed in mouse litters in which decrements in thyroid hormone occurred (Feng et al., 2017). However developmental effects appeared to be less sensitive than thyroid hormone perturbations in developing mice, with PODs ranging from 7.5–14.8 mg/kg-day compared to 4.2–8.9, respectively (see Table 8). Thus, the thyroid and kidney effects were carried forward for derivation of candidate RfDs.

When deriving the candidate subchronic RfD based on thyroid effects, the Feng et al. (2017) and NTP (2018) studies were both considered for potential principal study due to the observed sensitivity of thyroid hormone decrements. However, the biological significance of hypothyroxinemia (i.e., decreased T4) in nonpregnant adult animals is unclear; therefore, the thyroid effects from the NTP (2018) study were not selected as a critical effect. Instead, the gestational exposure study in mice was selected as the principal study for derivation of the candidate subchronic RfD based on thyroid effects. The gestational exposure study conducted by Feng et al. (2017) reports administration of K^+PFBS by gavage in ICR mice (10/dose) from GDs 1 to 20. This study was of good quality (i.e., high confidence) with adequate reporting and consideration for appropriate study design, methods, and conduct (click to see risk of bias analysis in HAWC). Feng et al. (2017) reported statistically significantly decreased total T3, total T4, and free T4, as well as increased TSH in dams and offspring (increased TSH PND 30 only) gestationally exposed to PFBS.

The critical effect from the Feng et al. (2017) study is decreased serum total thyroxine (T4) in newborn (PND 1) mice (Feng et al., 2017). T4 and T3 are essential for normal growth of developing offspring across animal species (for review see Forhead and Fowden (2014)). And, previous studies have shown that exposure to other PFAS during pregnancy results in lower T4 and T3 levels in pregnant women and fetuses or neonates (Yang et al., 2016; Wang et al., 2014). The selection of total T4 as the critical effect is based on a number of key considerations (see below) that account for cross-species correlations in thyroid physiology and hormone dynamics particularly within the context of a developmental life stage.

A key consideration for selection of total T4 is that this represents the aggregate of potential thyroid endocrine signaling (i.e., free T4 + protein bound T4) at any given time. Although T3 is the more active hormone form in respondent somatic tissues, the formation of T3 is contingent upon the deiodination of free T4. T4, not T3, is the thyroid hormone that crosses the placenta of humans and rodents. Although free T4 might be considered a suitable measure of thyroid hormone status in non-developmental (e.g., adult) life stages, there are some important factors associated with maintenance of the microenvironment for a developing offspring *in utero* that lends credence to the use of total T4 as the critical effect. A tightly regulated transfer of maternal thyroid hormone to a fetus is paramount to proper development of multiple tissues and organ systems (e.g., nervous system), especially during the early trimesters. The placenta has transporters and deiodinases that collectively act as a gatekeeper to maintain an optimal T4 microenvironment in the fetal compartment (Fisher, 1997; Koopdonk-Kool et al., 1996). For example, deiodinase 3 (D3) is highly expressed in human uterus, placenta, and amniotic membrane, where it serves a critical role of regulating thyroid hormone transfer to the fetus through the deiodination of T4 to

transcriptionally inactive reverse triiodothyronine (rT3) or T3 to inactive 3,5-diiodo-L-thyronine (T2). Similarly, Wasco et al. (2003) showed that D3 is highly expressed in rodent uterus and is highly induced during pregnancy. Further, the *Dio3* gene that encodes D3 has been shown to be imprinted in the mouse (Hernandez et al., 2002), suggesting a pivotal role for this specific deiodinase in the mouse as well. Indeed, the human and rodent placenta have been shown to be similarly permeable to T4 and T3 (Fisher, 1997; Calvo et al., 1992). Due to placental barrier functionality, free T4 levels in a pregnant dam might not be entirely representative of actual T4 status in a developing fetus. Thus, decreased total T4 in offspring is expected to be more representative of PFBS-mediated thyroid effects and potentially associative developmental effects.

There is some difference in HPT development and functional maturation and regulation during early life stages (e.g., timing of *in utero* and early postnatal thyroid development) between humans and rodents. For example, the human thyroid gland is not formed until the second trimester of pregnancy, whereas in rodents the fetal thyroid is not formed and functional until the 17th day of gestation, which is just days before birth (typically GD 20). As such, rodent neurodevelopment in the early postnatal phase is analogous to the third trimester of human development *in utero* (Gilbert et al., 2012). However, within the context of early developmental life stages, there are several commonalities in HPT dynamics between humans and rodents such as similar profiles of (1) thyroid hormone binding proteins, (2) hormone functional reserve, and (3) placental deiodinase. For example, two carrier proteins—thyroid binding globulin (TBG) and transthyretin (TTR)—are primarily responsible for storage and transit of T4 in mammals. TBG is the primary carrier of T4 in humans across all life stages (Savu et al., 1991). Importantly, in fetal and infant rats, TBG is also the primary carrier of T4 (Savu et al., 1989). As rats transition to adulthood, TTR takes over as the primary carrier of T4. In addition, as a relatively highly abundant carrier protein, albumin also plays a role in thyroid hormone binding and transit in humans and rodents; however, the relative affinity for binding is lower than either TBG or TTR.

Life stage-specific differences in thyroid hormone reserve capacity between adults and neonates have been noted. On average, intrathyroidal thyroglobulin stores in adults are on the order of months whereas in neonates the functional reserve is approximated at less than 1 day (Gilbert and Zoeller, 2010; Savin et al., 2003; Van Den Hove et al., 1999). This suggests that the adult thyroid has compensatory abilities not present in early life stages, making fetal/neonatal populations particularly sensitive to perturbations in thyroid hormone economy. As human and rodent neonates are posited to be more alike than different, the dynamic reserve capacity of T4 between species near birth and in early postpartum might not be significantly different. Human neonates have a serum half-life of T4 of approximately 3 days (Vulsma et al., 1989), and thyroid tissue stores of T4 are estimated to be less than 1 day (Van Den Hove et al., 1999). As the developing rodent thyroid does not begin producing its own hormone until late in gestation ($\geq$ GD 17), newborn rodent T4 levels are primarily a reflection of transplacentally translocated maternal hormone; and adult rats have been shown to have a serum T4 half-life of 0.5–1 day (Choksi et al., 2003). As such, significant differences in functional thyroid reserve capacity between human and rodent neonates is not anticipated.

Accounting for the information presented above, the candidate subchronic RfD for thyroid, based on the $BMDL_{20}$ (HED) of 4.2 mg/kg-day for serum total T4 in newborn (PND 1) mice, is derived as follows:

Candidate Subchronic RfD for K⁺PFBS (Thyroid)   = $BMDL_{20}$ (HED) ÷ UF_C
                                                                          =   4.2 mg/kg-day ÷ 100
                                                                          =   0.042 mg/kg-day
                                                                          =   **4×10^{-2} mg/kg-day**

Table 9 summarizes the UFs for the candidate subchronic RfD for K⁺PFBS based on effects in the thyroid.

Table 9. UFs for the candidate subchronic RfD for thyroid effects for K⁺PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for uncertainty in characterizing the toxicokinetic and toxicodynamic differences between mice and humans following oral K⁺PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the EPA's *Recommended Use of Body Weight³ᐟ⁴ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b); however, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, the EPA's guidance recommends use of a UF_A of 3.
UF_D	3	A UF_D of 3 is applied due to database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2018; Bijland et al., 2011; NTP, 2011; 3M, 2010; Lieder et al., 2009a; 3M, 2001, 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002). However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.
UF_H	10	A UF_H of 10 is applied for interindividual variability in the absence of quantitative information on the toxicokinetics and toxicodynamics of K⁺PFBS/PFBS in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR was selected based on evidence that it represented a minimal biologically significant response level in susceptible populations such as pregnant mice and developing offspring.
UF_S	1	A UF_S of 1 is applied because the POD comes from a developmental study in mice. The developmental period is recognized as a susceptible life stage in which exposure during certain time windows (e.g., gestational) is more relevant to the induction of developmental effects than lifetime exposure (U.S. EPA, 1991a).
UF_C	100	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

In the derivation of the candidate subchronic RfD based on kidney effects, the limited number of repeat-dose studies in experimental rats were considered and consistently identified the kidney as a target of PFBS toxicity in adults (Lieder et al., 2009a; Lieder et al., 2009b) with PODs ranging from 11.5─47.0 mg/kg-day (see Table 8). Based on the dose-response modeling presented in Table 8, the P0 female rats from the reproductive toxicity study by Lieder et al. (2009b) provided the most sensitive measure of kidney toxicity, that is, an increased incidence of mild to moderate

papillary epithelial tubular/ductal hyperplasia in the female rat. The Lieder et al. (2009b) study was peer-reviewed, applied established approaches, recommendations, and best practices, and employed an appropriate exposure design for evaluating systemic toxicity endpoints (Organisation for Economic Co-operation and Development [OECD] 416; the Office of Prevention, Pesticides and Toxic Substances [OPPTS] 870.3800), and was rated high confidence. Kidney effects observed, such as papillary epithelial tubular/ductal hyperplasia, could influence the ability of the kidney to filter waste. Therefore, the kidney endpoint was identified as a critical effect for derivation of a candidate subchronic RfD for K⁺PFBS based on the $BMDL_{10}$ (HED) of 11.5 mg/kg-day for papillary epithelial tubular/ductal hyperplasia in female P_0 rats (Lieder et al., 2009b). The candidate subchronic RfD for kidney is derived as follows:

Candidate Subchronic RfD for K⁺PFBS (Kidney)	=	$BMDL_{10}$ (HED) ÷ UF_C
	=	11.5 mg/kg-day ÷ 100
	=	0.12 mg/kg-day
	=	**1×10^{-1} mg/kg-day**

Table 10 summarizes the UFs for the candidate subchronic RfD for K⁺PFBS based on effects in the kidney.

Table 10. UFs for the candidate subchronic RfD for kidney effects for K⁺PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for toxicokinetic and toxicodynamic differences between rats and humans following oral K⁺PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the EPA's *Recommended Use of Body Weight³ᐟ⁴ as the Default Method in Derivation of the Oral Reference Dose* (U.S. EPA, 2011b); however, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's guidance recommends use of a UF of 3.
UF_D	3	A UF_D of 3 is applied due to database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals (NTP, 2018; Bijland et al., 2011; NTP, 2011; 3M, 2010; Lieder et al., 2009a; 3M, 2001; 2000d), a two-generation reproductive toxicity study in rats (Lieder et al., 2009b), and multiple developmental toxicity studies in mice and rats (Feng et al., 2017; York, 2002). However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.
UF_H	10	A UF_H of 10 is applied for interindividual variability in susceptibility in the absence of quantitative information on the toxicokinetics and toxicodynamics of K⁺PFBS/PFBS in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR was selected based on evidence that it represented a minimal biologically significant response level in adult rats.
UF_S	1	A UF_S of 1 is applied because the POD comes from a subchronic-duration study of rats.
UF_C	100	Composite Uncertainty Factor = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

Candidate subchronic RfDs derived for thyroid effects and kidney effects are presented in Table 11.

Table 11. Summary of candidate noncancer subchronic reference values for K⁺PFBS (CASRN 29420-49-3)

Critical effect	POD method	POD (HED) (mg/kg-d)	UF$_C$	Reference dose (mg/kg-d)
Decreased serum total T4 in newborn (PND 1) mice—Feng et al. (2017).	BMDL$_{20}$	4.2	100	4×10^{-2}
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia in P0 female rats—Lieder et al. (2009b)	BMDL$_{10}$	11.5	100	1×10^{-1}

The data for K⁺PFBS can be used to derive a subchronic RfD for the free acid (PFBS), as K⁺PFBS is fully dissociated in water at the environmental pH range of 4−9 (NICNAS, 2005). To calculate the subchronic RfD for the free acid, the subchronic RfD for the potassium salt is adjusted to compensate for differences in MW between K⁺PFBS (338.19) and PFBS (300.10). The subchronic RfD for PFBS (free acid) for thyroid and kidney effects are the same as the values for the K⁺PFBS salt. The calculations are as follows:

Subchronic RfD	=	RfD for K⁺PFBS salt × (MW free acid ÷ MW salt)
for PFBS (free acid)	=	0.042 mg/kg-day × (300.10 ÷ 338.19)
for Thyroid Effects	=	0.042 mg/kg-day × (0.89)
	=	0.037 mg/kg-day
	=	**4×10^{-2} mg/kg-day**

Subchronic RfD	=	RfD for K⁺PFBS salt × (MW free acid ÷ MW salt)
for PFBS (free acid)	=	0.12 mg/kg-day × (300.10 ÷ 338.19)
for Kidney Effects	=	0.12 mg/kg-day × (0.89)
	=	0.11 mg/kg-day
	=	**1×10^{-1} mg/kg-day**

The confidence in the candidate subchronic RfD for PFBS and K⁺PFBS for both thyroid and kidney effects is medium, as explained in Tables 12 and 13.

Table 12. Confidence descriptors for candidate subchronic RfD (Thyroid Effects) for PFBS (CASRN 375-73-5) and the related compound K⁺PFBS (CASRN 29420-49-3)

Confidence categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.
Confidence in database	M	Confidence in the oral toxicity database for derivation of the candidate subchronic RfD for thyroid effects is medium because although there are multiple developmental toxicity studies in mice and rats, no studies are available that have specifically evaluated neurodevelopmental effects.
Confidence in candidate subchronic RfD	M	The overall confidence in the candidate subchronic RfD for thyroid effects is medium.

Notes: H = high; M = medium

Table 13. Confidence descriptors for the candidate subchronic RfD (Kidney Effects) for PFBS (CASRN 375-73-5) and the related compound K⁺PFBS (CASRN 29420-49-3)

Confidence categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.
Confidence in database	M	Confidence in the oral toxicity database for derivation of the candidate subchronic RfD for kidney effects is medium because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, and one acceptable two-generation reproductive toxicity study in rats, the database lacks studies that have specifically evaluated neurodevelopmental effects.
Confidence in candidate subchronic RfD	M	The overall confidence in the candidate subchronic RfD for kidney effects is medium.

Notes: H = high; M = medium.

The subchronic RfD is derived to be protective of all types of effects across studies and species following oral subchronic exposure and is intended to protect sensitive subpopulations and life stages. In light of the consistent observation of the thyroid effects across life stages and the greater dose-response sensitivity, relative to the kidney, EPA is proposing to base the overall subchronic RfD on the thyroid effects. See the Federal Register Notice announcing the availability of the draft assessment for PFBS and requesting public review and comment on this proposal in addition to the approaches and conclusions in the PFBS assessment.

6.1.2 Derivation of Candidate Chronic RfDs

There are no chronic-duration studies available for PFBS and K⁺PFBS. Therefore, based on the same database and similar considerations for the subchronic RfD, candidate noncancer chronic RfDs were derived for thyroid effects and kidney effects.

The candidate chronic RfD for thyroid effects following exposure to K^+PFBS, based on the $BMDL_{20}$ (HED) of 4.2 mg/kg-day for serum total T4 in newborn (PND 1) mice ([Feng et al., 2017](#)), is derived as follows:

Candidate Chronic RfD for K^+PFBS (Thyroid)	=	$BMDL_{20}$ (HED) ÷ UF_C
	=	4.2 mg/kg-day ÷ 300
	=	0.014 mg/kg-day
	=	**1×10^{-2} mg/kg-day**

Table 14 summarizes the UFs for the candidate chronic RfD for K^+PFBS based on effects in the thyroid.

Table 14. UFs for the candidate chronic RfD for thyroid for K^+PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF_A	3	A UF_A of 3 ($10^{0.5}$) is applied to account for toxicokinetic and toxicodynamic differences between mice and humans following oral K^+PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the EPA's *Recommended Use of Body Weight[3/4] as the Default Method in Derivation of the Oral Reference Dose* ([U.S. EPA, 2011b](#)); however, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, the EPA's guidance recommends use of a UF_A of 3.
UF_D	10	A UF_D of 10 is applied to account for database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals ([NTP, 2018](#); [Bijland et al., 2011](#); [NTP, 2011](#); [Lieder et al., 2009a](#); [3M, 2001](#), [2000d](#)), a two-generation reproductive toxicity study in rats ([Lieder et al., 2009b](#)), and multiple developmental toxicity studies in mice and rats ([Feng et al., 2017](#); [York, 2002](#)). However, as thyroid hormone is known to be critical during developmental life stages, particularly for neurodevelopment, the database is limited by the lack of developmental neurotoxicity studies. Further, due to the lack of chronic duration studies, there is additional uncertainty regarding how longer-term exposures might impact hazard identification and dose-response assessment for PFBS via the oral route (e.g., potentially more sensitive effects). Lastly, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.
UF_H	10	A UF_H of 10 is applied for interindividual variability in susceptibility in the absence of quantitative information on the toxicokinetics and toxicodynamics of K^+PFBS/PFBS in humans.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR selected based on evidence that it represented a minimal biologically significant response level in susceptible populations such as pregnant mice and developing offspring.
UF_S	1	A UF_S of 1 is applied because the POD comes from a developmental study of mice. The developmental period is recognized as a susceptible life stage in which exposure during certain time windows (e.g., gestational) is more relevant to the induction of developmental effects than lifetime exposure ([U.S. EPA, 1991b](#)). The additional concern over potential hazards following longer-term (chronic) exposures is accounted for under the UF_D above.
UF_C	300	Composite UF = $UF_A \times UF_D \times UF_H \times UF_L \times UF_S$

The candidate chronic RfD for effects of exposure to K^+PFBS on the kidney, based on the BMDL$_{10}$ (HED) of 11.5 mg/kg-day for papillary epithelial tubular/ductal hyperplasia in female P$_0$ rats ([Lieder et al., 2009b](#)), is derived as follows:

Candidate Chronic RfD for K^+PFBS (Kidney)	=	BMDL$_{10}$ (HED) ÷ UF$_C$
	=	11.5 mg/kg-day ÷ 1,000
	=	0.12 mg/kg-day
	=	**1×10^{-2} mg/kg-day**

Table 15 summarizes the UFs for the candidate chronic RfD for K^+PFBS based on effects in the kidney.

Table 15. UFs for the candidate chronic RfD for kidney effects for K^+PFBS (CASRN 29420-49-3)

UF	Value	Justification
UF$_A$	3	A UF$_A$ of 3 ($10^{0.5}$) is applied to account for toxicokinetic and toxicodynamic differences between rats and humans following oral K^+PFBS/PFBS exposure. Some aspects of the cross-species extrapolation of toxicokinetic and toxicodynamic processes have been accounted for by calculating an HED by applying a DAF as outlined in the EPA's *Recommended Use of Body Weight[3/4] as the Default Method in Derivation of the Oral Reference Dose* ([U.S. EPA, 2011b](#)); however, some residual uncertainty remains. In the absence of chemical-specific data to quantify this uncertainty, EPA's guidance recommends use of a UF of 3.
UF$_D$	3	A UF$_D$ of 3 is applied due to database deficiencies. The oral exposure database contains multiple short-term and subchronic-duration toxicity studies of laboratory animals ([NTP, 2018](#); [Bijland et al., 2011](#); [NTP, 2011](#); [3M, 2010](#); [Lieder et al., 2009a](#); [3M, 2001](#); [2000d](#)), a two-generation reproductive toxicity study in rats ([Lieder et al., 2009b](#)), and multiple developmental toxicity studies in mice and rats ([Feng et al., 2017](#); [York, 2002](#)). However, the observation of decreased thyroid hormone is known to be a crucial element during developmental life stages, particularly for neurodevelopment, and the database is limited by the lack of developmental neurotoxicity studies. In addition, as immunotoxicity is an effect of increasing concern across several members of the larger PFAS family, the lack of studies evaluating this outcome following PFBS exposure is a limitation in the database.
UF$_H$	10	A UF$_H$ of 10 is applied for interindividual variability in susceptibility in the absence of quantitative information on the toxicokinetics and toxicodynamics of K^+PFBS/PFBS in humans.
UF$_L$	1	A UF$_L$ of 1 is applied for LOAEL-to-NOAEL extrapolation because the POD is a BMDL and the BMR was selected based on evidence that it represented a minimal biologically significant response level in adult rats.
UF$_S$	10	A UF$_S$ of 10 is applied to account for less than chronic-duration exposure because the POD comes from a subchronic duration study.
UF$_C$	1,000	Composite Uncertainty Factor = UF$_A$ × UF$_D$ × UF$_H$ × UF$_L$ × UF$_S$

Candidate chronic RfDs derived for thyroid effects and kidney effects are presented in Table 16.

Table 16. Summary of candidate noncancer chronic reference values for PFBS (CASRN 375-73-5) and related compound K⁺PFBS (CASRN 29420-49-3)

Critical effect	POD method	POD (HED) (mg/kg-d)	UF$_C$	Reference dose (mg/kg-d)
Decreased serum total T4 in newborn (PND 1) mice—Feng et al. (2017).	BMDL$_{20}$	4.2	300	1×10^{-2}
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia in P0 female rats—Lieder et al. (2009b)	BMDL$_{10}$	11.5	1000	1×10^{-2}

Using the same calculation used for adjusting K⁺PFBS to PFBS for the subchronic RfDs, the candidate chronic RfDs for PFBS (free acid) for thyroid and kidney effects are the same as the values for the K⁺PFBS salt, as shown in Table 16.

The confidence in the candidate chronic RfDs for PFBS and K⁺PFBS for both thyroid and kidney effects is low, as explained in Table 17 and 18 below.

Table 17. Confidence descriptors for candidate chronic RfD (Thyroid Effects) for PFBS (CASRN 375-73-5) and the related compound K⁺PFBS (CASRN 29420-49-3)

Confidence categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.
Confidence in database	L	Confidence in the oral toxicity database for derivation of the chronic RfD is low because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, one acceptable two-generation reproductive toxicity study in rats, and multiple developmental toxicity studies in mice and rats, the database lacks any chronic duration exposure studies or studies that have evaluated neurodevelopmental or immunological effects.
Confidence in candidate chronic RfD	L	The overall confidence in the candidate chronic RfD for thyroid effects is low.

Notes: H = high; L = low

Table 18. Confidence descriptors for the candidate chronic RfD (Kidney Effects) for PFBS (CASRN 375-73-5) and the related compound K+PFBS (CASRN 29420-49-3)

Confidence categories	Designation	Discussion
Confidence in study	H	Confidence in the principal study is high because the overall study design, performance, and characterization of exposure was good. Study details and risk of bias analysis can be found in HAWC.
Confidence in database	L	Confidence in the oral toxicity database for derivation of the chronic RfD is low because, although there are multiple short-term studies and a subchronic-duration toxicity study in laboratory animals, one acceptable two-generation reproductive toxicity study in rats, and multiple developmental toxicity studies in mice and rats, the database lacks any chronic duration exposure studies or studies that have evaluated neurodevelopmental or immunological effects.
Confidence in candidate chronic RfD	L	The overall confidence in the candidate chronic RfD for kidney effects is low.

Notes: H = high; L = low

The chronic RfD is derived to be protective of all types of effects across studies and species following oral chronic exposure and is intended to protect the population as a whole, including potentially susceptible populations and life stages (U.S. EPA, 2002). This value should be applied in general population risk assessments. Decisions concerning averaging exposures over time for comparison with the RfD should consider the types of toxicological effects and specific life stages of concern. For example, fluctuations in exposure levels that result in elevated exposures during development could potentially lead to an appreciable risk, even if average levels over the full exposure duration were less than or equal to the RfD. In light of the consistent observation of the thyroid effects across life stages and the greater dose-response sensitivity, relative to the kidney effects, EPA is proposing to base the overall chronic RfD on the thyroid effects. See the Federal Register Notice announcing the availability of the draft assessment for PFBS and requesting public review and comment on this proposal in addition to the approaches and conclusions in the PFBS assessment.

6.2   Derivation of Inhalation Reference Concentrations

No published studies investigating the effects of subchronic- or chronic-duration inhalation toxicity of PFBS and the related compound K$^+$PFBS in humans or animals have been identified.

6.3   Cancer Weight-of-Evidence Descriptor and Derivation of Cancer Risk Values

No studies evaluating the carcinogenicity of PFBS or K$^+$PFBS in humans or animals were identified. In accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), the EPA concluded that there is "inadequate evidence to assess carcinogenic potential" for PFBS and K$^+$PFBS by any route of exposure. Therefore, the lack of data on the carcinogenicity of PFBS and the related compound K$^+$PFBS precludes the derivation of quantitative estimates for either oral (oral slope factor) or inhalation (inhalation unit risk) exposure.

6.4   Susceptible Populations and Life Stages

Early life stages as well as pregnant women are potentially susceptible to PFBS exposure. PFBS has been detected in blood serum of nursing mothers, which might indicate a potential for

lactational exposure (Glynn et al., 2012); however, information on the kinetics of lactational transfer are lacking.

The available information suggests sex-specific variation in the toxicokinetics of PFBS in rodents. Studies in mice and rats report clearance and elimination half-life times to be faster for females than for males (see the "Toxicokinetics" section). For example, Chengelis et al. (2009) reported that the mean apparent clearance of PFBS from the serum was approximately eightfold higher for female rats (0.311 L/h/kg) than for male rats (0.0394 L/h/kg) and the mean apparent volume of distribution for PFBS in the serum was approximately 2.4-fold higher for female rats (0.288 L/kg) than for male rats (0.118 L/kg). Olsen et al. (2009) reported a statistically significant difference in the urinary clearance rates ($p \leq 0.01$) but not in serum half-lives, with female rats (469 ± 40 mL/hour) having faster clearance rates than male rats (119 ± 34 mL/h). Statistically significant sex-related differences in half-life or clearance were not observed between male and female monkeys Olsen et al. (2009). Differences in the toxicokinetics in rodents could result in sex-specific differences in toxicity studies.

In vivo toxicity studies report that PFBS exposure can alter thyroid hormone levels in parental and F1 generations animals (see "Thyroid Effects"). Thyroid hormones play a critical role in coordinating complex developmental processes for various organs/systems (e.g., reproductive and nervous system), and disruption of thyroid hormone production/levels in a pregnant woman or neonate can have persistent adverse health effects for the developing offspring (Ghassabian and Trasande, 2018; Foster and Gray, 2013; Julvez et al., 2013; Román et al., 2013).

Animal studies also provide evidence that gestationally exposed females might be a susceptible subpopulation because of potential effects on female reproduction, including evidence of altered ovarian follicle development and delayed vaginal opening (see "Reproductive Effects"). Furthermore, gestationally exposed females also had significantly reduced BWs and delayed eye opening. These findings suggest that developmental landmarks indicative of adverse responses can be affected after PFBS exposure (see "Offspring Growth and Early Development").

Appendix A: Literature Search Strategy

This appendix presents the full details of the literature search strategy used to identify primary, peer-reviewed literature pertaining to perfluorobutane sulfonic acid (PFBS) (Chemical Abstracts Service Registry Number [CASRN] 375-73-5) and/or the potassium salt (K⁺PFBS) (CASRN 45187-15-3). Initial database searches were conducted on July 18, 2017 using four online scientific databases (PubMed, Web of Science [WOS], Toxline, and TSCATS via Toxline) and last updated on February 28, 2018. The literature search focused on chemical name and synonyms (see Table A-1) with no limitations on publication type, evidence stream (i.e., human, animal, *in vitro*, and *in silico*) or health outcomes. Beyond database searches, references were also identified from studies submitted under the Toxic Substances Control Act (TSCA) and from review of other government documents (e.g., Agency for Toxic Substances and Disease Registry [ATSDR]) and combined with the results of the database search. Search results are retained in the EPA's Health and Environmental Research Online (HERO) database.

Table A-1. Synonyms and MESH terms

ChemID	375-73-5 1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid 1-Perfluorobutanesulfonic acid Nonafluoro-1-butanesulfonic acid Nonafluorobutanesulfonic acid Perfluorobutanesulfonic acid PFBS 1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid
PubMed (new only)	Perfluorobutane sulfonic acid Perfluorobutanesulfonate Perfluorobutane sulfonate
EPA Spreadsheet	1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid 1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro- 1-Butanesulfonic acid, nonafluoro- 1-Perfluorobutanesulfonic acid Nonafluoro-1-butanesulfonic acid Nonafluorobutanesulfonic acid PFBS Perfluoro-1-butanesulfonate Perfluorobutane Sulfonate Perfluorobutanesulfonate Perfluorobutanesulfonic acid Perfluorobutylsulfonate 45187-15-3

Note: MESH = Medical subject headings

A.1.   Literature Search Strings

PubMed

375-73-5[rn] OR 45187-15-3[rn] "nonafluorobutane-1-sulfonic acid"[nm] OR "1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid"[tw] OR "1-Perfluorobutanesulfonic acid"[tw] OR "Nonafluoro-1-butanesulfonic acid"[tw] OR "Nonafluorobutanesulfonic acid"[tw]

OR "Perfluorobutanesulfonic acid"[tw] OR "1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid"[tw] OR "Perfluorobutane sulfonic acid"[tw] OR "Perfluorobutanesulfonate"[tw] OR "Perfluorobutane sulfonate"[tw] OR "1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-"[tw] OR "1-Butanesulfonic acid, nonafluoro-"[tw] OR "Perfluoro-1-butanesulfonate"[tw] OR "Perfluorobutylsulfonate"[tw] OR "Eftop FBSA"[tw] OR (PFBS[tw] AND (fluorocarbon*[tw] OR fluorotelomer*[tw] OR polyfluoro*[tw] OR perfluoro-*[tw] OR perfluoroa*[tw] OR perfluorob*[tw] OR perfluoroc*[tw] OR perfluorod*[tw] OR perfluoroe*[tw] OR perfluoroh*[tw] OR perfluoron*[tw] OR perfluoroo*[tw] OR perfluorop*[tw] OR perfluoros*[tw] OR perfluorou*[tw] OR perfluorinated[tw] OR fluorinated[tw] OR PFAS[tw] OR PFOS[tw] OR PFOA[tw]))

WOS

TS="1,1,2,2,3,3,4,4,4-Nonafluoro-1-butanesulfonic acid" OR TS="1-Perfluorobutanesulfonic acid" OR TS="Nonafluoro-1-butanesulfonic acid" OR TS="Nonafluorobutanesulfonic acid" OR TS="Perfluorobutanesulfonic acid" OR TS="1,1,2,2,3,3,4,4,4-Nonafluorobutane-1-sulphonic acid" OR TS="Perfluorobutane sulfonic acid" OR TS="Perfluorobutanesulfonate" OR TS="Perfluorobutane sulfonate" OR TS="1-Butanesulfonic acid, 1,1,2,2,3,3,4,4,4-nonafluoro-" OR TS="1-Butanesulfonic acid, nonafluoro-" OR TS="Perfluoro-1-butanesulfonate" OR TS="Perfluorobutylsulfonate" OR TS="Eftop FBSA" OR (TS=PFBS AND TS=(fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro-* OR perfluoroa* OR perfluorob* OR perfluoroc* OR perfluorod* OR perfluoroe* OR perfluoroh* OR perfluoron* OR perfluoroo* OR perfluorop* OR perfluoros* OR perfluorou* OR perfluorinated OR fluorinated OR PFAS OR PFOS OR PFOA))

Toxline

((375-73-5 [rn] OR 45187-15-3 [rn] OR "1 1 2 2 3 3 4 4 4-nonafluoro-1-butanesulfonic acid" OR "1-perfluorobutanesulfonic acid" OR "nonafluoro-1-butanesulfonic acid" OR "nonafluorobutanesulfonic acid" OR "perfluorobutanesulfonic acid" OR "1 1 2 2 3 3 4 4 4-nonafluorobutane-1-sulphonic acid" OR "perfluorobutane sulfonic acid" OR "perfluorobutanesulfonate" OR "perfluorobutane sulfonate" OR "1-butanesulfonic acid 1 1 2 2 3 3 4 4 4-nonafluoro-" OR "1-butanesulfonic acid nonafluoro-" OR "perfluoro-1-butanesulfonate" OR "perfluorobutylsulfonate" OR "eftop fbsa" OR (pfbs AND (fluorocarbon* OR fluorotelomer* OR polyfluoro* OR perfluoro* OR perfluorinated OR fluorinated OR pfas OR pfos OR pfoa)))) AND (ANEUPL [org] OR BIOSIS [org] OR CIS [org] OR DART [org] OR EMIC [org] OR EPIDEM [org] OR HEEP [org] OR HMTC [org] OR IPA [org] OR RISKLINE [org] OR MTGABS [org] OR NIOSH [org] OR NTIS [org] OR PESTAB [org] OR PPBIB [org]) AND NOT PubMed [org] AND NOT pubdart [org]

TSCATS

375-73-5[rn] AND tscsats[org]
45187-15-3[rn] AND tscsats[org]

Appendix B: Detailed PECO Criteria

Table B-1. Population, exposure, comparator, and outcome criteria

PECO element	Evidence
Population	**Human**: Any population (occupational; general population including children, pregnant women, and other sensitive populations). The following study designs will be considered most informative: controlled exposure, cohort, case-control, or cross-sectional. Note: Case reports and case series are not the primary focus of this assessment and will be tracked as supplemental material during the study screening process. **Animal**: Nonhuman mammalian animal species (whole organism) of any life stage (including preconception, *in utero*, lactation, peripubertal, and adult stages). ***In vitro* models of genotoxicity**: The studies will be considered PECO-relevant. All other *in vitro* studies will be tagged as "not-PECO relevant, but supplemental material." **Nonmammalian model systems/*in vitro*/*in silico* NOT related to genotoxicity**: Nonmammalian model systems (e.g., fish, amphibians, birds, and *C. elegans*); studies of human or animal cells, tissues, or biochemical reactions (e.g., ligand binding assays) with *in vitro* exposure regimens; bioinformatics pathways of disease analysis; and/or high throughput screening data. These studies will be classified as non-PECO-relevant, but have supplemental information.
Exposure	**Human**: Studies providing qualitative or quantitative estimates of 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, job title or other relevant occupational information. Human "mixture" studies are considered PECO-relevant as long as they have the per- and polyfluoroalkyl substances (PFAS) of interest. **Animal**: Studies providing qualitative and quantitative estimates of exposure based on administered dose or concentration. Oral and inhalation studies are considered PECO-relevant. Nonoral and noninhalation studies are tagged as supplemental. Experimental mixture studies are included as PECO-relevant only if they include a perfluorobutane sulfonic acid- (PFBS-) only arm. Otherwise, mixture studies are tagged as supplemental. All studies must include exposure to PFBS, CASRN 375-73-5. Studies of precursor PFAS that identify any of the targeted PFAS as metabolites will also be included.
Comparator	**Human**: A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. For D-R purposes, exposure-response quantitative results must be presented in sufficient detail such as regression coefficients presented with statistical measure of variation such as RR, HR, OR, or SMR or observed cases vs. expected cases (common in occupational studies); slope or linear regression coefficient (i.e., per unit increase in a continuous outcome); difference in the means; or report means with results of t-test, mean comparison by regression, or other mean-comparing hypothesis test. **Animal**: Quantitative exposure versus lower or no exposure with concurrent vehicle control group.
Outcome	Cancer and noncancer health outcomes. In general, endpoints related to clinical diagnostic criteria, disease outcomes, histopathological examination, genotoxicity, or other apical/phenotypic outcomes will be prioritized for evidence synthesis. Based on preliminary screening work and other assessments, the systematic review is anticipated to focus on liver (including serum lipids), developmental, reproductive, neurological, developmental neurotoxicity, thyroid disease/disruption, immunological, cardiovascular, and musculoskeletal outcomes.

Notes: D-R = Dose-Response; HR = hazard ratio; OR = odds ratio; PECO = population, exposure, comparator, and outcome; RR = risk ratio; SMR = standardized mortality ratio.

Appendix C: Study Evaluation Methods

For each outcome in a study, in each domain, reviewers reached a consensus judgment of *good, adequate, deficient, not reported,* or *critically deficient*. Questions used to guide the development of criteria for each domain in epidemiology studies are presented in Table C-1 and experimental animal toxicology studies in Table C-3. These categories were applied to each evaluation domain for each study as follows:

- *Good* represents a judgment that the study was conducted appropriately in relation to the evaluation domain and any deficiencies, if present, are minor and would not be expected to influence the study results.
- *Adequate* indicates a judgment that there are methodological limitations relating to the evaluation domain, but that those limitations are not likely to be severe or to have a notable impact on the results.
- *Deficient* denotes identified biases or deficiencies that are interpreted as likely to have had a notable impact on the results or that prevent interpretation of the study findings.
- *Not reported* indicates that the information necessary to evaluate the domain was not available in the study. Generally, this term carries the same functional interpretation as *deficient* for the purposes of the study confidence classification. Depending on the number and severity of other limitations identified in the study, it may or may not be worth reaching out to the study authors for this information.
- *Critically deficient* reflects a judgment that the study conduct introduced a serious flaw that makes the observed effect(s) uninterpretable. Studies with a determination of critically deficient in an evaluation domain will almost always cause the study to be considered overall "uninformative".

Once the evaluation domains were rated, the identified strengths and limitations were considered to reach a study confidence rating of *high, medium, low*, or *uninformative* for a specific health outcome. This was based on the reviewer judgments across the evaluation domains and included consideration of the likely impact the noted deficiencies in bias and sensitivity, or inadequate reporting, have on the results. The ratings, which reflect a consensus judgment between reviewers, are defined as follows:

- *High*: A well-conducted study with no notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology. *High* confidence studies generally reflect judgments of *good* across all or most evaluation domains.
- *Medium*: A satisfactory (acceptable) study in which deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree. Generally, *medium* confidence studies will include *adequate* or *good* judgments across most domains, with the impact of any identified limitation not being judged as severe.
- *Low*: A substandard study in which 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. Typically, *low* confidence studies would have a *deficient* evaluation for one or more domains, although some *medium* confidence studies could have a *deficient* rating in domain(s) considered to have less influence on the magnitude or

direction of effect estimates. Generally, *low* confidence results are given less weight than *high* or *medium* confidence results during evidence synthesis and integration and are generally not used as the primary sources of information for hazard identification or derivation of toxicity values unless they are the only studies available. Studies rated as *low* confidence only because of sensitivity concerns about bias towards the null require additional consideration during evidence synthesis. Observing an effect in these studies could increase confidence, assuming the study was otherwise well-conducted.

- *Uninformative*: An unacceptable study in which serious flaw(s) make the study results unusable for informing hazard identification. Studies with *critically deficient* judgments in any evaluation domain will almost always be classified as *uninformative* (see explanation above). Studies with multiple *deficient* judgments across domains might also be considered *uninformative. Uninformative* studies will not be considered further in the synthesis and integration of evidence for hazard identification or dose response but might be used to highlight possible research gaps.

Table C-1. Questions used to guide the development of criteria for each domain in epidemiology studies

Core question	Prompting questions	Follow-up questions
Exposure measurement Does the exposure measure reliably distinguish between levels of exposure in a time window considered most relevant for a causal effect with respect to the development of the outcome?	For all: • Does the exposure measure capture the variability in exposure among the participants, considering intensity, frequency, and duration of exposure? • Does the exposure measure reflect a relevant time window? If not, can the relationship between measures in this time and the relevant time window be estimated reliably? • Was the exposure measurement likely to be affected by a knowledge of the outcome? • Was the exposure measurement likely to be affected by the presence of the outcome (i.e., reverse causality)? For case-control studies of occupational exposures: • Is exposure based on a comprehensive job history describing tasks, setting, time period, and use of specific materials? For biomarkers of exposure, general population: • Is a standard assay used? What are the intra- and inter-assay coefficients of variation? Is the assay likely to be affected by contamination? Are values less than the limit of detection dealt with adequately? What exposure time period is reflected by the biomarker? If the half-life is short, what is the correlation between serial measurements of exposure?	Is the degree of exposure misclassification likely to vary by exposure level? If the correlation between exposure measurements is moderate, is there an adequate statistical approach to ameliorate variability in measurements? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?

Core question	Prompting questions	Follow-up questions
Outcome ascertainment Does the outcome measure reliably distinguish the presence or absence (or degree of severity) of the outcome?	For all: • Is outcome ascertainment likely to be affected by knowledge of, or presence of, exposure (e.g., consider access to health care, if based on self-reported history of diagnosis)? For case-control studies: • Is the comparison group without the outcome (e.g., controls in a case-control study) based on objective criteria with little or no likelihood of inclusion of people with the disease? For mortality measures: • How well does cause of death data reflect occurrence of the disease in an individual? How well do mortality data reflect incidence of the disease? For diagnosis of disease measures: • Is diagnosis based on standard clinical criteria? If based on self-report of diagnosis, what is the validity of this measure? For laboratory-based measures (e.g., hormone levels): • Is a standard assay used? Does the assay have an acceptable level of inter-assay variability? Is the sensitivity of the assay appropriate for the outcome measure in this study population?	Is there a concern that any outcome misclassification is nondifferential, differential, or both? What is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Participant selection Is there evidence that selection into or out of the study (or analysis sample) was jointly related to exposure and to outcome?	For longitudinal cohort: • Did participants volunteer for the cohort based on knowledge of exposure and/or preclinical disease symptoms? Was entry into the cohort or continuation in the cohort related to exposure and outcome? For occupational cohort: • Did entry into the cohort begin with the start of the exposure? • Was follow-up or outcome assessment incomplete, and if so, was follow-up related to both exposure and outcome status? • Could exposure produce symptoms that would result in a change in work assignment/work status ("healthy worker survivor effect")? For case-control study: • Were controls representative of population and time periods from which cases were drawn? • Are hospital controls selected from a group whose reason for admission is independent of exposure? • Could recruitment strategies, eligibility criteria, or participation rates result in differential participation relating to both disease and exposure? For population-based survey: • Was recruitment based on advertisement to people with knowledge of exposure, outcome, and hypothesis?	Were differences in participant enrollment and follow-up evaluated to assess bias? If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)? Were appropriate analyses performed to address changing exposures over time in relation to symptoms? Is there a comparison of participants and nonparticipants to address whether differential selection is likely?

Core question	Prompting questions	Follow-up questions
Confounding Is confounding of the effect of the exposure likely?	Is confounding adequately addressed by considerations in… a. … participant selection (matching or restriction)? b. … accurate information on potential confounders, and statistical adjustment procedures? c. … lack of association between confounder and outcome, or confounder and exposure in the study? d. … information from other sources? Is the assessment of confounders based on a thoughtful review of published literature, potential relationships (e.g., as can be gained through directed acyclic graphing), minimizing potential overcontrol (e.g., inclusion of a variable on the pathway between exposure and outcome)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Analysis Do the analysis strategy and presentation convey the necessary familiarity with the data and assumptions?	• Are missing outcome, exposure, and covariate data recognized and, if necessary, accounted for in the analysis? • Does the analysis appropriately consider variable distributions and modeling assumptions? • Does the analysis appropriately consider subgroups of interest (e.g., based on variability in exposure level or duration, or susceptibility)? • Is an appropriate analysis used for the study design? • Is effect modification considered, based on considerations developed a priori? • Does the study include additional analyses addressing potential biases or limitations (i.e., sensitivity analyses)?	If there is a concern about the potential for bias, what is the predicted direction or distortion of the bias on the effect estimate (if there is enough information)?
Sensitivity Is there a concern that sensitivity of the study is not adequate to detect an effect?	• Is the exposure range adequate? • Was the appropriate population included? • Was the length of follow-up adequate? Is the time/age of outcome ascertainment optimal given the interval of exposure and the health outcome? • Are there other aspects related to risk of bias or otherwise that raise concerns about sensitivity?	
Selective reporting Is there reason to be concerned about selective reporting?	• Are the results needed for the IRIS analysis presented (based on a priori specification)? If not, can these results be obtained? • Are only statistically significant results presented?	

Note: IRIS = Integrated Risk Information System

C.1. Exposure measurement evaluation criteria

The criteria used to evaluate exposure measurement for PFBS (Table C-2) are adapted from the criteria developed by the National Toxicology Program (NTP) Office of Health Assessment and Translation for their assessment of the association between perfluorooctane sulfonic acid (PFOS) and perfluorooctanoic acid (PFOA) and immune effects (NTP, 2016, 2015) and were established prior to beginning study evaluation. Standard analytical methods for evaluating individual per- and polyfluoroalkyl substances (PFAS) in serum or whole-blood using quantitative techniques such as liquid chromatography-triple quadrupole mass spectrometry are preferred

(CDC, 2018; U.S. EPA, 2014d, e; ATSDR, 2009; CDC, 2009). The estimated serum half-life of PFBS is approximately 1 month (Lau, 2015; Olsen et al., 2009), so unlike for some other PFAS with longer half-lives, current exposure might not be indicative of past exposures. Little data is available on repeated measures of PFBS in humans over time, so the reliability of a single measure is unclear. The timing of the exposure measurement is considered in relation to the etiologic window for each outcome being reviewed.

Table C-2. Criteria for evaluation of exposure measurement in epidemiology studies

Exposure measurement rating	Criteria
Good	All of the following: • Evidence that exposure was consistently assessed using well-established methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma). • Exposure was assessed in a relevant time window for development of the outcome (i.e., temporality is established and sufficient latency occurred prior to disease onset). • There is evidence that a sufficient proportion of the exposure data measurements are above the limit of quantification for the assay so that different exposure groups can be distinguished based on the analyses conducted. • The laboratory analysis included standard quality control measures with demonstrated precision and accuracy. • There is sufficient specificity/sensitivity and range or variation in exposure measurements that would minimize potential for exposure measurement error and misclassification by allowing exposure classifications to be differentiated (i.e., can reliably categorize participants into groups such as high vs. low exposure).
Adequate	• Evidence that exposure was consistently assessed using well-established methods that directly measure exposure (e.g., measurement of PFAS in blood, serum, or plasma), but there were some minor concerns about quality control measures or other potential for nondifferential misclassification. OR • Exposure was assessed using indirect measures (e.g., drinking water concentrations and residential location/history, questionnaire, or occupational exposure assessment by a certified industrial hygienist) that have been validated or empirically shown to be consistent with methods that directly measure exposure (i.e., inter-methods validation: one method vs. another) Note: This could be *good* if the validation was sufficient. All studies for PFBS used direct measures. And all of the following: • Exposure was assessed in a relevant time window for development of the outcome. • There is evidence that a sufficient proportion of the exposure data measurements are above the limit of quantification for the assay. • There is sufficient specificity/sensitivity and range or variation in exposure measurements that would minimize potential for exposure measurement error and misclassification by allowing exposure classifications to be differentiated (i.e., can reliably categorize participants into groups such as high vs. low exposure), but there might be more uncertainty than in *good*.

Exposure measurement rating	Criteria
Deficient	Any of the following: • Some concern, but no direct evidence, that the exposure was assessed using poorly validated methods. • There is insufficient information provided about the exposure assessment, including precision, accuracy, and level of quantification, but no evidence for concern about the method used. • Exposure was assessed in a relevant time window for development of the outcome. There could be concerns about reverse causation between exposure and outcome, but there is no direct evidence that it is present. • There is some concern over insufficient specificity/sensitivity and range or variation in exposure measurements that may result in considerable exposure measurement error and misclassification when exposure classifications are compared (i.e., data do not lend themselves to reliably categorize participants into groups such as high vs. low exposure, and/or there is considerable uncertainty in exposure values that do not allow for confidence in the examination of small per unit changes in continuous exposures).
Critically deficient	Any of the following: • Exposure was assessed in a time window that is unknown or not relevant for development of the outcome. This could be due to clear evidence of reverse causation between exposure and outcome, or other concerns such as the lack of temporal ordering of exposure and disease onset, insufficient latency, or having exposure measurements that are not reliable measures of exposure during the etiologic window. • Direct evidence that bias was likely, since the exposure was assessed using methods with poor validity. • Evidence of differential exposure misclassification (e.g., differential recall of self-reported exposure). • There is evidence that an insufficient proportion of the exposure data measurements are above the limit of quantification for the assay.

Table C-3. Questions used to guide the development of criteria for each domain in experimental animal toxicology studies

Evaluation type	Domain– core question	Prompting questions	Basic considerations

Reporting Quality	**Reporting Quality –** Does the study report information for evaluating the design and conduct of the study for the endpoint(s)/outcome(s) of interest?
	
	Notes:
	Reviewers should reach out to authors to obtain missing information when studies are considered key for hazard evaluation and/or dose-response.
	This domain is limited to reporting. Other aspects of the exposure methods, experimental design, and endpoint evaluation methods are evaluated using the domains related to risk of bias and study sensitivity.
		Does the study report the following?
		
		• **Critical information** necessary to perform study evaluation:
		
		  o Species; test article name; levels and duration of exposure; route (e.g., oral; inhalation); qualitative or quantitative results for at least one endpoint of interest.
		
		• **Important information** for evaluating the study methods:
		
		  o Test animal: strain, sex, source, and general husbandry procedures.
		  o Exposure methods: source, purity, method of administration.
		  o Experimental design: frequency of exposure, animal age and life stage during exposure and at endpoint/outcome evaluation.
		  o Endpoint evaluation methods: assays or procedures used to measure the endpoints/outcomes of interest.
			These considerations typically do not need to be refined by assessment teams, although in some instances the **important information** may be refined depending on the endpoints/outcomes of interest or the chemical under investigation.
			A judgment and rationale for this domain should be given for the study. Typically, these will not change regardless of the endpoints/outcomes investigated by the study. **In the rationale, reviewers should indicate whether the study adhered to GLP, OECD, or other testing guidelines.**
			
			• *Good*: All critical and **important information** is reported or inferable for the endpoints/outcomes of interest.
			• *Adequate*: All **critical information** is reported but some **important information** is missing. However, the missing information is not expected to significantly impact the study evaluation.
			• *Deficient*: All **critical information** is reported but **important information** is missing that is expected to significantly reduce the ability to evaluate the study.
			• *Critically Deficient*: Study report is missing any pieces of **critical information**. Studies that are *Critically Deficient* for reporting are Uninformative for the overall rating and considered no further for evidence synthesis and integration.

Evaluation type		Domain– core question	Prompting questions	Basic considerations
Risk of Bias	Selection and performance bias	**Allocation –** Were animals assigned to experimental groups using a method that minimizes selection bias?	For each study: • Did each animal or litter have an equal chance of being assigned to any experimental group (i.e., random allocation)? • Is the allocation method described? • Aside from randomization, were any steps taken to balance variables across experimental groups during allocation?	These considerations typically do not need to be refined by assessment teams. A judgment and rationale for this domain should be given for each cohort or experiment in the study. • *Good*: Experimental groups were randomized and any specific randomization procedure was described or inferable (e.g., computer-generated scheme). [Note that normalization is not the same as randomization (see response for 'Adequate').] • *Adequate*: Authors report that groups were randomized but do not describe the specific procedure used (e.g., "animals were randomized"). Alternatively, authors used a nonrandom method to control for important modifying factors across experimental groups (e.g., body weight normalization). • *Not Reported* (interpreted as *Deficient*): No indication of randomization of groups or other methods (e.g., normalization) to control for important modifying factors across experimental groups. • *Critically Deficient*: Bias in the animal allocations was reported or inferable.
		Observational Bias/Blinding– Did the study implement measures to reduce observational bias?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the study report blinding or other methods/procedures for reducing observational bias? • If not, did the study use a design or approach for which such procedures can be inferred? • What is the expected impact of failure to implement (or report implementation) of these methods/procedures on results?	These considerations typically do not need to be refined by the assessment teams. [Note that it can be useful for teams to identify highly subjective measures of endpoints/outcomes where observational bias may strongly influence results prior to performing evaluations.] A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *Good*: Measures to reduce observational bias were described (e.g., blinding to conceal treatment groups during endpoint evaluation; consensus-based evaluations of histopathology lesions).[a]

Evaluation type	Domain– core question	Prompting questions	Basic considerations

			• *Adequate*: Methods for reducing observational bias (e.g., blinding) can be inferred or were reported but described incompletely.
			• *Not Reported*: Measures to reduce observational bias were not described.
			   ○ Interpreted as *Adequate*—The potential concern for bias was mitigated based on use of automated/computer driven systems, standard laboratory kits, relatively simple, objective measures (e.g., body or tissue weight), or screening-level evaluations of histopathology.
			   ○ Interpreted as *Deficient*—The potential impact on the results is major (e.g., outcome measures are highly subjective).
			• *Critically Deficient*: Strong evidence for observational bias that could have impacted results.

Confounding/ variable control	**Confounding–** Are variables with the potential to confound or modify results controlled for and consistent across all experimental groups?	For each study: • Are there differences across the treatment groups (e.g., co-exposures, vehicle, diet, palatability, husbandry, health status, and so forth) that could bias the results? • If differences are identified, to what extent are they expected to impact the results?	These considerations may need to be refined by assessment teams, as the specific variables of concern can vary by experiment or chemical. A judgment and rationale for this domain should be given for each cohort or experiment in the study, noting when the potential for confounding is restricted to specific endpoints/outcomes. • *Good*: Outside of the exposure of interest, variables that are likely to confound or modify results appear to be controlled for and consistent across experimental groups. • *Adequate*: Some concern that variables that were likely to confound or modify results were uncontrolled or inconsistent across groups, but are expected to have a minimal impact on the results. • *Deficient*: Notable concern that potentially confounding variables were uncontrolled or inconsistent across groups and are expected to substantially impact the results.

Evaluation type	Domain– core question	Prompting questions	Basic considerations
			• *Critically Deficient*: Confounding variables were presumed to be uncontrolled or inconsistent across groups and are expected to be a primary driver of the results.
Reporting and attrition bias	**Selective Reporting and Attrition–** Did the study report results for all prespecified outcomes and tested animals? *Note:* *This domain does **not** consider the appropriateness of the analysis/results presentation. This aspect of study quality is evaluated in another domain.*	For each study: *Selective reporting bias:* • Are all results presented for endpoints/outcomes described in the methods (see note)? *Attrition bias:* • Are all animals accounted for in the results? • If there are discrepancies, do authors provide an explanation (e.g., death or unscheduled sacrifice during the study)? • If unexplained results, omissions, and/or attrition are identified, what is the expected impact on the interpretation of the results?	These considerations typically do not need to be refined by assessment teams. A judgment and rationale for this domain should be given for each cohort or experiment in the study. • *Good*: Quantitative or qualitative results were reported for all prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Data not reported in the primary article is available from supplemental material. If results, omissions, or animal attrition is identified, the authors provide an explanation and these are not expected to impact the interpretation of the results. • *Adequate*: Quantitative or qualitative results are reported for most prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints. Omissions and/or attrition are not explained, but are not expected to significantly impact the interpretation of the results. • *Deficient*: Quantitative or qualitative results are missing for many prespecified outcomes (explicitly stated or inferred), exposure groups and evaluation timepoints and/or high animal attrition; omissions and/or attrition are not explained and may significantly impact the interpretation of the results. • *Critically Deficient*: Extensive results omission and/or animal attrition is identified and prevents comparisons of results across treatment groups.

Evaluation type		Domain–core question	Prompting questions	Basic considerations

Sensitivity	**Exposure methods sensitivity**	**Chemical Administration and Characterization–**
		
		Did the study adequately characterize exposure to the chemical of interest and the exposure administration methods?
		
		Note:
		Consideration of the appropriateness of the route of exposure is not evaluated at the individual study level. Relevance and utility of the routes of exposure are considered in the PECO criteria for study inclusion and during evidence synthesis.
			For each study:
			
			• Does the study report the source and purity and/or composition (e.g., identity and percent distribution of different isomers) of the chemical? If not, can the purity and/or composition be obtained from the supplier (e.g., as reported on the website)?
			• Was independent analytical verification of the test article purity and composition performed?
			• Did the authors take steps to ensure the reported exposure levels were accurate?
			
			  o For inhalation studies: Were target concentrations confirmed using reliable analytical measurements in chamber air?
			  o For oral studies: If necessary based on consideration of chemical-specific knowledge (e.g., instability in solution; volatility) and/or exposure design (e.g., the frequency and duration of exposure), were chemical concentrations in the dosing solutions or diet analytically confirmed?
			
			• Are there concerns about the methods used to administer the chemical (e.g., inhalation chamber type, gavage volume, etc.)?
				It is essential that these criteria are considered and potentially refined by assessment teams, as the specific variables of concern can vary by chemical.
				A judgment and rationale for this domain should be given for each cohort or experiment in the study.
				
				• *Good*: Chemical administration and characterization is complete (i.e., source, purity, and analytical verification of the test article are provided). There are no concerns about the composition, stability, or purity of the administered chemical or the specific methods of administration. For inhalation studies, chemical concentrations in the exposure chambers are verified using reliable analytical methods.
				• *Adequate*: Some uncertainties in the chemical administration and characterization are identified but these are expected to have minimal impact on interpretation of the results (e.g., source and vendor-reported purity are presented, but not independently verified; purity of the test article is suboptimal but not concerning). For inhalation studies, actual exposure concentrations are missing or verified with less reliable methods.
				• *Deficient*: Uncertainties in the exposure characterization are identified and expected to substantially impact the results (e.g., source of the test article is not reported; levels of impurities are substantial or concerning; deficient administration methods such as use of static inhalation chambers or a gavage volume considered too large for the species and/or life stage at exposure).
				• *Critically Deficient*: Uncertainties in the exposure characterization are identified, and there is reasonable certainty that the results are largely attributable to factors other than exposure to the chemical of interest (e.g., identified impurities are expected to be a primary driver of the results).

Evaluation type		Domain– core question	Prompting questions	Basic considerations
		Exposure Timing, Frequency and Duration– Was the timing, frequency, and duration of exposure sensitive for the endpoint(s)/outcome(s) of interest?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the exposure period include the critical window of sensitivity? • Was the duration and frequency of exposure sensitive for detecting the endpoint of interest?	Considerations for this domain are highly variable depending on the endpoint(s)/outcome(s) of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *Good*: The duration and frequency of the exposure was sensitive and the exposure included the critical window of sensitivity (if known). • *Adequate*: The duration and frequency of the exposure was sensitive and the exposure covered most of the critical window of sensitivity (if known). • *Deficient*: The duration and/or frequency of the exposure is not sensitive and did not include the majority of the critical window of sensitivity (if known). These limitations are expected to bias the results towards the null. • *Critically Deficient*: The exposure design was not sensitive and is expected to strongly bias the results towards the null. The rationale should indicate the specific concern(s).
	Outcome measures and results display	**Endpoint Sensitivity and Specificity–** Are the procedures sensitive and specific for evaluating the endpoint(s)/outcome(s) of interest? *Note: Sample size alone is not a reason to conclude an individual study is critically deficient.*	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Are there concerns regarding the specificity and validity of the protocols? • Are there serious concerns regarding the sample size (see note)? • Are there concerns regarding the timing of the endpoint assessment?	Considerations for this domain are highly variable depending on the endpoint(s)/outcome(s) of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. Examples of potential concerns include: • Selection of protocols that are insensitive or nonspecific for the endpoint of interest. • Use of unreliable methods to assess the outcome. • Assessment of endpoints at inappropriate or insensitive ages, or without addressing known endpoint variation (e.g., due to circadian rhythms, estrous cyclicity, etc.).

Evaluation type		Domain– core question	Prompting questions	Basic considerations
				• Decreased specificity or sensitivity of the response due to the timing of endpoint evaluation, as compared to exposure (e.g., short-acting depressant or irritant effects of chemicals; insensitivity due to prolonged period of nonexposure prior to testing).
		Results Presentation– Are the results presented in a way that makes the data usable and transparent?	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Does the level of detail allow for an informed interpretation of the results? • Are the data analyzed, compared, or presented in a way that is inappropriate or misleading?	Considerations for this domain are highly variable depending on the outcomes of interest and must be refined by assessment teams. A judgment and rationale for this domain should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. Examples of potential concerns include: • Nonpreferred presentation such as developmental toxicity data averaged across pups in a treatment group when litter responses are more appropriate. • Failing to present quantitative results. • Pooling data when responses are known or expected to differ substantially (e.g., across sexes or ages). • Failing to report on or address overt toxicity when exposure levels are known or expected to be highly toxic. • Lack of full presentation of the data (e.g., presentation of mean without variance data; concurrent control data are not presented).

Evaluation type	Domain– core question	Prompting questions	Basic considerations
Overall Confidence	**Overall Confidence–** Considering the identified strengths and limitations, what is the overall confidence rating for the endpoint(s)/outcome(s) of interest? *Note:* *Reviewers should mark studies that are rated lower than high confidence only due to low sensitivity (i.e., bias towards the null) for additional consideration during evidence synthesis. If the study is otherwise well-conducted and an effect is observed, the confidence may be increased.*	For each endpoint/outcome or grouping of endpoints/outcomes in a study: • Were concerns (i.e., limitations or uncertainties) related to the reporting quality, risk of bias, or sensitivity identified? • If yes, what is their expected impact on the overall interpretation of the reliability and validity of the study results, including (when possible) interpretations of impacts on the magnitude or direction of the reported effects?	The overall confidence rating considers the likely impact of the noted concerns (i.e., limitations or uncertainties) in reporting, bias, and sensitivity on the results. A confidence rating and rationale should be given for each endpoint/outcome or group of endpoints/outcomes investigated in the study. • *High Confidence*: No notable concerns are identified (e.g., most or all domains rated *Good*). • *Medium Confidence*: Some concerns are identified, but expected to have minimal impact on the interpretation of the results (e.g., most domains rated *Adequate* or *Good*; may include studies with *Deficient* ratings if concerns are not expected to strongly impact the magnitude or direction of the results). Any important concerns should be carried forward to evidence synthesis. • *Low Confidence*: Identified concerns are expected to significantly impact on the study results or their interpretation (e.g., generally, *Deficient* ratings for one or more domains). The concerns leading to this confidence judgment must be carried forward to evidence synthesis (see note). • *Uninformative*: Serious flaw(s) that make the study results unusable for informing hazard identification (e.g., generally, *Critically Deficient* rating in any domain; many *Deficient* ratings). *Uninformative* studies are considered no further in the synthesis and integration of evidence.

Notes: GLP =good laboratory practices; OECD = Organisation for Economic Cooperation and Development.
[a] For nontargeted or screening-level histopathology outcomes often used in guideline studies, blinding during the initial evaluation of tissues is generally not recommended as masked evaluation can make "the task of separating treatment-related changes from normal variation more difficult" and "there is concern that masked review during the initial evaluation may result in missing subtle lesions." Generally, blinded evaluations are recommended for targeted secondary review of specific tissues or in instances when there is a predefined set of outcomes that is known or predicted to occur (Crissman et al., 2004).

Appendix D: HAWC User Guide and Frequently Asked Questions

D.1. What is HAWC and What is its Purpose?

HAWC (Health Assessment Workspace Collaborative) is an interactive expert-driven content management system for human health assessments that is intended to promote transparency, trackability, data usability, and understanding of the data and decisions supporting an environmental and human health assessment. Specifically, HAWC is an interface that allows the data and decisions supporting an assessment to be managed in modules (e.g., study evaluation, summary study data, etc.) that can be publicly accessed on-line (see #2 below and Figure D-1). Following literature search and screening that are conducted using HERO and DistillerSR, HAWC manages each study included in an assessment and makes the extracted information available via a web link that takes a user to a web page displaying study specific details and data (e.g., study evaluation, experimental design, dosing regime, endpoints evaluated, dose response data, etc., described in further detail below in #s 3–6). Finally, all data managed in HAWC is fully downloadable using the blue "Download datasets" link (highlighted in the red box below) also located in the grey navigation bar located on the assessment home page (discussed in # 7 below). Note that a user may quickly navigate HAWC by clicking on the file path (highlighted in orange dashed box below) given in the grey row below the HAWC icon and Login bar (Figure D-1). HAWC aims to facilitate team collaboration by scientists who develop these assessments and enhance transparency of the process by providing online access (no user account required) to the data and expert decisions used to evaluate potential human health hazard and risk of chemical exposures.
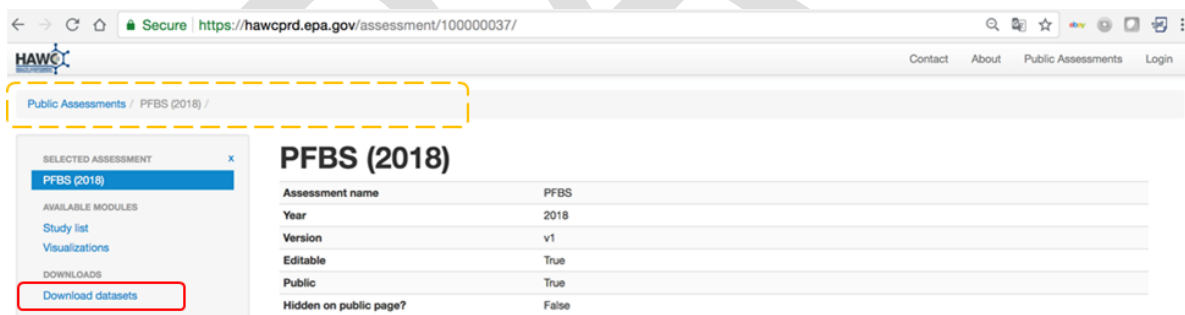
Figure D-1. HAWC homepage for the public PFBS assessment.

D.2. How Do I Access HAWC?

HAWC is an open-source online application that may be accessed using the following link: https://hawcprd.epa.gov/assessment/public/ and then selecting an available assessment. The following browsers are fully supported for accessing HAWC: Google chrome (preferred), Mozilla Firefox, and Apple Safari. There are errors in functionality when viewed with Internet Explorer. No user account is required for access to public HAWC assessments. The assessments located in HAWC are meant to accompany a textual expert synthesis of the data managed in HAWC. Each written assessment document contains embedded URL links to the evidence in HAWC (e.g., study evaluation, summary study data, visualizations, etc) supporting the

assessment text. The links embedded in an assessment document can be accessed by a mouse click (or hover while pressing CTRL+right click).

D.3. What Can I Find in HAWC?

HAWC contains a comprehensive landscape of study details and data supporting an assessment. Note that links are provided in the assessment text to guide the reader, but a user may also navigate to the HAWC homepage for an assessment on their own. Once a user lands on an assessment homepage all studies included in an assessment can be viewed by clicking the blue "Study list" link (highlighted in the red box below) in the grey navigation pane (Figure D-2). By clicking the study name listed in blue (under "Short citation") a user can view the full study details, study evaluation, and experimental details and data. For example, in Figure D-2, a user may click on 3M (2000d) (highlighted in orange dashed box below). This will take the user to the 3M (2000d) study details page that includes a link to the study in HERO along with study details, study evaluation, and available experimental (animal) and study population (epidemiologic) groups.
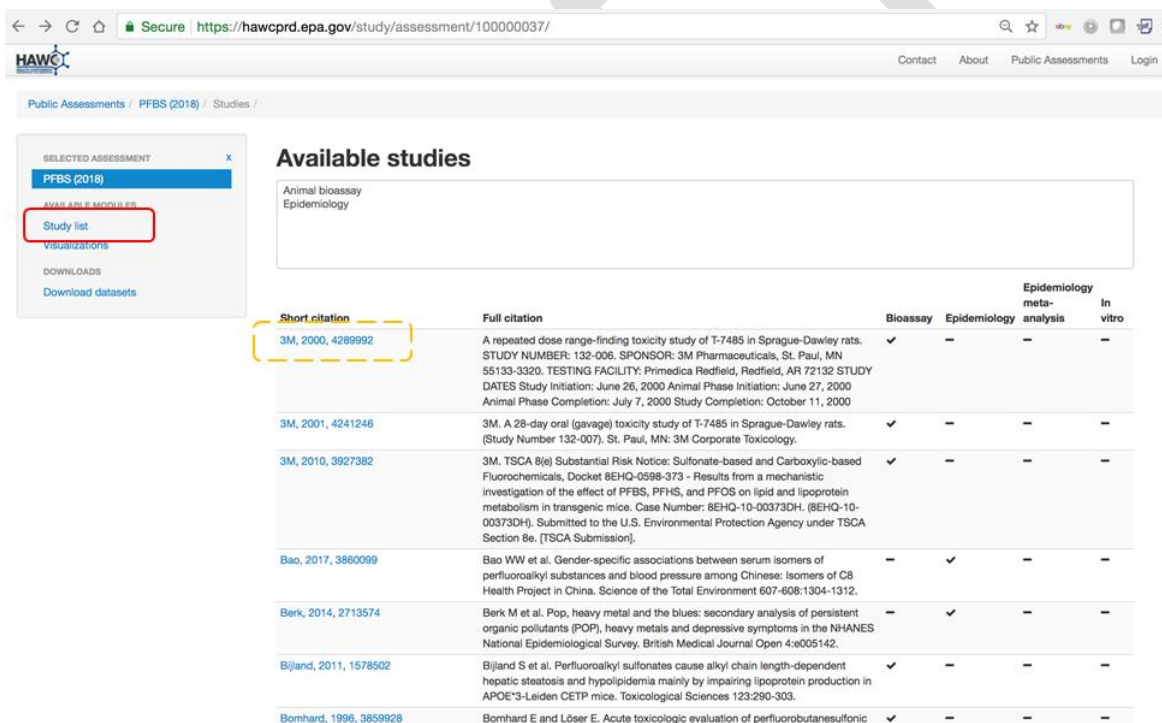
Figure D-2. Representative study list.

D.4. How Do I Access Study Evaluation(S)?

Study evaluation is performed to ensure that the studies used in the assessment are conducted in such a manner that the results are credible for each outcome and the ratings are outcome specific. The study evaluation criteria and decisions are fully documented in HAWC and displayed for each study on the study details page. Study evaluation is depicted as a pie chart with each domain and rating making up a piece of the pie that is colored according to the rating. A user may hover over each piece of the pie that causes rating metric text to populate to the right of the

pie graph (Figure D-3). For full domain and rating details the user may click the blue "View details" button (highlighted in the red box below). (Note that this example is given for the 3M (2000d)).
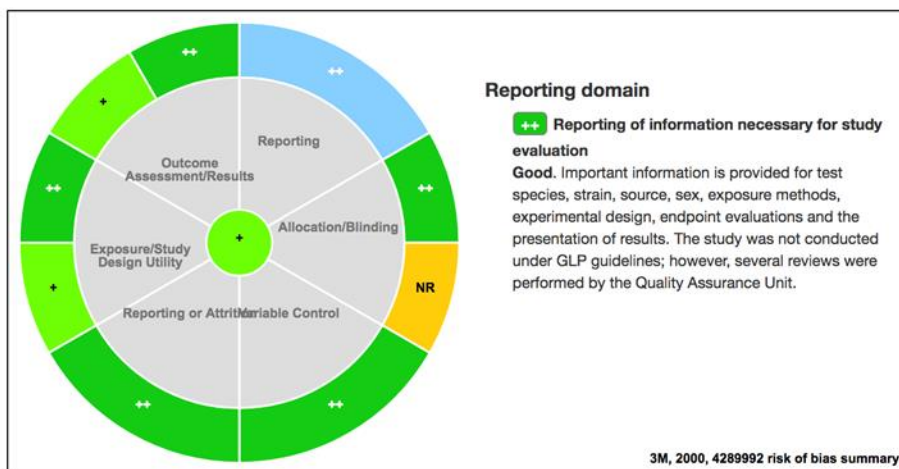
Figure D-3. Representative study evaluation pie chart with the reporting domain selected and text populating to the right of pie chart.

D.5. How Do I Access Study Specific Information on Experimental and Study Population Details, and Extracted Endpoint Data?

Specific information on experimental design, dosing (if animal bioassay), outcomes and exposure (if epidemiology) and extracted endpoint data can be accessed from the study details page by clicking on (for the 3M (2000d) study) Available animal bioassay experiments at the bottom of the study details page. A user may click on the experiment name (highlighted in blue, 10 Day Oral) to view dosing/exposure details and available groups. Clicking on available animal groups (e.g., Male Sprague-Dawley or Female Sprague-Dawley) will take the reader to a new page with experimental group information (e.g., species/strain/sex , dosing regime information, and available/additional endpoints information for animal studies; and outcome and exposure information for epidemiologic studies. If a study reports data then the data are extracted and managed as "available endpoints". If study authors include endpoints in the methods and results, but do not report data the endpoint is listed under "additional endpoints" without dose-response data. All endpoints are also clickable and contain an endpoint description, methods, and (if data are reported) a clickable data plot (e.g., Alanine Aminotransferase (ALT)). The description of endpoints, methods, and data are often copied directly from the study report and, therefore, can contain study author judgments and may not necessarily include EPA judgments on the endpoint data that would be included in the assessment.

D.6. What Are Visualizations and How Do I Access Them?

The data managed in HAWC is displayed using visualizations that are intended to support textual descriptions within an assessment. All visualizations can be accessed using the blue "Visualizations" link (highlighted in the red box below) also found in the grey navigation pane

(Figure D-4A). *Note that the available visualizations are at the discretion of the chemical manager and are meant to accompany the assessment text*. Visualizations are fully interactive. Hovering and clicking on records in the rows and columns and data points on a plot will cause a pop-up window to appear (Figure D-4B). This pop-up window is also interactive and clicking on blue text within this pop-up will open a new web page with descriptive data.

Figure D-4A. Visualization example for PFBS. (Note that the records listed under each column (study, experiment endpoint, units, study design, observation time, dose) and data within the plot are interactive.)
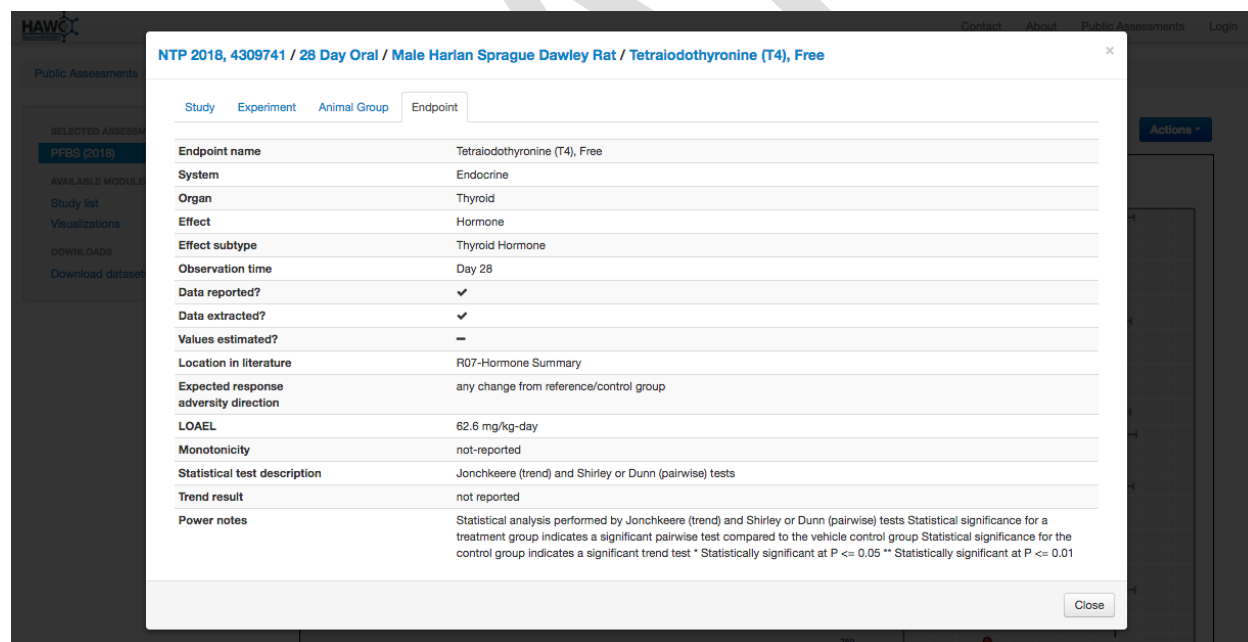
Figure D-4B. Example pop-up window after clicking on interactive visualization links. (In Figure D-4A the red circle for study NTP (2011); male at a dose of 500 mg/kg-day was clicked leading to the pop-up shown above. Clicking on blue text will open a new window with descriptive data.)

D.7. How do I download datasets?

A user may download any available dataset by first clicking on the blue "Download datasets" link (highlighted in the red box below) in the grey navigation pane on the assessment homepage. This takes the user to a new page where the desired data set may be selected for download as an excel file (See representative image in Figure D-5).
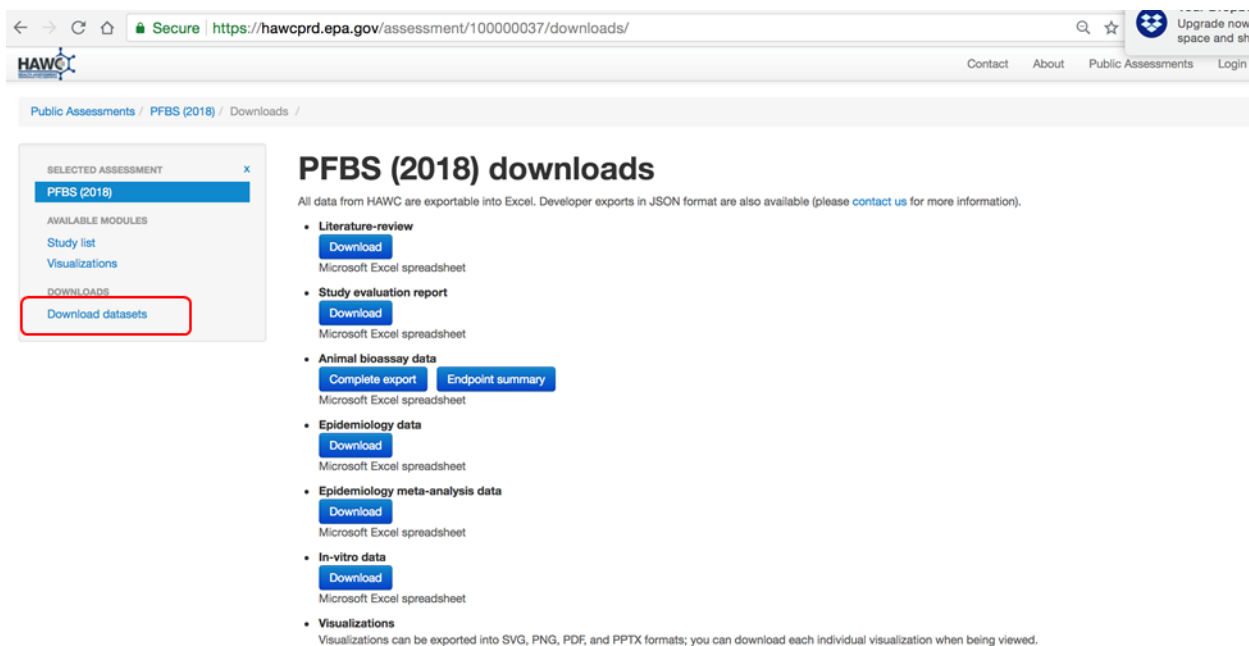
Figure D-5. Representative data download page.

D.8. How Do I Access the Benchmark Dose Modeling Outputs?

Benchmark dose (BMD) modeling is performed on an endpoint by endpoint basis at the discretion of the chemical manager. Those endpoints for which BMD modeling has been completed are referenced in the assessment text and are available for viewing. To access BMD modeling outputs the user can click on links included in the assessment text. Alternatively, the user may navigate to the BMD modeling outputs by clicking on a study (e.g., Feng et al. (2017)) of interest from the Study list, an available animal bioassay experiment (in this example the 20 Day Oral Gestation), an available animal group (P0 Female ICR Mice), and an endpoint of interest (Tetraiodothyronine (T4), Free). Next navigate to the blue Actions button, click, and scroll to "View session" (highlighted in the red box below) under BMD Modeling (Figure D-6A). The BMD setup, Results, and Model recommendation and selection (highlighted in orange dashed box below) are available for viewing (Figure D-6B). Selecting the BMD setup tab will display the modeled dose-response data, the selected models and options, and all benchmark modeling responses (BMRs). The results tab will display the BMD modeling output summary for all models. A user may hover over a selected model row to visualize the model fit to the data. In addition, a user may obtain the Benchmark Dose Software (BMDS) Output text by clicking the "View" button under the "Output" column for each model that was run. The Model recommendation and selection tab displays all models, warnings when appropriate, and the recommendation for which models are valid, questionable, or failed to fit.
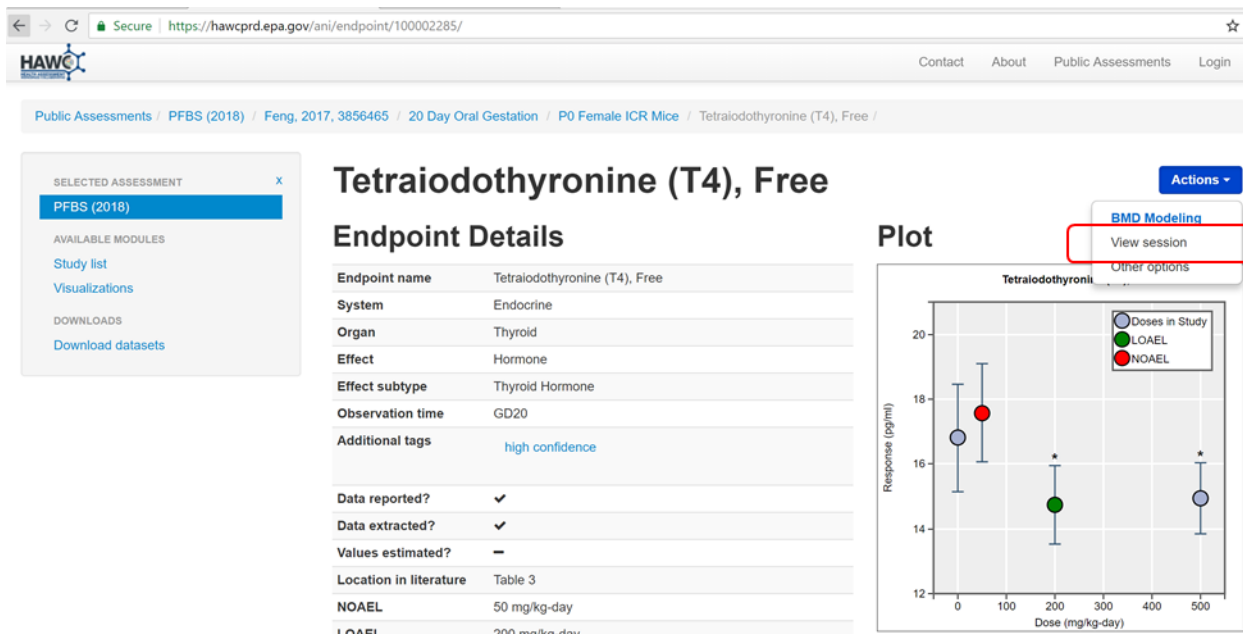
Figure D-6A. Example BMD modeling navigation.
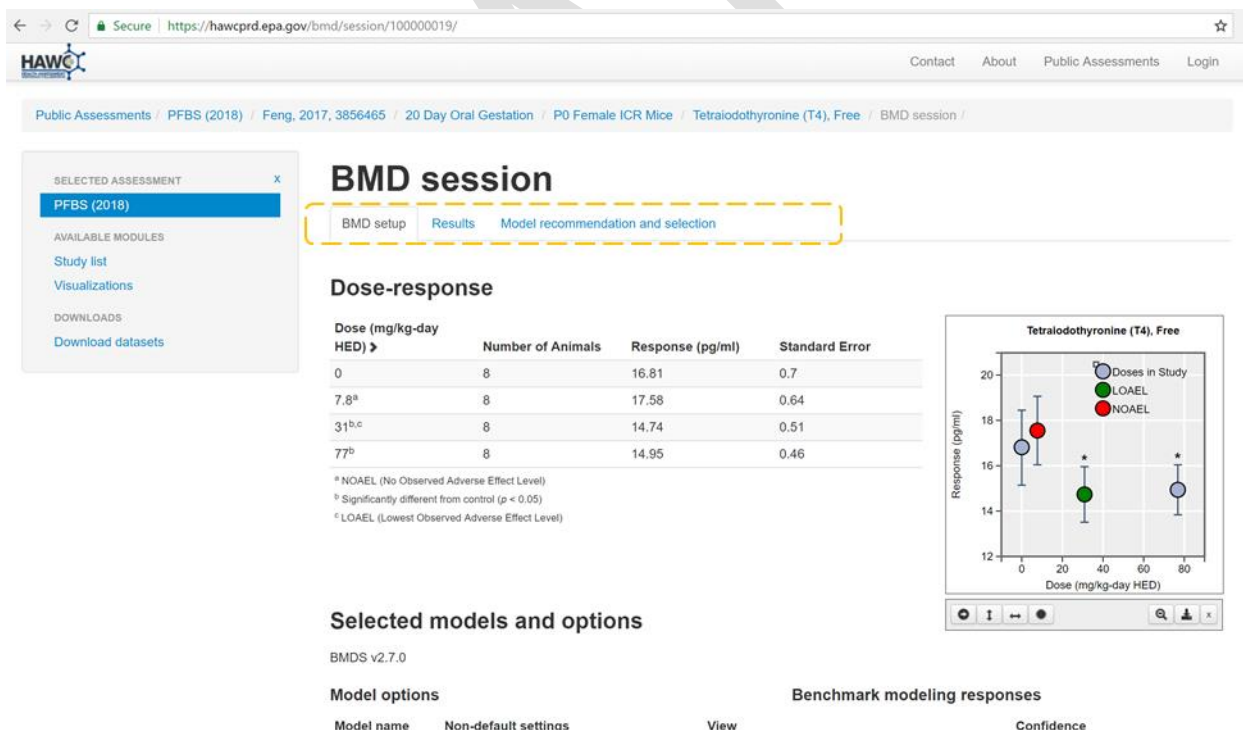
Figure D-6B. Example BMD session.

Appendix E. Additional Data Figures
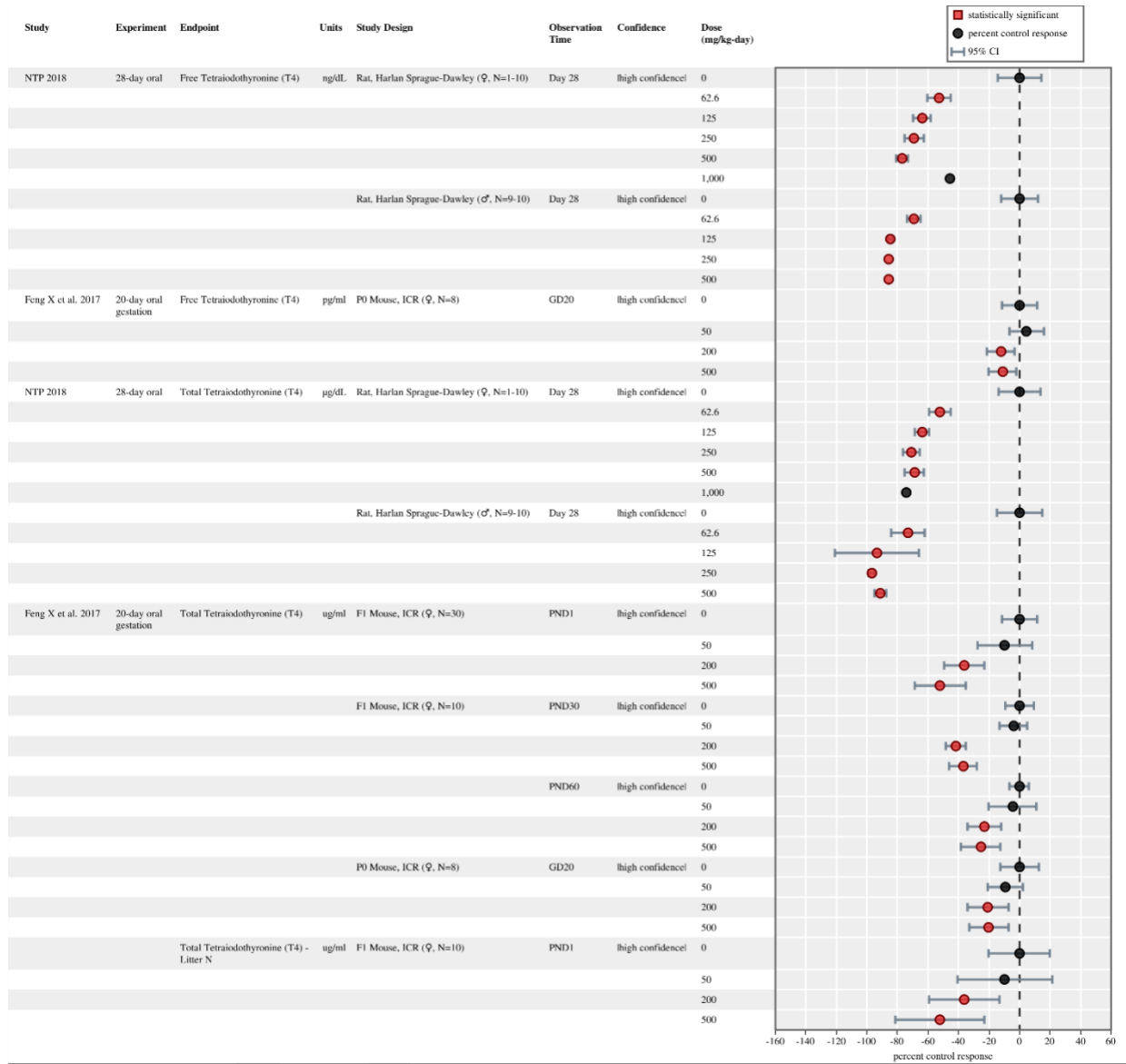
Figure E-1. Serum free and total thyroxine (T4) response in animals following K$^+$PFBS exposure (click to see interactive data graphic).
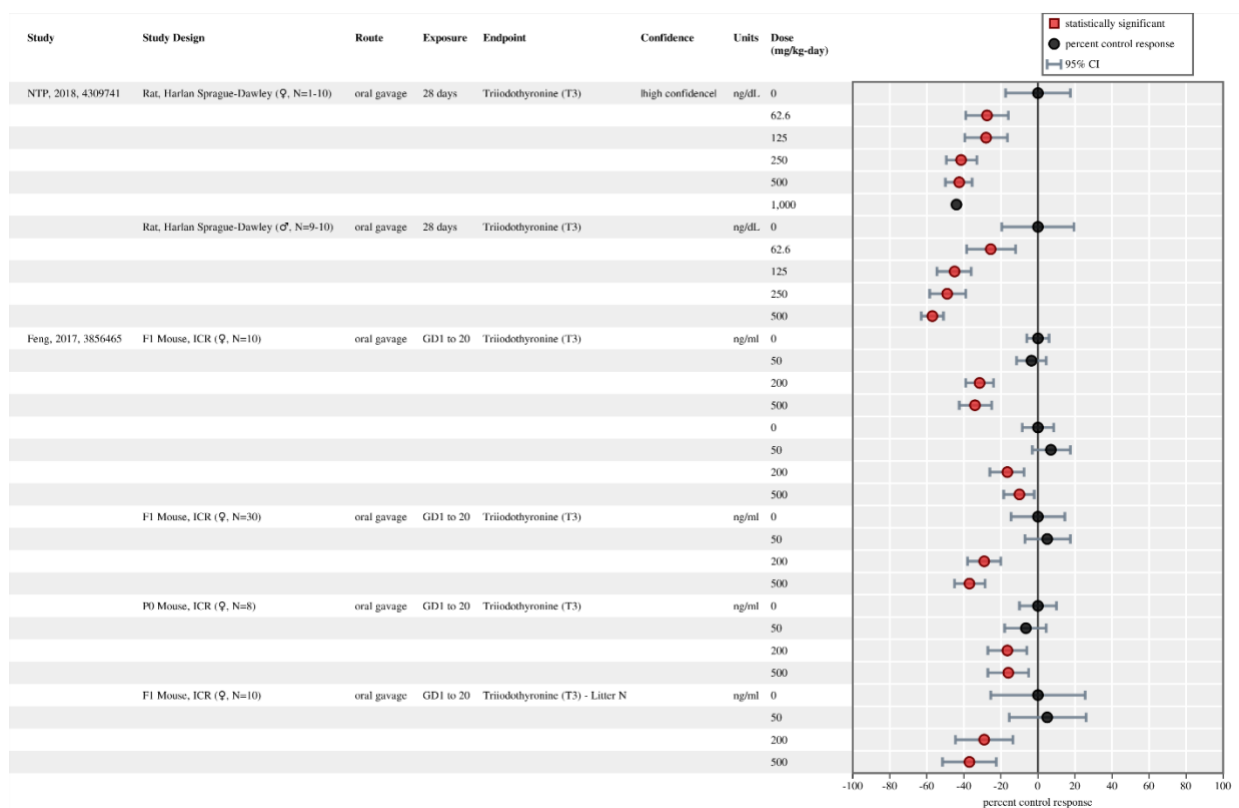
Figure E-2. Serum total triiodothyronine (T3) response in animals following K⁺PFBS exposure (click to see interactive data graphic).
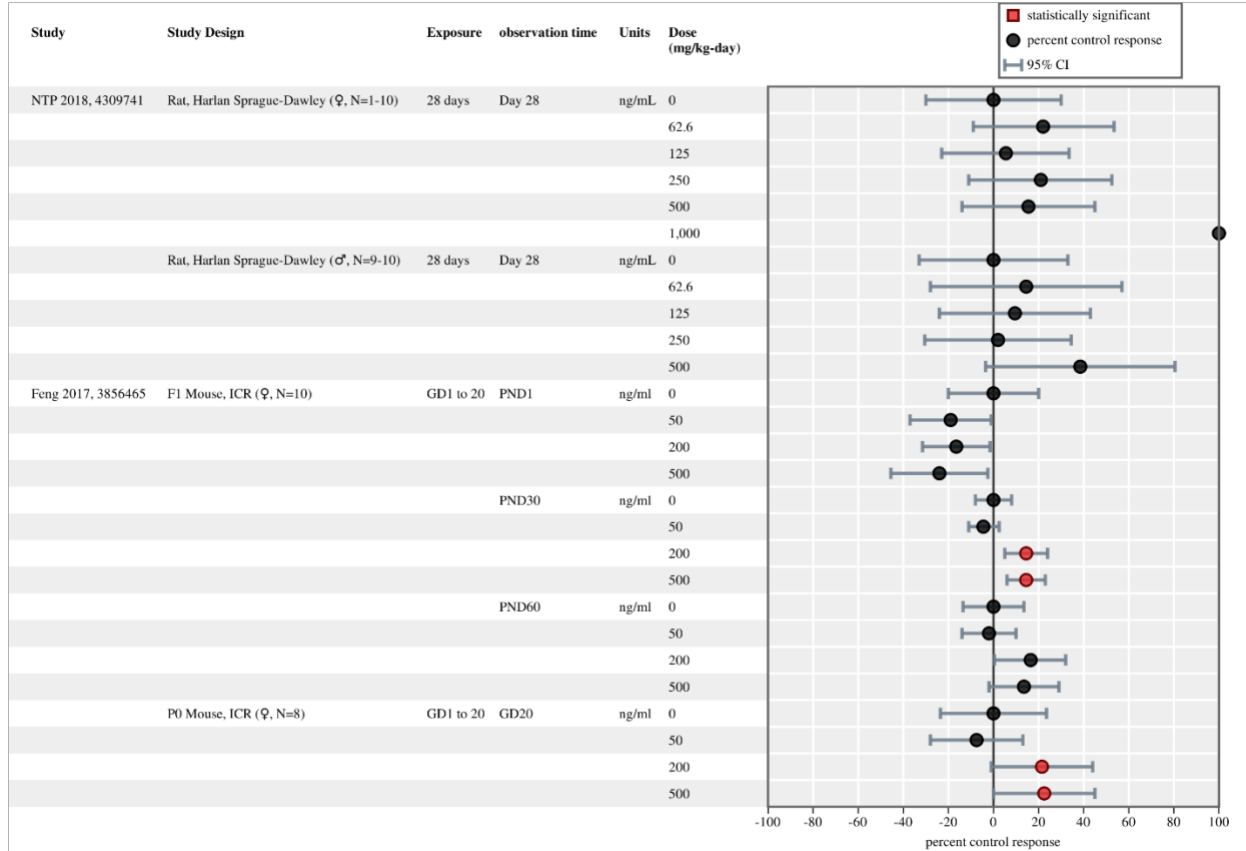
Figure E-3. Serum thyroid-stimulating hormone (TSH) response in animals following K+PFBS exposure (click to see interactive data graphic).
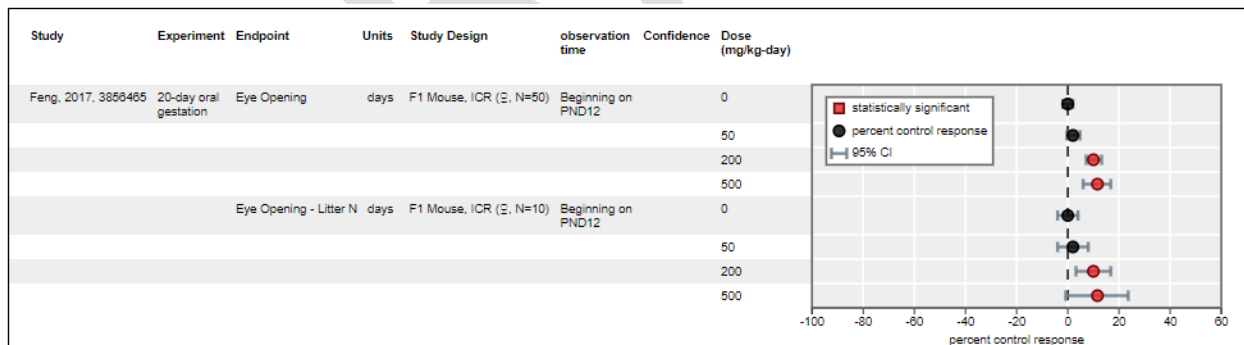
Figure E-4. Developmental effects (eye opening) following K+PFBS in rats (click to see interactive data graphic).
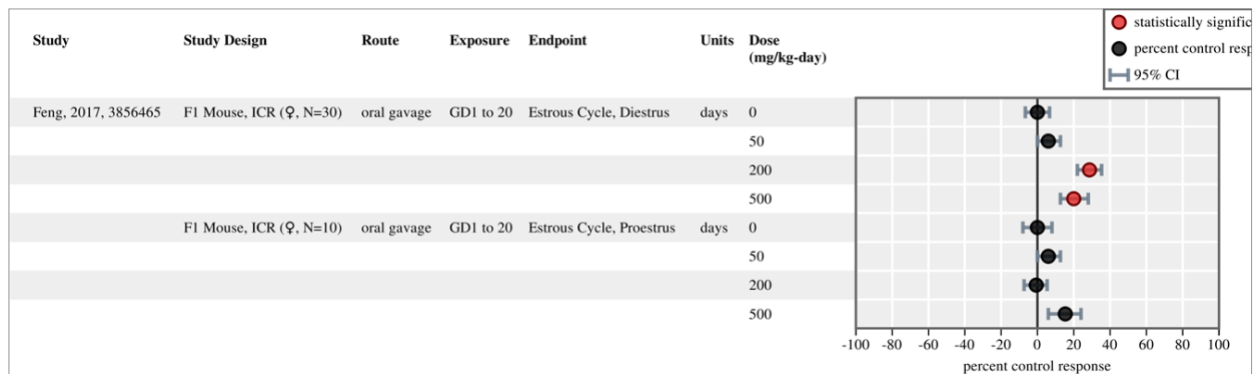
Figure E-5. Developmental effects (first estrus) following K^+PFBS in rats
(click to see interactive data graphic).
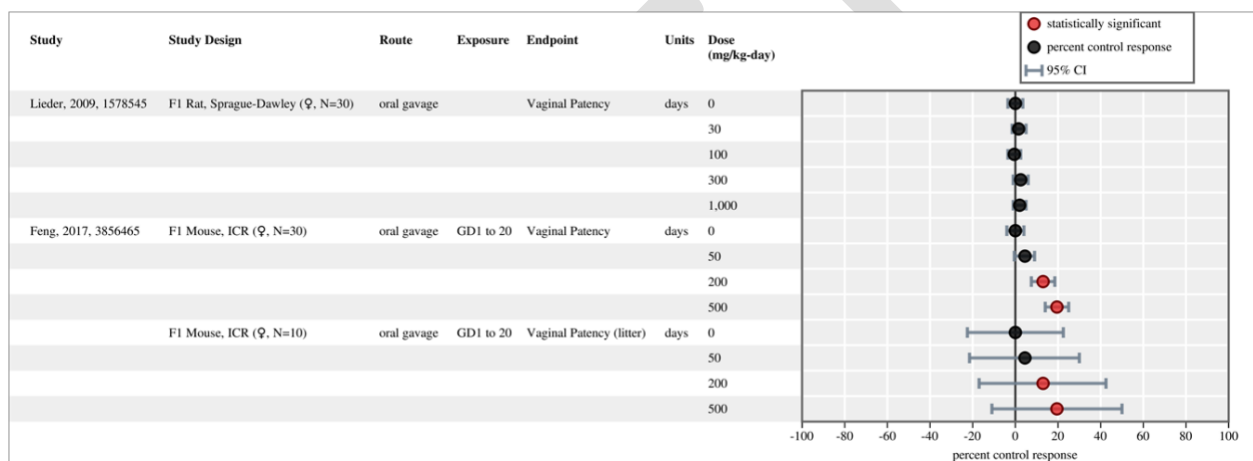
Figure E-6. Developmental effects (vaginal patency) following K^+PFBS in rats
(click to see interactive data graphic).

Study	Animal description	Route	Study Duration	Endpoint	Incidence	Dose (mg/kg-day)	
Lieder, 2009, 1578546	Rat, Crl:Cd@(Sd)Igs Br Vaf/Plustm (♀, N=10)	oral gavage	subchronic (90 days)	Kidney, Edema, Focal Papillary	0/10 (0.0%)	0	
						60	
						200	
					3/10 (30.0%)	600	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	0/10 (0.0%)	0	
						60	
					1/10 (10.0%)	200	
					6/10 (60.0%)	600	
	Rat, Crl:Cd@(Sd)Igs Br Vaf/Plustm (♂, N=10)	oral gavage	subchronic (90 days)	Kidney, Edema, Focal Papillary	0/10 (0.0%)	0	
						60	
						200	
					3/10 (30.0%)	600	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	1/10 (10.0%)	0	
						200	
					8/10 (80.0%)	600	
Lieder, 2009, 1578545	F1 Rat, Sprague-Dawley (♀, N=30)	oral gavage	reproductive	Kidney, Edema, Focal Papillary	0/30 (0.0%)	0	
					7/30 (23.3%)	300	
					4/30 (13.3%)	1,000	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	2/30 (6.7%)	0	
					13/30 (43.3%)	300	
					15/30 (50.0%)	1,000	
	F1 Rat, Sprague–Dawley (♂, N=30)	oral gavage	reproductive	Kidney, Edema, Focal Papillary	1/30 (3.3%)	0	
					0/30 (0.0%)	300	
					9/30 (30.0%)	1,000	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	3/30 (10.0%)	0	
					5/30 (16.7%)	300	
					21/30 (70.0%)	1,000	
	P0 Rat, Sprague-Dawley (♀, N=30)	oral gavage	reproductive	Kidney, Edema, Focal Papillary	1/30 (3.3%)	0	
					8/30 (26.7%)	300	
					7/30 (23.3%)	1,000	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	3/30 (10.0%)	0	
					16/30 (53.3%)	300	
					21/30 (70.0%)	1,000	
	P0 Rat, Sprague-Dawley (♂, N=30)	oral gavage	reproductive	Kidney, Edema, Focal Papillary	1/30 (3.3%)	0	
					2/30 (6.7%)	300	
					6/30 (20.0%)	1,000	
				Kidney, Hyperplasia, Papillary Tubular/Ductal Epithelium	0/30 (0.0%)	0	
					9/30 (30.0%)	300	
					19/30 (63.3%)	1,000	

Figure E-7. Kidney histopathological effects following K^+PFBS in rats
(click to see interactive data graphic).
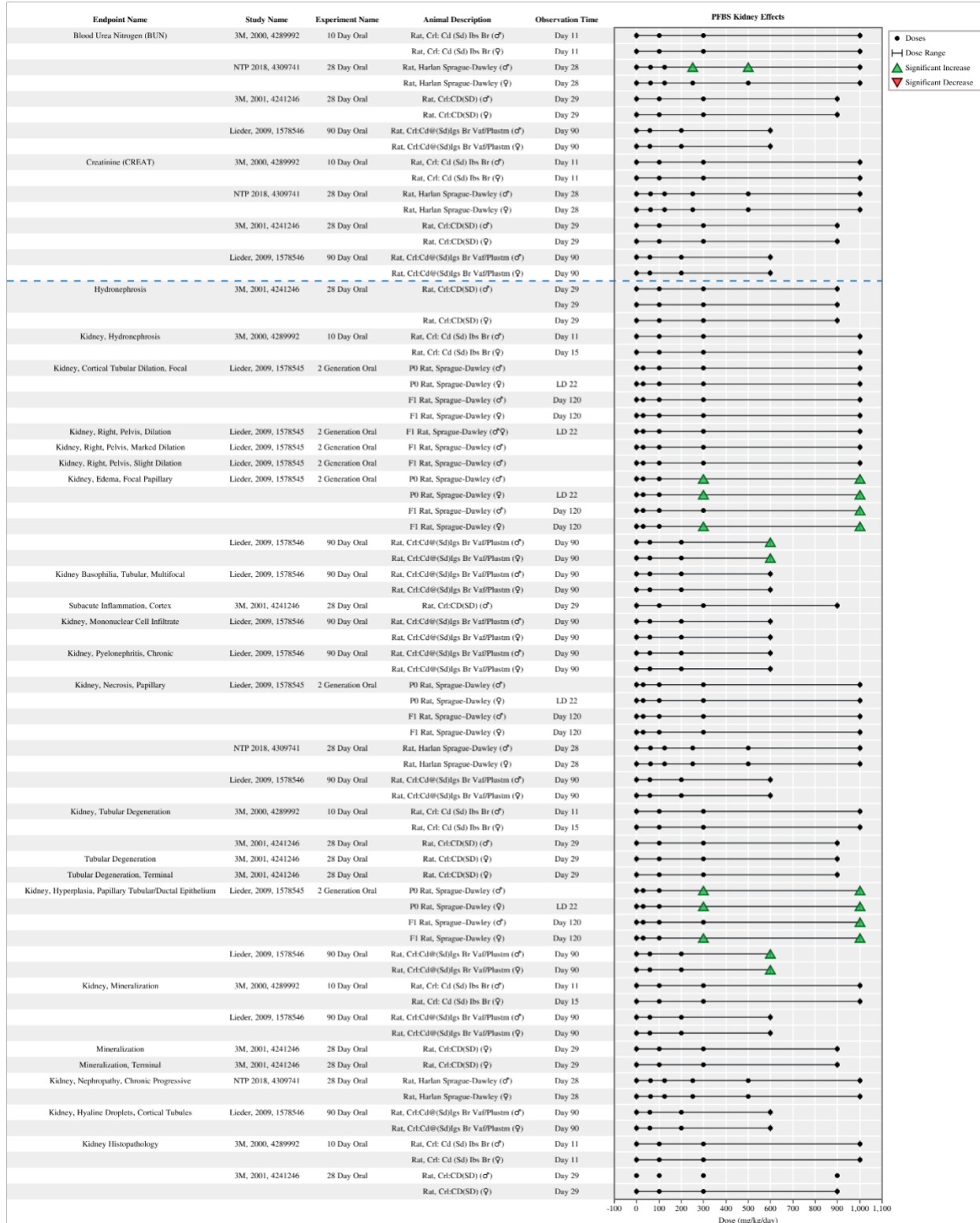
Figure E-8. Renal effects following K^{+}PFBS in rats (click to see interactive data graphic).
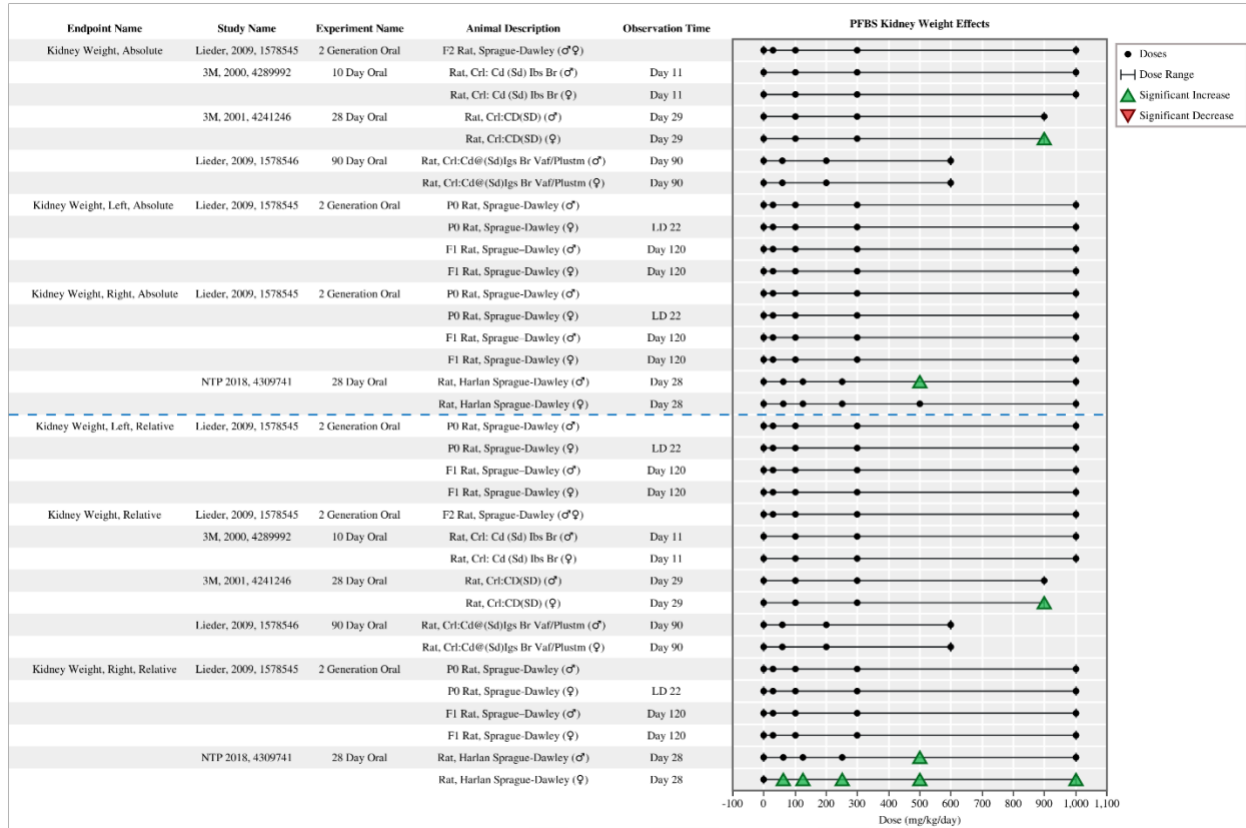
Figure E-9. Kidney weight effects following K$^+$PFBS in rats (click to see interactive data graphic).
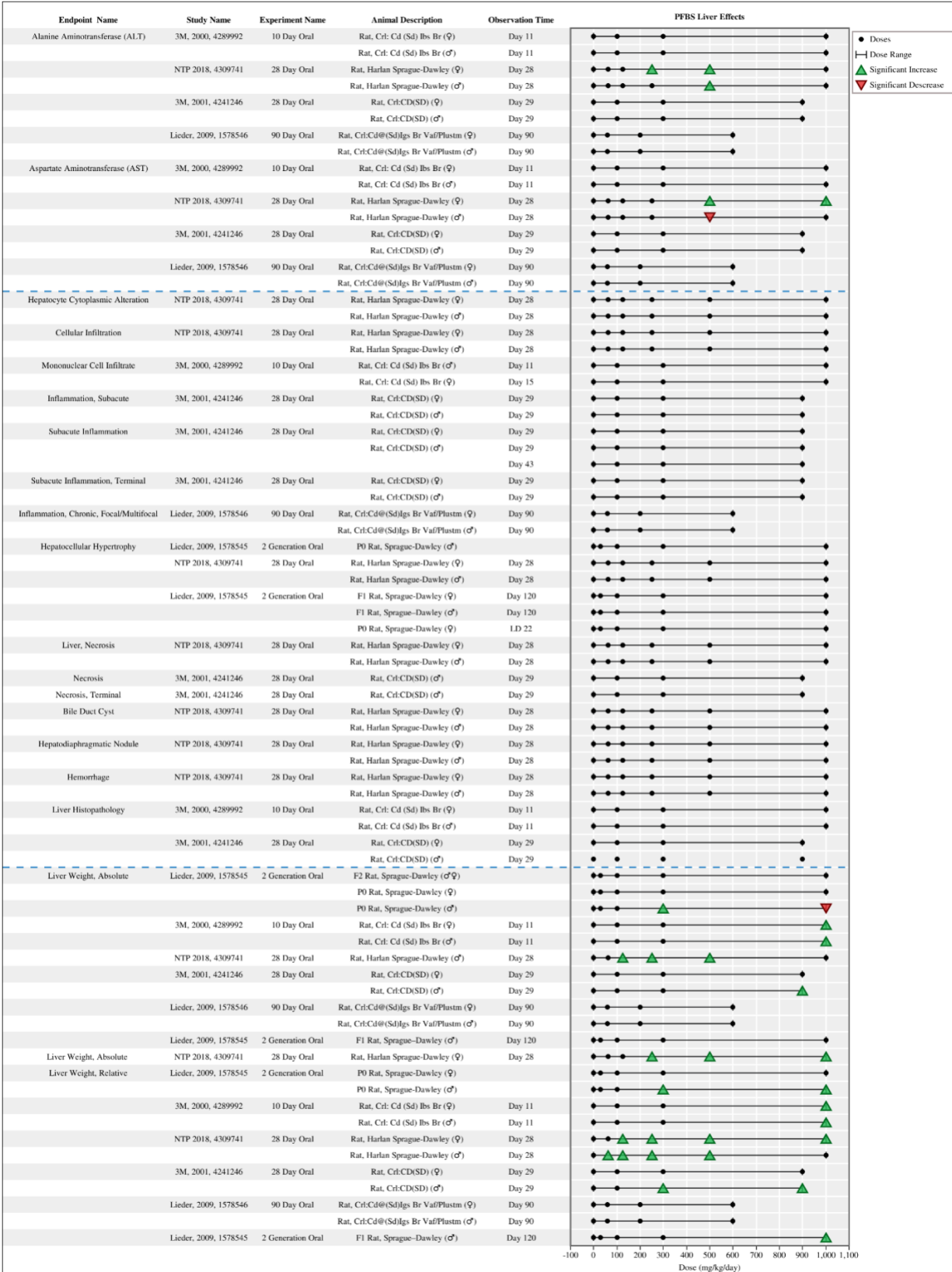
Figure E-10. Liver effects following K⁺PFBS in rats (click to see interactive data graphic).
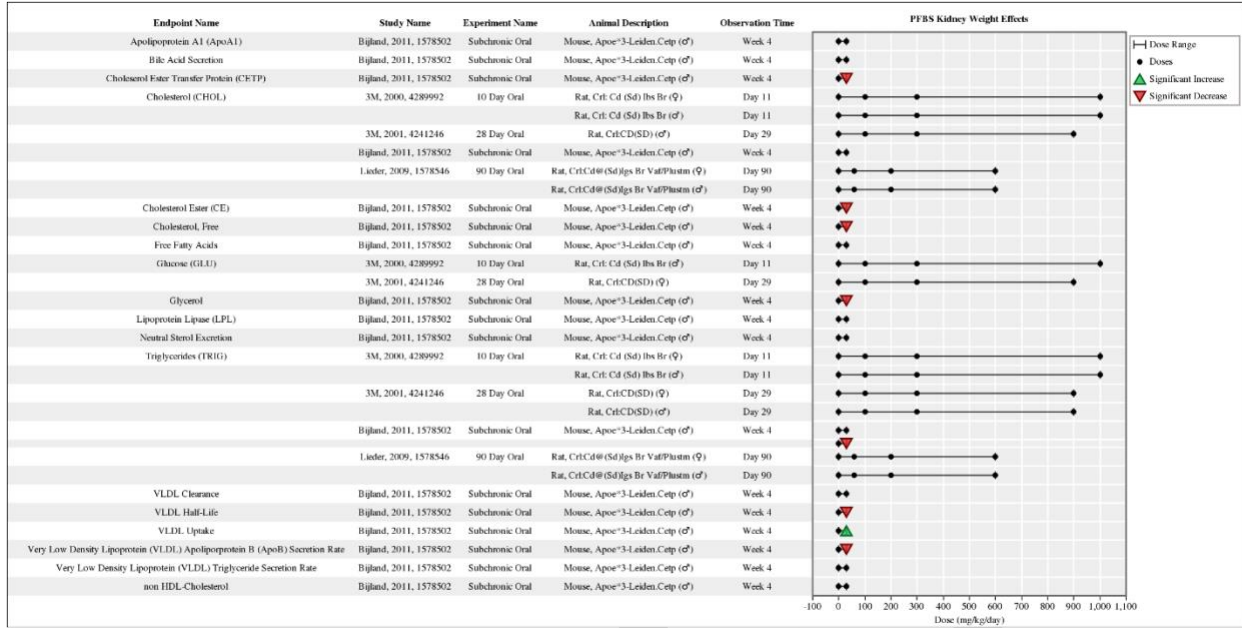
Figure E-11. Effects on lipids and lipoproteins following K$^+$PFBS in rats and mice
(click to see interactive data graphic).

Appendix F. Benchmark Dose Modeling Results

F.1.   Modeling of Noncancer Endpoints

As discussed in the body of the report under "Derivation of Oral Reference Doses," the endpoints selected for benchmark dose (BMD) modeling were incidence of renal papillary epithelial tubular/ductal hyperplasia in rats from Lieder et al. (2009a) and Lieder et al. (2009b); thyroid hormones in pregnant mice and offspring at postnatal day (PND) 1, PND 30, and PND 60 from Feng et al. (2017) and adult rats from NTP (2018); and developmental effects (i.e., eye opening, first estrus, vaginal opening) from Feng et al. (2017). The animal doses in the study, converted to human equivalent doses (HEDs), were used in the BMD modeling; the data are available for download in Health Assessment Workspace Collaborative (HAWC). BMD modeling was conducted by experts in quantitative Benchmark Dose Software (BMDS) analysis and interpretation. Links to the data and modeling output are included in Table F-1. The selected point of departure (POD) (HED) listed in Table F-1 represents the best fitting model for each endpoint; if the data were determined to not be amenable to BMD modeling, the no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) is listed. Figure F-1 illustrates the doses examined and NOAEL, LOAEL, BMD, and benchmark dose lower confidence limit (BMDL) values for the potential critical effects.

Table F-1. Candidate PODs for the derivation of the subchronic and chronic RfDs for PFBS (CASRN 375-73-5) and the related compound K$^+$PFBS (CASRN 29420-49-3)

Endpoint/reference	Species/life stage—sex	Selected POD (HED)[a] (mg/kg-d)
Kidney effects		
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009a)	Rat/Male	BMDL$_{10}$ = 47.0
	Rat/Female	BMDL$_{10}$ = 12.6
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/P$_0$—Male	BMDL$_{10}$ = 20.0
	Rat/P$_0$—Female	BMDL$_{10}$ = 11.5
Kidney histopathology—papillary epithelial tubular/ductal hyperplasia—Lieder et al. (2009b)	Rat/F$_1$—Male	BMDL$_{10}$ = 42.8
	Rat/F$_1$—Female	BMDL$_{10}$ = 20.02
Thyroid effects		
Total T4 – NTP (2018)	Rat—Male	LOAEL = 15.5
	Rat—Female	BMDL$_{1SD}$ = 1.6
Free T4 – NTP (2018)	Rat—Male	LOAEL = 15.5
	Rat—Female	LOAEL = 14.3
Total T4—Feng et al. (2017)	Mouse/P$_0$—Female	BMDL$_{20}$ = 7.8
Free T4—Feng et al. (2017)	Mouse/P$_0$—Female	NOAEL = 7.5
TSH—Feng et al. (2017)	Mouse/P$_0$—Female	NOAEL = 7.5
Total T4 PND 1 (fetal *n*)—Feng et al. (2017)	Mouse/F$_1$—Female	NOAEL = 7.5
Total T4 PND 1 (litter *n*)—Feng et al. (2017)	**Mouse/F$_1$—Female**	**BMDL$_{20}$ = 4.2**
Total T4 PND 30—Feng et al. (2017)	Mouse/F$_1$—Female	BMDL$_{20}$ = 7.8

Endpoint/reference	Species/life stage—sex	Selected POD (HED)[a] (mg/kg-d)
Total T4 PND 60—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5
TSH PND 30—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5
Developmental effects		
Eyes opening (fetal *n*)—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5
Eyes opening (litter *n*)—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 14.8
Vaginal opening (fetal *n*)—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 12.4
Vaginal opening (litter *n*)—Feng et al. (2017)	Mouse/F_1—Female	$BMDL_{1SD}$ = 7.9
First estrous (fetal *n*)—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5
First estrous (litter *n*)—Feng et al. (2017)	Mouse/F_1—Female	NOAEL = 7.5

Notes: BW = body weight; RfD = reference dose; PFBS = perfluorobutane sulfonic acid; CASRN = Chemical Abstracts Service Registry Number; K^+PFBS = potassium perfluorobutane sulfonate; T3 = total triiodothyronine; T4 = total thyroxine; TSH = thyroid-stimulating hormone.

[a] Following U.S. EPA (2011b) guidance, animal doses from candidate principal studies were converted to HEDs through the application of a dosimetric adjustment factor (DAF). DAFs for each dose are calculated as follows: $DAF = (BW_a^{1/4} \div BW_h^{1/4})$, where BW_a = animal BW and BW_h = human BW. For all DAF calculations, a reference human BW (BW_h) of 80 kilogram (kg) (U.S. EPA, 1988) was used. See Table 9 in assessment for full details. Links are to the HAWC BMDS session containing full modeling results for that endpoint.

[b] Data from offspring,
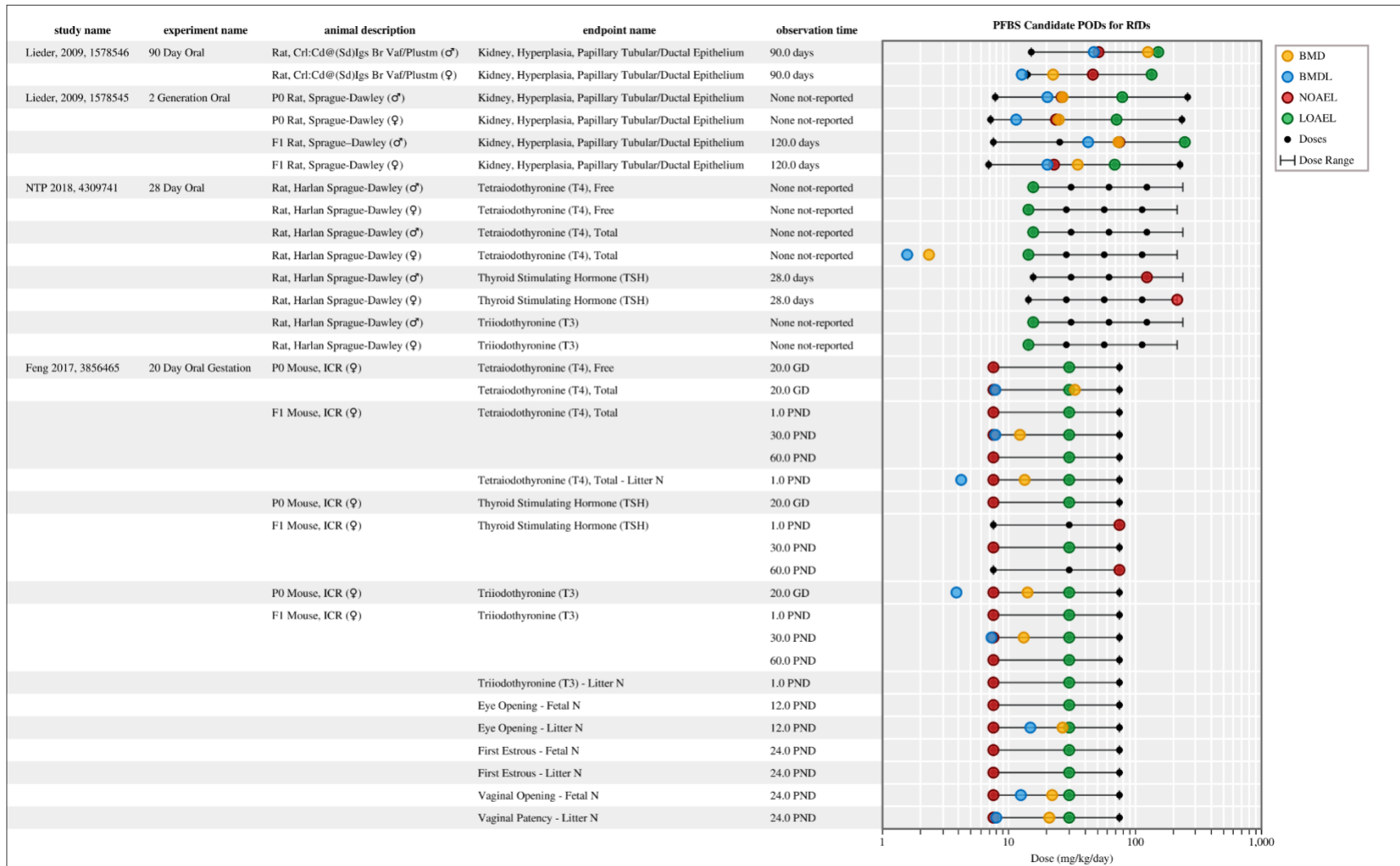
Figure F-1. Candidate PODs for the derivation of the subchronic and chronic RfDs for PFBS
(click to see interactive data graphic).

F.2.   Modeling Procedure for Continuous Noncancer Data

BMD modeling of continuous data was conducted on the HAWC website using the U.S. Environmental Protection Agency's (EPA's) BMDS (Version 2.7). All continuous models available within the software were fit using a benchmark response (BMR) of 1 standard deviation (SD) when no toxicological information was available to determine an adverse level of response. When toxicological information was available, the BMR was based on relative deviation (e.g., BMR 20% relative deviation [RD] for T4 effects), as outlined in the *Benchmark Dose Technical Guidance* (U.S. EPA, 2012). An adequate fit is judged based on the $\chi 2$ goodness-of-fit *p*-value ($p > 0.1$), magnitude of the scaled residuals in the vicinity of the BMR, and visual inspection of the model fit. In addition to these three criteria for judging adequacy of model fit, a determination is made as to whether the variance across dose groups is homogeneous. If a homogeneous variance model is deemed appropriate based on the statistical test provided by BMDS (i.e., Test 2), the final BMD results are estimated from a homogeneous variance model. If the test for homogeneity of variance is rejected ($p < 0.1$), the model is run again while modeling the variance as a power function of the mean to account for this nonhomogeneous variance. If this nonhomogeneous variance model does not adequately fit the data (i.e., Test 3; $p < 0.1$), the data set is considered unsuitable for BMD modeling. In cases in which a model with # parameters = # dose-groups was fit to the data set and all parameters were estimated and no p-value was calculated, that model was not considered for estimation of a POD unless no other model provided adequate fit. Among all models providing adequate fit, the BMDL from the model with the lowest Akaike's information criterion (AIC) was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD from which to derive the oral reference dose/inhalation reference concentration (RfD/RfC).

Modeling Predictions for Serum Total T$_4$ in PND 1 Female Offspring (litter n)

The modeling results for total T4 in PND 1 female offspring (litter n) exposed gestation days (GDs) 1−20 are shown in Table F-2. The Exponential 4 model (Figure F-2) was selected given appropriate fit to the data and that the BMDL values differed by greater than threefold. The output for the EPA's BMDS model run is also provided below.

Table F-2. Modeling results for total T4 in PND 1 female offspring (litter n) exposed GDs 1−20[a]

Model	Global p-value	AIC	BMD_{1SD} (HED) (mg/kg-d)	$BMDL_{1SD}$ (HED) (mg/kg-d)	BMD_{20} (HED) (mg/kg-d)	BMDL20 (HED) (mg/kg-d)	Residual of interest
Linear	0.558	-4.72314	54.547	35.8919	27.9861	20.2211	0.349
Polynomial	0.558	-4.72314	54.547	35.8919	27.9861	20.2211	0.349
Power	0.558	-4.72314	54.547	35.8919	27.9861	20.2211	0.349
Hill	-999	-1.89	30.1637	8.14952	14.2873	3.26417	-0.0000023
Exponential-M2	0.7627	-5.34819	43.9284	23.3838	20.8317	12.5215	-0.5885
Exponential-M3	0.7627	-5.34819	43.9284	23.3838	20.8317	12.5215	-0.5885
Exponential-M4[b]	0.8421	-3.85031	30.3302	8.85171	13.2799	4.22705	-0.09388
Exponential-M5	-999	-1.89	30.136	8.93443	14.8157	4.26686	4.453e-7

Notes: BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., 20 = exposure concentration associated with 20% relative deviation from control).
[a] Feng et al. (2017).
[b] Selected model. Exponential 4 model was selected given appropriate fit to the data and that the BMDL values differed by greater than threefold. The Exponential 5 model was not selected because it did not return a p-value.
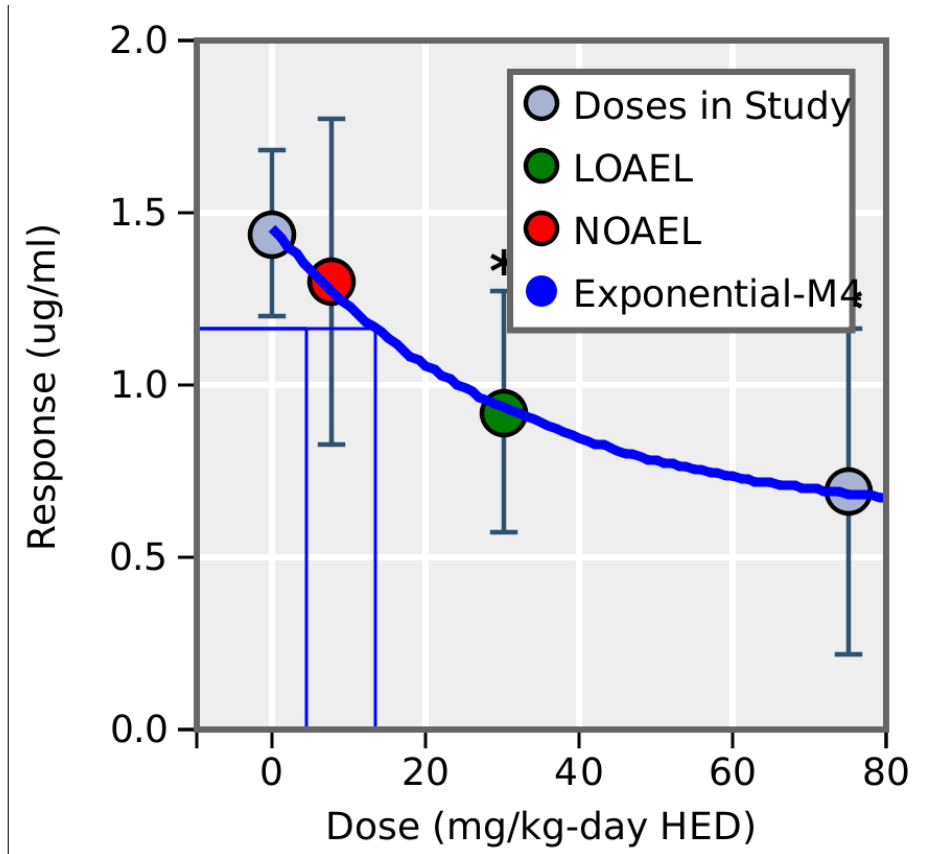
Figure F-2. Exponential (Model 4) for total T4 in PND 1 female offspring (litter n) exposed GDs 1−20 (Feng et al. (2017).

```
========================================================================
        Exponential Model. (Version: 1.11;  Date: 03/14/2017)
        Input Data File: C:\Windows\TEMP\bmds-dfile-bc5js794.(d)
        Gnuplot Plotting File:
                                              Thu Aug 09 08:49:17 2018
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BMDS_Model_Run

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  The form of the response function by Model:
    Model 2:     Y[dose] = a * exp{sign * b * dose}
    Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
    Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
    Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]
```

```
 Note: Y[dose] is the median response for exposure = dose;
        sign = +1 for increasing trend in data;
        sign = -1 for decreasing trend.


   Model 2 is nested within Models 3 and 4.
   Model 3 is nested within Model 5.
   Model 4 is nested within Model 5.


Dependent variable = Response
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.


Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


MLE solution provided: Exact


                Initial Parameter Values

                Variable           Model 4
                --------           --------
                  lnalpha          -1.29725
                      rho                 0 Specified
                        a             1.512
                        b         0.0428586
                        c          0.434618
                        d                 1 Specified


                  Parameter Estimates

                Variable           Model 4          Std. Err.
                --------           -------          ---------
                  lnalpha          -1.29626          0.0611684
                        a            1.4541           0.148456
                        b         0.0316353          0.0322218
                        c          0.416958           0.222524
```

```
NC = No Convergence


        Table of Stats From Input Data

   Dose       N         Obs Mean      Obs Std Dev
   -----      ---       ----------    -------------
      0       10          1.44          0.329
    7.5       10           1.3          0.657
   29.9       10          0.92          0.493
     75       10          0.69          0.657


          Estimated Values of Interest

   Dose      Est Mean      Est Std      Scaled Residual
   ------    ----------    ---------    ----------------
      0        1.454        0.523          -0.08525
    7.5        1.275        0.523            0.151
   29.9       0.9355        0.523          -0.09388
     75       0.6853        0.523           0.02816


Other models for which likelihoods are calculated:

  Model A1:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma^2

  Model A2:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = Sigma(i)^2

  Model A3:        Yij = Mu(i) + e(ij)
           Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:        Yij = Mu + e(i)
           Var{e(ij)} = Sigma^2


                    Likelihoods of Interest

            Model      Log(likelihood)      DF        AIC
            -------    -----------------    ----    -----------
              A1          5.944999            5      -1.889998
              A2          8.698072            8      -1.396144
              A3          5.944999            5      -1.889998
               R          0.3138778           2       3.372244
               4          5.925156            4      -3.850311
```

```
Additive constant for all log-likelihoods =      -36.76. This constant added to
the above values gives the log-likelihood including the term that does not
depend on the model parameters.


                         Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2:  Are Variances Homogeneous? (A2 vs. A1)

Test 3:  Are variances adequately modeled? (A2 vs. A3)


Test 6a: Does Model 4 fit the data? (A3 vs 4)


                         Tests of Interest

   Test          -2*log(Likelihood Ratio)        D. F.         p-value
 --------        ------------------------        ------       --------------

   Test 1                     16.77                6            0.01017

   Test 2                     5.506                3            0.1383

   Test 3                     5.506                3            0.1383

  Test 6a                    0.03969               1            0.8421


  The p-value for Test 1 is less than .05. There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1. A homogeneous
  variance model appears to be appropriate here.

  The p-value for Test 3 is greater than .1. The modeled
  variance appears to be appropriate here.

  The p-value for Test 6a is greater than .1. Model 4 seems
  to adequately describe the data.

Benchmark Dose Computations:

  Specified Effect = 1.000000

        Risk Type = Estimated standard deviations from control

  Confidence Level = 0.950000

            BMD =      30.3302

           BMDL =      8.85171

           BMDU =       750000
```

```
  =====================================================================

            Exponential Model. (Version: 1.11;  Date: 03/14/2017)
            Input Data File: C:\Windows\TEMP\bmds-dfile-nq3ld_k8.(d)
            Gnuplot Plotting File:

                                         Thu Aug 09 08:49:17 2018

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BMDS_Model_Run

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   The form of the response function by Model:
      Model 2:     Y[dose] = a * exp{sign * b * dose}
      Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
      Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
      Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

    Note: Y[dose] is the median response for exposure = dose;
          sign = +1 for increasing trend in data;
          sign = -1 for decreasing trend.

      Model 2 is nested within Models 3 and 4.
      Model 3 is nested within Model 5.
      Model 4 is nested within Model 5.


   Dependent variable = Response
   Independent variable = Dose
   Data are assumed to be distributed: normally
   Variance Model: exp(lnalpha +rho *ln(Y[dose]))
   rho is set to 0.
   A constant variance model is fit.


   Total number of dose groups = 4
   Total number of records with missing values = 0
   Maximum number of iterations = 500
   Relative Function Convergence has been set to: 1e-008
   Parameter Convergence has been set to: 1e-008


   MLE solution provided: Exact
```

```
              Initial Parameter Values

           Variable            Model 4
           --------            --------
             lnalpha            -1.29725
                rho                    0 Specified
                  a              1.512
                  b          0.0428586
                  c           0.434618
                  d                    1 Specified


              Parameter Estimates

           Variable            Model 4          Std. Err.
           --------            -------          ---------
             lnalpha           -1.29626          0.0611684
                  a             1.4541           0.148456
                  b          0.0316353           0.0322218
                  c           0.416958           0.222524


   NC = No Convergence

          Table of Stats From Input Data

    Dose      N       Obs Mean     Obs Std Dev
    -----    ---     ----------    -------------
       0     10         1.44          0.329
     7.5     10          1.3          0.657
    29.9     10         0.92          0.493
      75     10         0.69          0.657


             Estimated Values of Interest

    Dose      Est Mean      Est Std      Scaled Residual
    ------   ----------    ---------    ----------------
       0        1.454        0.523          -0.08525
     7.5        1.275        0.523            0.151
    29.9       0.9355        0.523          -0.09388
      75       0.6853        0.523           0.02816


   Other models for which likelihoods are calculated:

     Model A1:        Yij = Mu(i) + e(ij)
              Var{e(ij)} = Sigma^2
```

```
   Model A2:           Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

   Model A3:           Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

   Model  R:           Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2

                        Likelihoods of Interest

                Model      Log(likelihood)      DF        AIC
                -------    -----------------    ----    ------------
                  A1           5.944999           5       -1.889998
                  A2           8.698072           8       -1.396144
                  A3           5.944999           5       -1.889998
                   R           0.3138778          2        3.372244
                   4           5.925156           4       -3.850311


Additive constant for all log-likelihoods =     -36.76. This constant added to
the above values gives the log-likelihood including the term that does not
depend on the model parameters.


                          Explanation of Tests

Test 1:  Does response and/or variances differ among Dose levels? (A2 vs. R)

Test 2:  Are Variances Homogeneous? (A2 vs. A1)

Test 3:  Are variances adequately modeled? (A2 vs. A3)


Test 6a: Does Model 4 fit the data? (A3 vs 4)


                           Tests of Interest

   Test         -2*log(Likelihood Ratio)        D. F.         p-value
 --------       ------------------------        ------      --------------
   Test 1                  16.77                   6            0.01017
   Test 2                  5.506                   3            0.1383
   Test 3                  5.506                   3            0.1383
  Test 6a                  0.03969                 1            0.8421


  The p-value for Test 1 is less than .05. There appears to be a
  difference between response and/or variances among the dose
  levels, it seems appropriate to model the data.

  The p-value for Test 2 is greater than .1. A homogeneous
  variance model appears to be appropriate here.
```

```
     The p-value for Test 3 is greater than .1. The modeled
     variance appears to be appropriate here.

     The p-value for Test 6a is greater than .1. Model 4 seems
     to adequately describe the data.

  Benchmark Dose Computations:

    Specified Effect = 0.100000

           Risk Type = Relative deviation

    Confidence Level = 0.950000

                 BMD =      5.94765

                BMDL =      1.83718

                BMDU =      18.5178

 =====================================================================

          Exponential Model. (Version: 1.11;  Date: 03/14/2017)
          Input Data File: C:\Windows\TEMP\bmds-dfile-1lwh9peo.(d)
          Gnuplot Plotting File:
                                            Thu Aug 09 08:49:17 2018

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BMDS_Model_Run

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   The form of the response function by Model:
     Model 2:     Y[dose] = a * exp{sign * b * dose}
     Model 3:     Y[dose] = a * exp{sign * (b * dose)^d}
     Model 4:     Y[dose] = a * [c-(c-1) * exp{-b * dose}]
     Model 5:     Y[dose] = a * [c-(c-1) * exp{-(b * dose)^d}]

  Note: Y[dose] is the median response for exposure = dose;
        sign = +1 for increasing trend in data;
        sign = -1 for decreasing trend.

    Model 2 is nested within Models 3 and 4.
    Model 3 is nested within Model 5.
    Model 4 is nested within Model 5.
```

```
Dependent variable = Response
Independent variable = Dose
Data are assumed to be distributed: normally
Variance Model: exp(lnalpha +rho *ln(Y[dose]))
rho is set to 0.
A constant variance model is fit.


Total number of dose groups = 4
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


MLE solution provided: Exact


                Initial Parameter Values

                Variable            Model 4
                --------            --------
                  lnalpha           -1.29725
                      rho                  0 Specified
                        a              1.512
                        b          0.0428586
                        c           0.434618
                        d                  1 Specified


                  Parameter Estimates

                Variable            Model 4           Std. Err.
                --------            -------           ---------
                  lnalpha           -1.29626          0.0611684
                        a             1.4541          0.148456
                        b          0.0316353          0.0322218
                        c           0.416958          0.222524


   NC = No Convergence


         Table of Stats From Input Data

   Dose      N         Obs Mean     Obs Std Dev
   -----    ---       ----------    -------------
      0      10          1.44         0.329
    7.5      10           1.3         0.657
```

```
    29.9      10           0.92          0.493
     75       10           0.69          0.657


                Estimated Values of Interest

    Dose      Est Mean      Est Std     Scaled Residual
    ------    ----------    ---------   ----------------
       0         1.454        0.523        -0.08525
     7.5         1.275        0.523          0.151
    29.9        0.9355        0.523        -0.09388
      75        0.6853        0.523         0.02816


Other models for which likelihoods are calculated:

  Model A1:          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma^2

  Model A2:          Yij = Mu(i) + e(ij)
            Var{e(ij)} = Sigma(i)^2

  Model A3:          Yij = Mu(i) + e(ij)
            Var{e(ij)} = exp(lalpha + log(mean(i)) * rho)

  Model  R:          Yij = Mu + e(i)
            Var{e(ij)} = Sigma^2


                    Likelihoods of Interest

            Model      Log(likelihood)      DF         AIC
            -------    -----------------    ----    ------------
              A1          5.944999           5       -1.889998
              A2          8.698072           8       -1.396144
              A3          5.944999           5       -1.889998
               R          0.3138778          2        3.372244
               4          5.925156           4       -3.850311
```

Additive constant for all log-likelihoods =   -36.76. This constant added to the
above values gives the log-likelihood including the term that does not
depend on the model parameters.

                    Explanation of Tests

Test 1: Does response and/or variances differ among Dose levels? (A2 vs. R)
Test 2: Are Variances Homogeneous? (A2 vs. A1)
Test 3: Are variances adequately modeled? (A2 vs. A3)

```
Test 6a: Does Model 4 fit the data? (A3 vs 4)


                          Tests of Interest

   Test           -2*log(Likelihood Ratio)      D. F.         p-value
 --------         ------------------------      ------       --------------

   Test 1                        16.77             6             0.01017

   Test 2                        5.506             3             0.1383

   Test 3                        5.506             3             0.1383

  Test 6a                      0.03969             1             0.8421


   The p-value for Test 1 is less than .05. There appears to be a
   difference between response and/or variances among the dose
   levels, it seems appropriate to model the data.

   The p-value for Test 2 is greater than .1. A homogeneous
   variance model appears to be appropriate here.

   The p-value for Test 3 is greater than .1. The modeled
   variance appears to be appropriate here.

   The p-value for Test 6a is greater than .1. Model 4 seems
   to adequately describe the data.


 Benchmark Dose Computations:

   Specified Effect = 0.200000

         Risk Type = Relative deviation

   Confidence Level = 0.950000

               BMD =        13.2799

              BMDL =        4.22705

              BMDU =        39.2189
```

F.3.   Modeling Procedure for Dichotomous Noncancer Data

BMD modeling of dichotomous noncancer data was conducted on the HAWC website using the
EPA's BMDS Version 2.7. For these data, the Gamma, Logistic, Log-Logistic, Log-Probit,
Multistage, Probit, and Weibull dichotomous models available within the software were fit using
a BMR of 10% extra risk. The Multistage model is run for all polynomial degrees up to $n-2$,
where n is the number of dose groups including control. Adequacy of model fit was judged based
on the $\chi 2$ goodness-of-fit p-value ($p > 0.1$), scaled residuals at the data point (except the control)
closest to the predefined BMR (absolute value < 2.0), and visual inspection of the model fit. In
the cases where no best model was found to fit to the data, a reduced data set without the
high-dose group was further attempted for modeling and the result was presented along with that
of the full data set. In cases in which a model with # parameters = # dose-groups was fit to the

data set and all parameters were estimated and no *p*-value was calculated, that model was not considered for estimation of a POD *unless* no other model provided adequate fit. Among all models providing adequate fit, the BMDL from the model with the lowest AIC was selected as a potential POD when BMDL values were sufficiently close (within threefold). Otherwise, the lowest BMDL was selected as a potential POD.

Modeling Predictions for Papillary Tubular/Ductal Epithelium Hyperplasia in P_0 Female Rats

The modeling results papillary tubular/ductal epithelium hyperplasia in P0 female rats are shown in Table F-3. The Dichotomous Hill model (Figure F-3) was selected based on the lowest AIC. The output for the EPA's BMDS model run is also provided below.

Table F-3. Modeling results for papillary tubular/ductal epithelium hyperplasia in P_0 female rats[a]

Model	Global p-value	AIC	BMD_{10} (HED) (mg/kg-d)	$BMDL_{10}$ (HED) (mg/kg-d)	Residual of interest
Logistic	0.0099	154.462	44.8115	35.0795	-0.34
LogLogistic	0.2488	148.137	16.852	8.27226	-0.503
Probit	0.0121	153.975	42.7597	34.2962	-0.294
LogProbit	0.1977	147.751	29.4015	21.4928	0.193
Multistage	0.2216	147.724	18.1972	13.3757	-0.391
Gamma	0.2216	147.724	18.1971	13.3757	-0.391
Weibull	0.2216	147.724	18.1973	13.3757	-0.391
Dichotomous-Hill[b]	**0.5993**	**147.623**	**24.915**	**11.4888**	**0.063**

Notes: BMD = maximum likelihood estimate of the exposure concentration associated with the selected BMR; BMDL = 95% lower confidence limit on the BMD (subscripts denote BMR: i.e., 10 = exposure concentration associated with 10% extra risk).
[a] Lieder et al. (2009b)
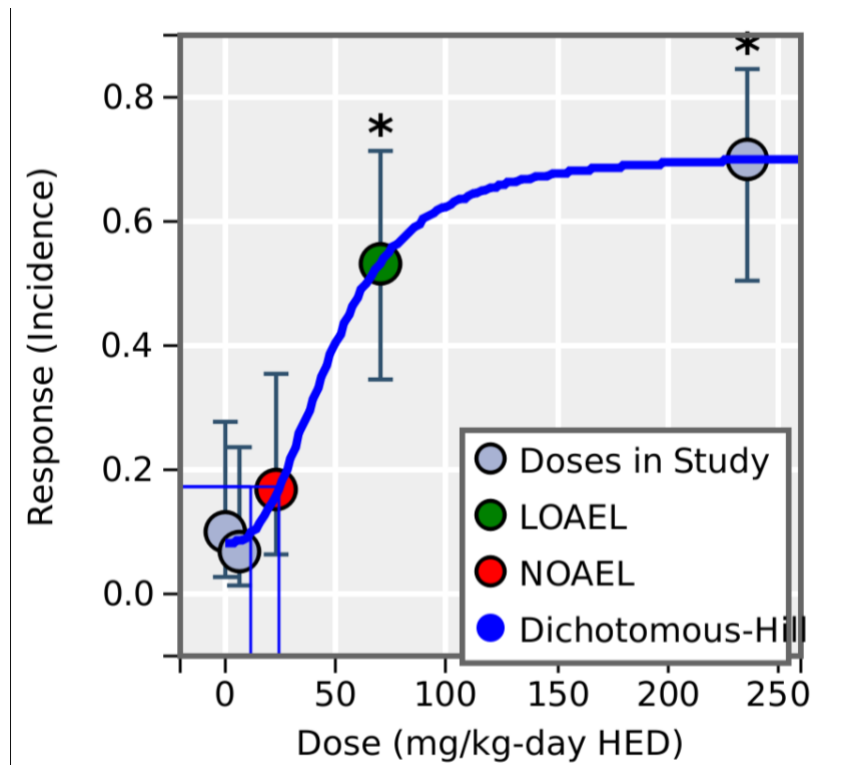[b] Selected model. Dichotomous Hill model was selected based on lowest AIC.

Figure F-3. Dichotomous-Hill model for papillary tubular/ductal epithelium hyperplasia in P$_0$ female rats Lieder et al. (2009b).

```
=======================================================================
        Dichotomous Hill Model. (Version: 1.3; Date: 02/28/2013)
        Input Data File: C:\Windows\TEMP\bmds-dfile-lohmpk31.(d)
        Gnuplot Plotting File:  C:\Windows\TEMP\bmds-dfile-lohmpk31.plt
                                        Thu Aug 09 11:03:14 2018

================================================================
BMDS_Model_Run

~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~

  The form of the probability function is:

  P[response] = v*g +(v-v*g)/[1+EXP(-intercept-slope*Log(dose))]

      where: 0 <= g < 1, 0 < v <= 1

            v is the maximum probability of response predicted by the model,

            and v*g is the background estimate of that probability.
```

```
Dependent variable = Incidence
Independent variable = Dose
Slope parameter is restricted as slope >= 1


Total number of observations = 5
Total number of records with missing values = 0
Maximum number of iterations = 500
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008


                Default Initial Parameter Values
                        v =              1
                        g =            0.1
                intercept =       -6.87307
                    slope =        1.44728


        Asymptotic Correlation Matrix of Parameter Estimates

                    v           g      intercept        slope

        v           1       -0.34         0.36        -0.45

        g       -0.34           1        -0.47         0.45

intercept        0.36       -0.47            1        -0.99

    slope       -0.45        0.45        -0.99            1


                        Parameter Estimates

            95.0% Wald Confidence Interval
Variable      Estimate        Std. Err.     Lower Conf. Limit    Upper Conf. Limit
    v         0.709341        0.0972023            0.518828             0.899854
    g         0.116633        0.0558081           0.00725113            0.226015
intercept     -10.1333         4.57076             -19.0918             -1.1748
  slope        2.60307         1.20367             0.243915             4.96223


                    Analysis of Deviance Table

     Model      Log(likelihood)  # Param's  Deviance  Test d.f.   P-value
    Full model       -69.6709         5
   Fitted model      -69.8116         4      0.281529      1        0.5957
  Reduced model      -93.2613         1      47.1808       4       <.0001


        AIC:          147.623
```

```
                       Goodness   of   Fit

                                                      Scaled
     Dose      Est._Prob.     Expected    Observed    Size      Residual

   -------------------------------------------------------------------

     0.0000      0.0827         2.482       3.000     30.000      0.343
     7.1000      0.0868         2.604       2.000     30.000     -0.392
    23.4000      0.1624         4.872       5.000     30.000      0.063
    70.9000      0.5357        16.072      16.000     30.000     -0.026
   236.0000      0.6990        20.971      21.000     30.000      0.012


  Chi^2 = 0.28      d.f. = 1        P-value = 0.5993


    Benchmark Dose Computation

 Specified effect =           0.1

 Risk Type        =       Extra risk

 Confidence level =           0.95

          BMD =            24.915

         BMDL =          11.4888
```

Appendix G. References

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