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IRIS Toxicological Review of Perfluorodecanoic Acid [PFDA, CASRN 335-76-2] and Related Salts

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Integrated Risk Information System
Center for Public Health and Environmental Assessment
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ABBREVIATIONS AND ACRONYMS

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

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EXECUTIVE SUMMARY

Summary of Occurrence and Health Effects

Perfluorodecanoic acid (PFDA, CASRN 335-76-2)[1], and its related salts are members of the group per- and polyfluoroalkyl substances (PFAS). This assessment applies to PFDA as well as salts (including non-metal or alkali metal salts) of PFDA that would be expected to fully dissociate in aqueous solutions of pH ranging from 4–9 (e.g., in the human body). Thus, while this assessment would not necessarily apply to non-alkali metal salts of PFDA due to the possibility of PFDA-independent contributions of toxicity, it does apply to PFDA salts including ammonium perfluorodecanoate (PFDA NH4, CASRN 3108-42-7) and sodium perfluorodecanoate (PFDA-Na, CASRN 3830-45-3), and other non-metal or alkali metal salts of PFDA. The synthesis of evidence and toxicity value derivation presented in this assessment focuses on the free acid of PFDA, given the currently available toxicity data[2].

Concerns about PFDA and other PFAS stem from the resistance of these compounds to hydrolysis, photolysis, and biodegradation, which leads to their persistence in the environment. PFAS are not naturally occurring in the environment; they are synthetic compounds that have been used widely over the past several decades in industrial applications and consumer products because of their resistance to heat, oil, stains, grease, and water. PFAS in the environment are linked to industrial sites, military fire training areas, wastewater treatment plants, and commercial products (see Section 1.1.3. Environmental Fate and Transport for information specific to PFDA).

The Integrated Risk Information System (IRIS) Program is developing a series of five PFAS assessments (i.e., perfluorobutanoic acid [PFBA], perfluorohexanoic acid [PFHxA], perfluorohexanesulfonic acid [PFHxS], perfluorononanoic acid [PFNA], perfluorodecanoic acid [PFDA], and their associated salts) (see December 2018 IRIS Program Outlook) at the request of EPA National Programs. Specifically, the development of human health toxicity assessments for exposure to these individual PFAS represents only one component of the broader PFAS strategic roadmap at the EPA (https://www.epa.gov/pfas/pfas-strategic-roadmap-epas-commitments-

[1] The CASRN given here is for linear PFDA; the source PFDA used in the animal toxicity study NTP (2018) was reported to be >97% pure, giving this CASRN. For the human studies [e.g., Valvi et al. (2017)] the purity of the PFDA source was not provided by the study authors. None of the available studies explicitly state that only the linear form was used. Therefore, there is the possibility that some proportion of the PFDA used in the studies were branched isomers and thus observed health effects may apply to the total linear and branched isomers in a given exposure source.

[2] Candidate values for different salts of PFDA were also calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

action-2021-2024). The systematic review protocol (Appendix A) for these five PFAS assessments outlines the related scoping and problem formulation efforts, including a summary of other federal and state assessments of PFDA. The protocol also lays out the systematic review and dose-response methods used to conduct this review (see also Section 1.2). The systematic review protocol was released for public comment in November 2019 and was updated based on those public comments. Appendix A links to the updated version of the protocol which summarizes the history of the revisions.

Human epidemiological studies have examined possible associations between PFDA exposure and health outcomes, in particular liver serum biomarkers, antibody responses, sensitization and allergic responses, fetal growth restrictions, semen parameters, reproductive hormones, pubertal development, neurodevelopment, thyroid hormones, urinary effects, serum lipids, adiposity, cardiovascular disease, atherosclerosis, and cancer. With the exception of immune [i.e., decreased antibody responses] and developmental [i.e., decreased birth weight], the ability to draw judgments regarding these associations based on the available human evidence is limited by the overall quality of the epidemiological studies (studies were generally low confidence), the few studies per health outcome, and, in some studies, the lack of a quantifiable measure of exposure.

Animal studies of PFDA exposure exclusively examined the oral exposure route, and therefore no inhalation assessment was conducted nor was an RfC derived (Section 5.2.3). The available animal studies of oral PFDA exposure examined a variety of noncancer endpoints, including those relevant to liver, immune, developmental, male, and female reproductive, endocrine, urinary, cardiometabolic and other health effects. Limited evidence was identified evaluating PFDA-induced carcinogenicity in animals.

Overall, the available ***evidence indicates*** that PFDA exposure is likely to cause liver, immune, developmental, and male and female reproductive effects in humans, given sufficient exposure conditions[3]. Specifically, for liver effects, the primary support for this hazard conclusion included evidence of increased relative liver weights, altered serum biomarkers of liver injury (e.g., serum enzymes) and histopathology (including necrosis) in rats. For immune effects, the primary supporting evidence included decreased antibody responses in children. Developmental effects were identified as a hazard based primarily on consistent findings of dose-dependent decreases in fetal weight in mice supported by evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy. The primary basis for the hazard judgment on male reproductive effects involved coherent responses across sperm counts, testosterone levels, and male reproductive histopathology and organ weights in adult male rats. For female reproductive effects, the primary hazard judgement was based on decreased uterus weight and estrous cycle effects in adult female rats. Selected quantitative data from these identified

[3] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

hazards were used to derive lifetime and subchronic organ-specific reference doses (osRfDs) (see Table ES-1) and the overall lifetime and subchronic RfDs (see Table ES-2).

The available ***evidence suggests*** that PFDA exposure might have the potential to cause cardiometabolic and neurodevelopmental effects in humans under sufficient exposure conditions[4] based on findings from human studies; however, due to issues regarding inconsistency, imprecision and/or sensitivity, these health hazards were not used in the derivation of toxicity values. Likewise, some human and animal evidence was also identified for endocrine, urinary, and other health effects (e.g., hematological), but the ***evidence is inadequate*** to assess whether PFDA may cause these health effects in humans and was not advanced for the derivation of toxicity values.

Table ES-1. Organ-specific RfDs for health effects with evidence available to synthesize and draw summary judgments for the derivation of toxicity values

Organ/ System	Integration judgment	Toxicity value	Value (mg/kg-day)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Immune	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	4×10^{-10}	*Medium*	1	10	1	1	3	30	Decreased serum antibody concentrations for both tetanus and diphtheria in children at age 7 years and PFDA measured at age 5 years Grandjean et al. (2012); (Budtz-Jørgensen and Grandjean, 2018a)
Developmental	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	3×10^{-10}	*Medium-low*	1	10	1	1	3	30	Decreased birth weight in male and female children (Wikström et al., 2020)
Liver	Evidence indicates (likely)	Lifetime osRfD	ND[a]								
		Subchronic osRfD	7×10^{-7}	*Medium*	3	10	10	1	3	1,000	Increased relative liver weight in SD female rats (NTP, 2018)
Male Reproductive	Evidence indicates (likely)	Lifetime osRfD	ND[a]								
		Subchronic osRfD	5×10^{-6}	*Medium-Low*	3	10	10	1	3	1,000	Decreased absolute whole epididymis weight in SD rats (NTP, 2018)
Female Reproductive	Evidence indicates (likely)	Lifetime osRfD	ND[a]								

[4] Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advanced for dose-response analysis in Section 5).

Organ/ System	Integration judgment	Toxicity value	Value (mg/kg-day)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
		Subchronic osRfD	3×10^{-6}	*Medium-Low*	3	10	10	1	3	1,000	Increased number of days spent in diestrus in SD rats (NTP, 2018)

[a]For hepatic, male reproductive, and female reproductive effects, derivation of candidate lifetime values was not attempted given the high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting.

ND = not determined; RfD = reference dose (in mg/kg-d) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposure; osRfD = organ- or system-specific reference dose (in mg/kg-d); UFA = animal to human uncertainty factor; UFC = composite uncertainty factor; UFD = evidence base deficiencies uncertainty factor; UFH = human variation uncertainty factor; UFL = LOAEL to NOAEL uncertainty factor; UFS = subchronic to chronic uncertainty factor.

Table ES-2. Overall Lifetime and subchronic RfDs

Organ/ System	Integration judgment	Toxicity value	Value (mg/kg-day)	Confidence	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Basis
Immune/developmental	Evidence indicates (likely)	Lifetime osRfD and subchronic osRfD	4×10^{-10}	*Medium*	1	10	1	1	3	30	Decreased serum antibody concentrations for tetanus and diphtheria in children at age 7 years and PFDA measured at age 5 years Grandjean et al. (2012); (Budtz-Jørgensen and Grandjean, 2018a) Decreased birth weight in male and female children (Wikström et al., 2020)

ND = not determined; RfD = reference dose (in mg/kg-d) for lifetime exposure; subchronic RfD = reference dose (in mg/kg-d) for less-than-lifetime exposure; osRfD = organ- or system-specific reference dose (in mg/kg-d); UFA = animal to human uncertainty factor; UFC = composite uncertainty factor; UFD = evidence base deficiencies uncertainty factor; UFH = human variation uncertainty factor; UFL = LOAEL to NOAEL uncertainty factor; UFS = subchronic to chronic uncertainty factor.

1

Lifetime and Subchronic Oral Reference Dose (RfD) for Noncancer Effects

Both of the identified hazards with quantitative information to support the derivation of candidate lifetime values (i.e., immune, and developmental), specifically decreased serum antibody concentrations in children (male and female) (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and decreased birth weight (male and female) (Wikström et al., 2020) were selected as the basis for the RfD of 4×10^{-10} mg/kg-day.[5,6] The PODs for these two osRfDs were nearly identical (i.e., 1.07×10^{-8} and 9.6×10^{-9}, respectively), amounting to a rounding difference once the identical UFs were applied. The marginally higher osRfD for immune effects was the value used for the RfD as confidence in that values were higher than confidence in the value for decreased birth weight. $BMDL_{1/2SD(HED)}$ values for decreased antibody concentrations for both tetanus and diphtheria at age 7 years and PFDA measured at age 5 years were nearly identical (1.07×10^{-8} and 1.06×10^{-8} mg/kg-day, respectively) and were used as the point of departure (POD) for this endpoint. For decreased birth weight in males and females (Wikström et al., 2020), a $BMDL_{5RD(HED)}$ of 9.6×10^{-9} mg/kg-day was identified for this endpoint and was used as the POD. The osRfDs for both outcomes were calculated by dividing the POD_{HED} by an identical composite uncertainty factor of 30 to account for interindividual differences in human susceptibility ($UF_H = 10$), and deficiencies in the toxicity evidence base ($UF_D = 3$). It is important to emphasize that both critical effects supporting this RfD are observed during the developmental period.

The same approach was selected as the basis for the subchronic RfD of 4×10^{-10} mg/kg-day. The subchronic and lifetime RfDs are identical given that the duration extrapolation uncertainty factor (UF_S) is 1 for both values. A UF_S of 1 was selected since the immune and developmental osRfDs are based on effects observed during the developmental period after exposure during gestation, which is recognized as a susceptible lifestage; therefore, exposure during this time window can be considered more relevant to the induction of sensitive effects on these outcomes than chronic and subchronic exposures (see section 5.2.1 and 5.2.2 for more details).

Confidence in the Oral Reference Dose (RfD) and subchronic RfD

The overall confidence in the RfD and subchronic RfD is **medium** and is driven by *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental (Wikström et al.,

[5] The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

[6] Note that the RfD for the free acid presented in this document and an RfD for the anion of PFDA (perfluorodecanoate, $C_{10}F_{19}O_2^-$, CASRN 73829-36-4) would be practically identical given the molecular weights between the two compounds differ by less than 0.5% (i.e., by the weight of a single hydrogen atom).

2020) endpoints using BMD modeling. (Budtz-Jørgensen and Grandjean, 2018a);(Grandjean et al., 2012).

Noncancer Effects Following Inhalation Exposure

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

Evidence for Carcinogenicity

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

1. OVERVIEW OF BACKGROUND INFORMATION AND ASSESSEMENT METHODS

1.1. BACKGROUND INFORMATION ON PERFLUORODECANOIC ACID (PFDA)

Section 1.1 provides a brief overview of aspects of the physicochemical properties, human exposure, and environmental fate characteristics of perfluorodecanoic acid (PFDA; CASRN 335-76-2), and its related salts that might provide useful context for this assessment. This overview is not intended to provide a comprehensive description of the available information on these topics. The reader is encouraged to refer to source materials cited below, more recent publications on these topics, and the assessment systematic review protocol (see Appendix A).

1.1.1. Physical and Chemical Properties

PFDA and its related salts are members of the group per- and polyfluoroalkyl substances (PFAS). Buck et al. (2011) define PFAS as fluorinated substances that "contain 1 or more C atoms on which all the H substituents (present in the nonfluorinated analogues from which they are notionally derived) have been replaced by F atoms, in such a manner that they contain the perfluoroalkyl moiety C_nF_{2n+1}–)." More specifically, PFDA is classified as a perfluoroalkyl carboxylic acid (PFCA) (OECD, 2018). PFCAs containing seven or more perfluorinated carbon groups are considered long-chain PFAS (ATSDR, 2018b). Thus, PFDA is a long-chain PFAS. The chemical structures of PFDA and some of its related salts are presented in Figure 1-1[7]. The physical-chemical properties of PFDA and these related salts are provided in Table 1-1.

[7] While this figure shows the linear structures, the assessment may also apply to other non-linear isomers of PFDA and related salts as described in the Executive Summary.

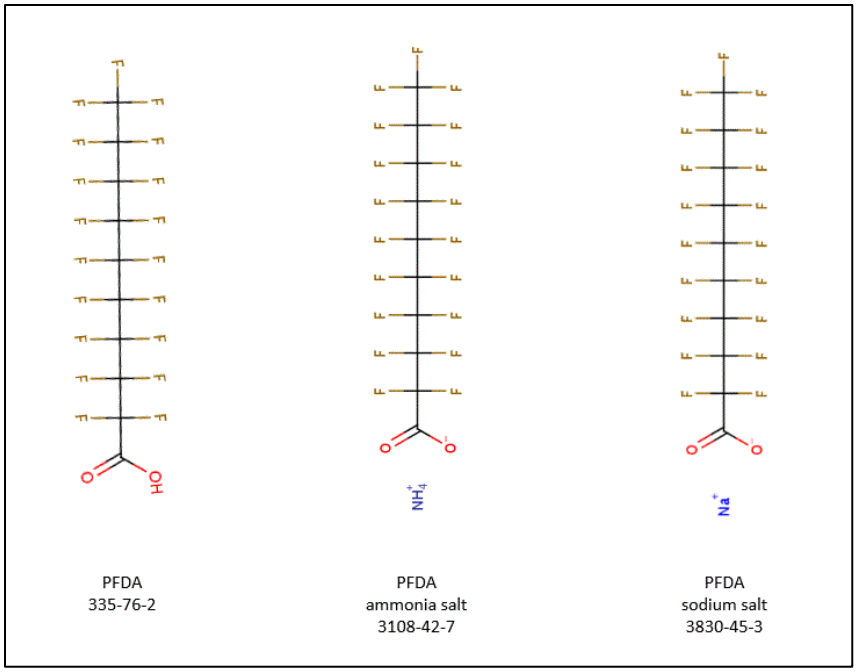


Figure 1-1. Chemical structure of PFDA and related salts.

Table 1-1. Physical-chemical properties of PFDA and related salts

Property (unit)	Value		
	PFDA 335-76-2	**PFDA NH_4^+ salt 3108-42-7**	**PFDA Na salt 3830-45-3**
Molecular weight (g/mol)	514[a]	531[c]	536[d]
Melting point (°C)	79.5[a]	82.6[a*]	84.4[a*]
Boiling point (°C)	218[a]	212[a*]	212[a*]
Density (g/cm^3)	1.79[a*]	1.76[a*]	1.76[a*]
Vapor pressure (mm Hg)	1.73–3[a]	2.39e–02[a*]	2.39e–02[a*]
Henry's law constant (atm-m^3/mole)	1.5e–10[a*]	1.5e–10[a*]	1.5e–10[a*]
Water solubility (mol/L)	5.25e–3[a]	1.86[a*]	1.86[a*]
PKa	–0.17[b*]	ND	ND
LogP	4.15[a]	7.39[a*]	7.39[a*]
Soil adsorption coefficient (L/kg)	397[a*]	397[a*]	397[a*]
Bioconcentration factor (BCF)	39.3[a]	29.8[a*]	29.8[a*]

[a] U.S. EPA (2019b) U.S. EPA CompTox Chemicals Dashboard: https://comptox.epa.gov/dashboard/dsstoxdb/results?search=PFDA. Median experimental values used where available; otherwise, median, or average predicted values used. All values from the U.S. EPA CompTox Chemicals Dashboard were accessed on May 24, 2022.

[b] ATSDR (2018a)

ND = no data.

*Predicted value.

1.1.2. Sources, Production, and Use

PFAS are not naturally occurring in the environment (ATSDR, 2018a). They are synthetic compounds that have been used widely over the past several decades in consumer products and industrial applications because of their resistance to heat, oil, stains, grease, and water. This class of chemicals has been used in consumer products including stain-resistant fabrics for clothing, carpets, and furniture; nonstick cookware; and personal care products (e.g., dental floss, cosmetics, and sunscreen) (ATSDR, 2018a, b).

PFDA has been used in stain and grease-proof coatings on food packaging, furniture, upholstery, and carpet (Harbison et al., 2015). Kotthoff et al. (2015) analyzed a variety of consumer products for PFAS. PFDA was detected in nano- and impregnation-sprays, outdoor textiles, carpet, gloves, paper-based food contact materials, ski wax, and leather.

EPA has been working with companies in the fluorochemical industry since the early 2000s to phase out the production and use of long-chain PFAS such as PFDA (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS). However, the production and use of PFAS has resulted in their release to the environment through various waste streams. Also, because products containing PFAS are still in use, they may continue to be a source of environmental contamination due to disposal or breakdown in the environment (Kim and Kannan, 2007).

No Chemical Reporting Data (CDR) on production volume are available in EPA's ChemView (U.S. EPA, 2019a) for PFDA or its salts. As part of the National Defense Authorization Act for Fiscal Year 2020 (Section 7321), 172 per- and polyfluoroalkyl substances including PFDA were added to the EPA's Toxic Release Inventory (TRI) list (https://www.epa.gov/toxics-release-inventory-tri-program/tri-listed-chemicals). The reporting requirements apply to a de minimus limit of 1% and a manufacture, process, or otherwise use threshold of 100 lbs. Currently, there is no quantitative information on PFDA releases to the environment from facilities manufacturing, processing, or otherwise available in EPA's Toxic Release Inventory.

Wang et al. (2014b) estimated global emissions of PFDA from direct and indirect (i.e., formation degradation of precursors) sources between 1951 and 2030 to be 8 metric tons based on a lower estimate and 222 metric tons based on a higher estimate. The lower estimate assumes that producers cease production and use of long-chain PFCAs and their precursors in line with global transition trends. The higher estimate assumes that the emission scenario in 2015 remains constant until 2030.

1.1.3. Environmental Fate and Transport

Long-chain PFAS, including PFDA, are considered very stable and persistent in the environment (ATSDR, 2018a; Harbison et al., 2015), and can be found world-wide in the environment, wildlife, and humans (https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/risk-management-and-polyfluoroalkyl-substances-PFAS). Long-chain PFAS, including

PFDA, have been found at private and federal facilities, and have been associated with various sources, including aqueous film forming foam (AFFF) for fire suppression, and PFAS manufacturers and industries that use PFAS (e.g., textiles) (ATSDR, 2018a).

Although specific data on PFDA are lacking, PFAS that are released to air have been found exist in the vapor phase in the atmosphere and resist photolysis, but particle-bound concentrations have also been measured (Kim and Kannan, 2007). Wet and dry deposition are potential removal processes for particle-bound PFAS in air (ATSDR, 2018a).

In soil, the mobility of PFAS will vary depending on their soil adsorption coefficients (see Table 1-1), with PFDA being moderately mobile. Uptake of soil PFAS to plants has been shown to occur for similar, long-chain PFAS such as PFOA (ATSDR, 2018a). Yoo et al. (2011) estimated a grass-soil accumulation factor (grass concentration divided by soil concentration) of 0.10 for PFDA, based on samples collected from a site with bio-solids-amended soil.

The potential for PFAS to bioaccumulate in aquatic organisms is dependent on their bioconcentration factors (see Table 1-1), with the potential for PFDA to bioaccumulate being high compared to most of the other PFAS for which these data are available.

1.1.4. Potential for Human Exposure, including Populations and Lifestages with Potentially Greater Exposure

The general population may be exposed to PFAS via inhalation of indoor or outdoor air, ingestion of drinking water and food, and dermal contact with PFAS-containing products (ATSDR, 2018a; NLM, 2017, 2013). Exposure may also occur via hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018a). However, the oral route of exposure has been considered the most important route of exposure among the general population. This conclusion is based on several studies that have investigated the various routes of PFAS exposure (Klaunig et al., 2015). Other authoritative sources on exposure assessment (e.g., ATSDR) continue to do human biomonitoring studies on PFAS, including PFDA, and those sources should be consulted for the most up-to-date information on PFDA exposure in humans.

Gebbink et al. (2015) modelled exposure to PFDA among the adult general population. 'Intermediate' exposure (i.e., based on median inputs for all exposure parameters) from direct and indirect (i.e., precursor) sources was estimated to be 67 pg/kg-day. Of the pathways evaluated (i.e., ingestion of dust, food, water; inhalation of air), direct intake of PFDA in the diet accounted for the largest portion of exposure for the intermediate scenario.

The presence of PFAS in human blood provides evidence of exposure among the general population. PFAS have been monitored in the human population as part of the National Health and Nutrition Examination Survey (NHANES). PFDA was measured in serum samples collected in 2013–2014 from more than 2,000 survey participants (CDC, 2022). The results of these analyses are presented in Table 1-2. Olsen et al. (2017) analyzed human plasma samples from 616 American Red Cross (AMC) donors for PFAS in 2015. The results were compared to results of similar analyses conducted in 2002–2001, 2006, and 2010. Geometric mean concentrations of PFDA

declined 50% from 2000–2001 to 2015. PFDA has also been detected in cord blood and human milk (ATSDR, 2018a). For example, Lankova et al. (2013) detected PFDA in 10% of human milk samples collected from 50 Czech women at concentrations ranging from <6 to 12 pg/mL indicating that breastmilk is a potential route of exposure for infants. Exposure can also occur through hand-to-mouth transfer of materials containing these compounds (ATSDR, 2018b) or in infants through ingestion of formula reconstituted with contaminated drinking water.

Populations that may experience exposures greater than those of the general population may include individuals in occupations that require frequent contact with PFAS-containing products, such as firefighters or individuals who install and treat carpets (ATSDR, 2018a). Also, because PFDA can be found in ski wax, individuals who engage in professional ski waxing may be more highly exposed because PFAS in dust may become airborne and inhaled during this process (Harbison et al., 2015). Nilsson et al. (2010) observed a significant correlation between the number of years individuals had worked as ski wax technicians and their blood levels of PFDA.

Populations living near fluorochemical facilities where environmental contamination has occurred may also be more highly exposed (ATSDR, 2018a). Yamada et al. (2014) estimated exposure to PFDA and other PFAS among high seafood consumers and high freshwater fish consumers in France. Depending on how non-detects were handled (set to zero or the limit of detection), mean estimates for PFDA were 0.16 to 0.73 ng/kg-day for high seafood consumers, and 0.42 to 0.96 ng/kg-day for high freshwater fish consumers, as compared to the adult general population (0.00 to 0.34 ng/kg-day). Thus, populations with a large portion of their diet from fish, including some tribal groups, may experience disproportionally greater PFDA exposure.

Table 1-2. Serum PFDA concentrations based on NHANES 2013–2014 data (µg/L)

Population group[a]	Value
Total population (N = 2,168)	
Geometric mean	0.185
50th percentile	0.200
95th percentile	0.700
3 to 5 years (N = 181)	
Geometric mean	–[b]
50th percentile	0.100
95th percentile	0.370
6 to 11 years (N = 458)	
Geometric mean	–[a]
50th percentile	<LOD[c]
95th percentile	0.350
12 to 19 years (N = 402)	
Geometric mean	0.136
50th percentile	0.100
95th percentile	0.400

Population group[a]	Value
20 years and older (N = 1,766)	
Geometric mean	0.193
50th percentile	0.200
95th percentile	0.800

[a]This table provides only general context on serum PFDA levels from a single study and within a narrow time-period (environmental PFDA levels are changing over time). Note that PFDA is expected to bioaccumulate over a lifetime (see Sections 1.1.3 and 3.1). Up-to-date information from authoritative bodies should be used in any decisional context.
[b]Not calculated because the proportion of results below the limit of detection was too high to provide a valid result.
[c]Limit of detection was 0.1.
LOD = limit of detection.
Source: CDC (2022). Fourth National Report on Human Exposure to Environmental Chemicals.

Air and Dust

PFDA is not currently listed as a hazardous air pollutant under the Clean Air Act and has not been evaluated under the National Air Toxics Assessment (https://www.epa.gov/national-air-toxics-assessment) nor the Air Toxics Screening Assessment (https://www.epa.gov/AirToxScreen). However, PFDA was measured at concentrations ranging from 0.13 to 1.56 pg/m^3 in the vapor phase and 0.13 to 0.49 pg/m^3 in the particle phase of air samples collected from an urban area of Albany, New York in 2006 (Kim and Kannan, 2007).

PFAS, including PFDA, have also been measured in indoor air and dust and may be associated with the indoor use of consumer products such as PFAS-treated carpets or other textiles (ATSDR, 2018a). For example, Strynar and Lindstrom (2008) analyzed dust samples from 110 homes and 10 daycare centers in North Carolina and Ohio in 2000-2001 and detected PFDA in 30.4% of the samples. Similar analyses were conducted by Karásková et al. (2016) who collected 56 dust samples from 41 homes in the Czech Republic, Canada, and the U.S in 2013. PFDA was detected in more than 80% of the samples with mean concentrations of 5.2, 8.5, and 6.9 ng/g for the Czech Republic, Canada, and the U.S., respectively. Knobeloch et al. (2012) collected vacuum cleaner dust from 39 homes in Wisconsin in 2008 and detected PFDA in 72% of the samples at a median concentration of 5.7 ng/g. Fraser et al. (2013) analyzed dust samples collected from offices (n = 31), homes (n = 30), and vehicles (n = 13) in Boston, MA in 2009. PFDA was detected in 97% of the office samples at concentrations ranging from 5.3 to 492 ng/g, 43% of the home samples at concentrations ranging from 7.0 to 26.8 ng/g, and 69% of the vehicle samples at concentrations ranging from 5.4 to 70.1 ng/g. Indoor air samples (n = 4) from a town in Norway collected between 2005-2006 had a mean concentration of 3.4 pg/m^3 for PFDA (Barber et al., 2007).

Water

U.S. EPA conducted monitoring for several PFAS in drinking water as part of the third Unregulated Contaminant Monitoring Rule (UCMR) (U.S. EPA, 2016c). However, PFDA was not among the 30 contaminants monitored. Kim and Kannan (2007) analyzed lake water, rainwater,

snow, and surface water from Albany, New York, and reported concentrations of PFDA ranging from non-detect to 8.39 ng/L. Konwick et al. (2008) observed elevated PFDA concentrations (30–113 ng/L) in a river in Georgia near the site of a wastewater land application system associated with carpet manufacturing. Washington et al. (2010) analyzed soil samples from agricultural fields in Decatur, AL where wastewater treatment sludges had been applied. PFDA was the PFAS with the highest concentration with a maximum of 990 ng/g.

AFFF Training Sites

PFDA was detected at an Australian training ground where AFFFs had been used (Baduel et al., 2015), and Bräunig et al. (2017) suggested that PFAS were distributed via groundwater to biotic and abiotic matrices in an Australian town impacted by PFAS from a nearby fire-fighting training site. Mean concentrations of PFDA were 0.12 µg/L in water, 0.4 µg/kg dry weight in soil, <0.2 µg/kg wet weight in grass, 0.24 ng/g in egg yolk, 0.21–9.7 µg/L in cow, sheep, and horse serum, and 0.4 µg/L in human serum.

Military Sites

PFDA was detected at 10 U.S. military sites in 67.0% of the surface soil samples, and 48.5% of the sediment samples (ATSDR, 2018a; Anderson et al., 2016). Table 1-3 provides the concentrations of PFDA in soil, sediment, surface water, and groundwater at these military sites.

Table 1-3. PFDA levels at 10 military installations

Media	Value
Surface soil	
Frequency of detection (%)	67.03
Median (µg/kg)	0.980
Maximum (µg/kg)	15.0
Subsurface soil	
Frequency of detection (%)	12.50
Median (µg/kg)	1.40
Maximum (µg/kg)	9.40
Sediment	
Frequency of detection (%)	48.48
Median (µg/kg)	1.90
Maximum (µg/kg)	59.0
Surface water	
Frequency of detection (%)	52.00
Median (µg/kg)	0.067
Maximum (µg/kg)	3.20
Groundwater	
Frequency of detection (%)	34.78
Median (µg/kg)	0.023
Maximum (µg/kg)	1.80

Source: Anderson et al. (2016); ATSDR (2018a).

Other Exposures

Schecter et al. (2012) collected 31 food samples from 5 grocery stores in Texas in 2009 and analyzed them for persistent organic pollutants, including PFDA, which was not detected (LOD = 0.2 ng/mL) in any of the foods. Chen et al. (2018b) analyzed PFAS, including PFDA, in foods in Taiwan. PFDA was detected in a wide range of foods at geometric mean concentrations ranging from 0.94 ng/mL in milk to 22.2 ng/g in eggs. Heo et al. (2014) analyzed a variety of foods and beverages in Korea for PFAS. PFDA was detected in 1.3% of the fruit and vegetable samples at a mean concentration of 0.0002 ng/g; 12.8% of the meat samples at a mean concentration of 0.132 ng/g; 13.5% of the dairy samples at a concentration of 0.041 ng/g; 19.0% of the beverage samples at a mean concentration of 0.019 ng/L; and 45.5% of the fish and shellfish samples at a mean concentration of 0.056 ng/g. Heo et al. (2014) also detected PFDA in tap water and bottled water in Korea at mean concentrations of 1.19 and 0.014 ng/L, respectively. Pérez et al. (2014) analyzed PFAS in 283 food items (38 from Brazil, 35 from Saudi Arabia, 36 from Serbia, and 174 from Spain). PFDA was detected in 4.5, 3.4, and 2.1% of the samples from Brazil, Serbia, and Spain, respectively. The mean concentrations of PFDA in foods from these countries were 170, 267, and 772 pg/g, respectively. Stahl et al. (2014) characterized PFAS in freshwater fish from 164 U.S. urban river sites and 157 near-shore Great Lakes sites. PFDA was detected in fish from 20% of the urban river samples (median = <method detection limit; maximum = 28.5 ng/g) and 92% of the Great Lakes samples (median = 0.68 ng/g; maximum = 13.0 ng/g).

1.2. SUMMARY OF ASSESSMENT METHODS

Section 1.2 summarizes the methods used for developing this assessment. A more detailed description of the methods for each step of the assessment development process is provided in the systematic review protocol (see Appendix A). The protocol includes additional problem formulation details, including the specific aims and key science issues identified for this assessment.

1.2.1. Literature Search and Screening

The detailed search approach, including the query strings and populations, exposures, comparators, and outcomes (PECO) criteria (Table 1-4), are provided in Appendix A. The results of the current literature search and screening efforts are documented in Section 2.1. Briefly, a literature search was first conducted in 2017 and regular yearly updates are performed. The most recent literate search update that was fully incorporated into the assessment is from April 2022. The literature from the past year (through March 2023) is in the process of being screened while the document is undergoing public comment. The results of this literature update and any additional unscreened studies identified during public comment will be screened against the PECO criteria and presented in a table that will be included as an Appendix to the assessment. The table will provide the identified studies that met PECO criteria or certain supplemental evidence categories (i.e., in vivo mechanistic or MOA studies, including non-PECO routes of exposure and

populations; in vitro and in silico models; and ADME and pharmacokinetic studies) and EPA's judgment on whether the studies would have a material impact on the assessment conclusions (i.e., identified hazards or toxicity values) presented in the public comment draft. The external peer reviewers are asked to consider EPA's disposition of these newly identified studies and make recommendations, as appropriate (see Charge Question 1).

The literature search queries the following databases (no date or language restrictions were applied):

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

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

- Review of studies cited in any PECO-relevant studies and published journal reviews; finalized or draft U.S. state, U.S. federal, and international assessments (e.g., the draft Agency for Toxic Substances and Disease Registry [ATSDR] assessment released publicly in 2018). In addition, studies included in ongoing IRIS PFAS assessments (PFHxA, PFHxS, PFNA, PFDA) were also scanned for any studies that met PFBA PECO criteria.
- Searches of published PFAS SEMs (Carlson et al., 2022; Pelch et al., 2022) starting in 2021.
- Review of studies submitted to federal regulatory agencies and brought to the attention of EPA. For example, studies submitted to EPA by the manufacturers in support of requirements under the Toxic Substances Control Act (TSCA).
- Identification of studies during screening for other PFAS. For example, epidemiology studies relevant to PFDA were sometimes identified by searches focused on one of the other four PFAS currently being assessed by the Integrated Risk Information System (IRIS) Program.
- Other gray literature (e.g., primary studies not indexed in typical databases, such as technical reports from government agencies or scientific research groups; unpublished laboratory studies conducted by industry; or working reports/white papers from research groups or committees) brought to the attention of EPA.

All literature is tracked in the U.S. EPA Health and Environmental Research Online (HERO) database (https://heronet.epa.gov/heronet/index.cfm/project/page/project_id/2614). The PECO criteria (see Table 1-4) identify the evidence that addresses the specific aims of the assessment and to guide the literature screening process.

Table 1-4. Populations, exposures, comparators, and outcomes (PECO) criteria

PECO element	Evidence
Populations	Human: Any population and lifestage (occupational or general population, including children and other sensitive populations). The following study designs will be included: controlled exposure, cohort, case control, and cross-sectional. (Note: Case reports and case series will be tracked as potential supplemental material.) Animal: Nonhuman mammalian animal species (whole organism) of any lifestage (including preconception, in utero, lactation, peripubertal, and adult stages). Other: In vitro, in silico, or nonmammalian models of genotoxicity. (Note: Other in vitro, in silico, or nonmammalian models will be tracked as potential supplemental material.)
Exposures	Human: Studies providing quantitative estimates of PFDA exposure based on administered dose or concentration, biomonitoring data (e.g., urine, blood, or other specimens), environmental or occupational setting measures (e.g., water levels or air concentrations, residential location and/or duration, job title, or work title). (Note: Studies that provide qualitative, but not quantitative, estimates of exposure will be tracked as supplemental material.) Animal: Oral or inhalation studies including quantified exposure to PFDA based on administered dose, dietary level, or concentration. (Note: Nonoral and noninhalation studies will be tracked as potential supplemental material.) PFDA mixture studies are included if they employ an experimental arm that involves exposure to a single PFDA. (Note: Other PFDA mixture studies are tracked as potential supplemental material.) Studies must address exposure to the following: PFDA (CASRN 335-76-2), or PFDA ammonium salt (CASRN 3108-42-7) or PFDA sodium salt (CASRN 3830-45-3).
Comparators	Human: A comparison or reference population exposed to lower levels (or no exposure/exposure below detection levels) or for shorter periods of time. Animal: Includes comparisons to historical controls or a concurrent control group that is unexposed, exposed to vehicle only or air only exposures. (Note: Experiments including exposure to PFDA across different durations or exposure levels without including one of these control groups will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol].)
Outcomes	All cancer and noncancer health outcomes. (Note: Other than genotoxicity studies, studies including only molecular endpoints [e.g., gene or protein changes; receptor binding or activation] or other nonphenotypic endpoints addressing the potential biological or chemical progression of events contributing towards toxic effects will be tracked as potential supplemental material [e.g., for evaluating key science issues; Section 2.4 of the protocol]).

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

- In vivo mechanistic or mode of action studies, including nonPECO routes of exposure (e.g., intraperitoneal injection) and populations (e.g., nonmammalian models)
- In vitro and in silico models
- Absorption, distribution, metabolism, and excretion (ADME) and pharmacokinetic studies (excluding models)[8]
- Exposure assessment or characterization (no health outcome) studies
- Human case reports or case series studies

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

1.2.2. Evaluation of Individual Studies

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

The key concerns for the review of epidemiology and animal toxicological studies are potential bias (systematic errors or deviations from the truth related to internal validity that affect the magnitude or direction of an effect in either direction) and insensitivity (factors that limit the ability of a study to detect a true effect and can lead to a false negative). For example, any types of random measurement error that may lead to attenuation of study results (i.e., bias towards the null). In evaluating individual studies, two or more reviewers independently arrived at judgments regarding the reliability of the study results (reflected as study confidence determinations; see below) regarding each outcome or outcome grouping of interest; thus, different judgments were possible for different outcomes within the same study. The results of these reviews were tracked within EPA's version of the Health Assessment Workplace Collaborative (HAWC). To develop these judgments, each reviewer assigned a rating of *good*, *adequate*, *deficient* (or *not reported*, which generally carried the same functional interpretation as *deficient*), or *critically deficient* (listed from

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

best to worst methodological conduct; see Appendix A.6 for definitions) related to each evaluation domain representing the different characteristics of the study methods that were evaluated based on the criteria outlined in HAWC.

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

- *High* confidence: No notable deficiencies or concerns were identified; the potential for bias is unlikely or minimal, and the study used sensitive methodology.
- *Medium* confidence: Possible deficiencies or concerns were noted, but the limitations are unlikely to be of a notable degree or to have a notable impact on the results.
- *Low* confidence: Deficiencies or concerns were noted, and the potential for bias or inadequate sensitivity could have a significant impact on the study results or their interpretation. *Low* confidence results were given less weight than *high* or *medium* confidence results during evidence synthesis and integration (see Sections 1.2.4 and 1.2.5).
- *Uninformative*: Serious flaw(s) were identified that make the study results unusable. *Uninformative* studies were not considered further, except to highlight possible research gaps.

Using the HAWC platform the two reviewers reached a consensus judgment regarding each evaluation domain and overall (confidence) determination with conflict resolution by an additional reviewer, as needed. The specific limitations identified during study evaluation were carried forward to inform the synthesis (see Section 1.2.4) within each body of evidence for a given health effect.

1.2.3. Additional Epidemiology Considerations

While the detailed methods for epidemiology study evaluation are described in the systematic review protocol (see Appendix A.6.2.1), a few considerations have been developed further; these are described here.

As noted above, study sensitivity is an important consideration given that it could lead to false negative (i.e., null) result (Type II error) if a study is underpowered or not designed with adequate sensitivity to detect an association that may exist. A key element for study sensitivity, along with others described in the systematic review protocol, is whether exposure contrasts/gradients are sufficient across populations to detect differences in risk. For example, if measurement error results in inaccurate exposure estimates this can lead to exposure misclassification but also influence the ability to detect an association as well as an exposure-response relationship which may be evidence of a biologic gradient.

Confounding across PFAS is a potential source of uncertainty when interpreting the results of epidemiology studies of individual PFAS (e.g., quantifying the effect of an individual PFAS can

potentially be confounded by other PFAS). In order for confounding to occur, co-pollutants would have to be associated with PFAS of interest, associated with the endpoint, and not act as an intermediate in the causal pathway. One way to begin to assess whether co-exposure is occurring is through examination of correlations. In a preliminary analysis of 22 studies in the inventory reporting correlations, correlations differed across the PFAS (see Appendix A.6, Figure 6-2). While some pairs have correlation coefficients consistently above 0.6 (e.g., PFNA and PFDA), the correlations for most vary from 0.1 to 0.6 depending on the study. For this reason, it was not considered appropriate to assume that co-exposure to other PFAS was necessarily an important confounder in all studies. The potential for confounding across PFAS is incorporated in individual study evaluations and assessed across studies in evidence synthesis. In most studies, it is difficult to determine the likelihood of confounding without considering additional information not typically included in individual study evaluation (e.g., associations of other PFAS with the outcome of interest and correlation profiles of PFAS within and across studies). In addition, even when this information is considered or the study authors perform analyses to adjust for other PFAS, it is often not possible to fully disentangle the associations due to high correlations. This stems from the potential for amplification bias in which bias can occur following adjustment of highly correlated PFAS (Weisskopf et al., 2018). Thus, in most studies, there may be some residual uncertainty about the risk of confounding by other PFAS. A "Good" rating for the confounding domain is reserved for situations where there is minimal concern for substantial confounding across PFAS as well as other sources of confounding. Examples of this include results for a PFAS that predominates in a population (such as a contamination event) or studies that demonstrate robust results following multi-PFAS adjustment which would also indicate minimal concern for amplification bias. Because of the challenge in evaluating individual studies for confounding across PFAS, this issue is also assessed across studies during the evidence synthesis phase, as described in the systematic review protocol (Appendix A, Section 9), primarily when there is support for an association with adverse health effects in the epidemiology evidence (i.e., moderate, or robust evidence in humans, as described below). Analyses used include comparing results across studies in populations with different PFAS exposure mixture profiles, considering results of multi-pollutant models when available, and examining strength of associations for other correlated PFAS. In situations where there is considerable uncertainty regarding the impact of residual confounding across PFAS, this is captured as a factor that decreases the overall strength of evidence (see Appendix A.10).

1.2.4. Data Extraction

The detailed data extraction approach is provided in Appendix A.8. Briefly, data extraction and content management were carried out using HAWC for all health effects for animal studies and some health effects for epidemiological studies. Data extraction elements that were collected from epidemiological, animal toxicological, and in vitro studies is described in HAWC (https://hawcprd.epa.gov/about/). Not all studies that meet the PECO criteria went through data extraction: studies evaluated as being *uninformative* were not used to inform assessment

judgments and therefore did not undergo full data extraction. All findings from informative studies were considered for extraction, regardless of the statistical significance of their findings. The level of extraction for specific outcomes within a study may differ (i.e., ranging from a narrative to full extraction of dose-response effect size information). For quality control, data extraction was performed by one member of the evaluation team and independently verified by at least one other member. Discrepancies in data extraction were resolved by discussion or consultation within the evaluation team.

1.2.5. Evidence Synthesis and Integration

For the purposes of this assessment, evidence synthesis and integration are considered distinct but related processes (see Appendix A, Sections 9 and 10 for full details). For each assessed health effect, the evidence syntheses provide a summary discussion of each body of evidence considered in the review that directly informs the integration across evidence to draw an overall judgment for each health effect. The available human and animal evidence pertaining to the potential health effects are synthesized separately, with each synthesis providing a summary discussion of the available evidence that addresses considerations regarding causation that are adapted from Hill (1965). Mechanistic evidence is also synthesized as necessary to help inform key decisions regarding the human and animal evidence; processes for synthesizing mechanistic information are covered in detail in Appendix A, Section 9.2.

The syntheses of the human and animal health effects evidence focus on describing aspects of the evidence that best inform causal interpretations, including the exposure context examined in the sets of studies. Thus, data permitting, the evidence synthesis emphasizes studies of high and medium confidence. Correspondingly, during data extraction when a relative abundance of medium and high confidence studies was available for a given health outcome the low confidence studies did not generally undergo full data extraction. Documentation of when this approach was taken is noted in the specific health effect sections. When possible, results across studies are compared using graphs and charts or other data visualization strategies. The synthesis of mechanistic information informs the integration of health effects evidence for both hazard identification (e.g., biological plausibility or coherence of the available human or animal evidence; inferences regarding human relevance, or the identification of susceptible populations and lifestages across the human and animal evidence) and dose-response evaluation (e.g., selection of benchmark response levels, selection of uncertainty factors). Evaluations of mechanistic information typically differ from evaluations of phenotypic evidence (e.g., from routine toxicological studies). This is primarily because mechanistic data evaluations consider the support for and involvement of specific events or sets of events within the context of a broader research question (e.g., support for a hypothesized mode of action; consistency with known biological processes), rather than evaluations of individual apical endpoints considered in relative isolation.

Following the synthesis of human and animal health effects data, and mechanistic data, integrated judgments are drawn across all lines of evidence for each assessed health effect. During evidence integration, a structured and documented two-step process is used, as follows:

- Building from the separate syntheses of the human and animal evidence, the strength of the evidence from the available human and animal health effect studies is summarized in parallel, but separately, using a structured evaluation of an adapted set of considerations first introduced by Sir Bradford Hill (Hill, 1965). This process is conceptually similar to that used by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) (Morgan et al., 2016; Guyatt et al., 2011; Schünemann et al., 2011), which arrives at an overall integration conclusion based on consideration of the body of evidence. These summaries incorporate the relevant mechanistic evidence (or mode of action [MOA] understanding) that informs the biological plausibility and coherence within the available human or animal health effect studies. The terms associated with the different strength of evidence judgments within evidence streams are *robust*, *moderate*, *slight*, *indeterminate*, and *compelling evidence of no effect*.

- The animal, human, and mechanistic evidence judgments are then combined to draw an overall judgment that incorporates inferences across evidence streams. Specifically, the inferences considered during this integration include the human relevance of the animal and mechanistic evidence, coherence across the separate bodies of evidence, and other important information (e.g., judgments regarding susceptibility). Note that without evidence to the contrary, the human relevance of animal findings is assumed. The final output is a summary judgment of the evidence base for each potential human health effect across evidence streams. The terms associated with these summary judgments are *evidence demonstrates*, *evidence indicates (likely)*, *evidence suggests*, *evidence inadequate*, and *strong evidence of no effect*. The decision points within the structured evidence integration process are summarized in an evidence profile table for each considered health effect.

As discussed in the protocol (Appendix A), the methods for evaluating the potential carcinogenicity of PFAS follow processes laid out in the EPA cancer guidelines (U.S. EPA, 2005); however, for PFDA, data relevant to cancer were sparse which limited the extent of analysis that was possible (see Section 3.3).

1.2.6. Dose-Response Analysis

The details for the dose-response employed in this assessment can be found in Appendix A.11. Briefly, a dose-response assessment was performed for noncancer health hazards, following exposure to PFDA via the oral route, as supported by existing data. For oral noncancer hazards, oral reference doses (RfDs) are derived when possible. An RfD is an estimate, with uncertainty spanning perhaps an order of magnitude, of an exposure to the human population (including susceptible subgroups) that is likely to be without an appreciable risk of deleterious health effects over a lifetime (U.S. EPA, 2002). The derivation of a reference value like the RfD

depends on the nature of the health hazard conclusions drawn during evidence integration. For noncancer outcomes, a dose-response assessment was conducted for evidence integration conclusions of *evidence demonstrates* or *evidence indicates (likely)*. In general, toxicity values are not developed for noncancer hazards with *evidence suggests* conclusions (see Appendix A, Section 10.2 for exceptions). Consistent with EPA practice, the PFDA assessment applied a two-step approach for dose-response assessment that distinguishes analysis of the dose-response data in the range of observation from any inferences about responses at lower environmentally relevant exposure levels (U.S. EPA, 2012a, 2005):

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

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

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

For dose-response purposes, EPA has developed a standard set of models (http://www.epa.gov/bmds) that can be applied to typical data sets, including those that are nonlinear. In situations where there are alternative models with significant biological support (e.g., toxicodynamic models), those models are included as alternatives in the assessment(s) along with a discussion of the models' strengths and uncertainties. EPA has developed guidelines on modeling dose-response data, assessing model fit, selecting suitable models, and reporting modeling results [see the *EPA Benchmark Dose Technical Guidance* (U.S. EPA, 2012a)]. For each modeled response, a POD from the observed data was estimated to mark the beginning of extrapolation to lower doses. The POD is an estimated dose (expressed in human-equivalent terms) near the lower end of the observed range without significant extrapolation to lower doses.

The POD is used as the starting point for subsequent extrapolations and analyses. For noncancer effects, the POD is used in calculating the RfD.

2. LITERATURE SEARCH RESULTS

2.1. LITERATURE SEARCH AND SCREENING RESULTS

The database searches yielded 1057 unique records, with 536 records identified from additional sources, such as posted National Toxicology Program (NTP) study tables, review of reference lists from other authoritative sources (ATSDR, 2018b) and searches of published PFAS SEMs (Pelch et al., 2022) (see Figure 2-1). No unique studies were identified in submissions to EPA. Of the 1057 studies identified, 595 were excluded during title and abstract screening, and 443 were reviewed at the full-text level. Of the 443 studies screened at the full-text level, 262 were considered to meet the populations, exposures, comparators, and outcomes (PECO) eligibility criteria (see Table 1-4). The PECO criteria identify the evidence that addresses the specific aims of the assessment and focuses the literature screening, including study inclusion/exclusion. In addition to those studies meeting the PECO criteria, studies containing supplemental material potentially relevant to the specific aims of the assessment were tagged during the literature screening process. Although these studies did not meet PECO criteria, they were not excluded. Rather, they were considered for use in addressing the identified key science issues and other major scientific uncertainties identified during assessment development but unanticipated at the time of protocol posting. Studies categorized as "potentially relevant supplemental material" included the following:

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

The studies meeting PECO at the full-text level included 234 epidemiologic studies, 14 animal studies, 1 PBPK model, and 8 in vitro/in vivo genotoxicity studies. Of the 1057 studies screened, 374 were identified as supplemental material during title and abstract or full text screening and tagged by topic area (e.g., mechanistic or MOA, non-PECO route of exposure, etc.). High-throughput screening data on perfluorodecanoic acid (PFDA) are currently available from the EPA's Chemicals Dashboard (U.S. EPA, 2021a), data were retrieved on November 2022) and

relevant information is presented and analyzed in Appendix E. The last literature search update used for the Toxicological Review was June 2022.

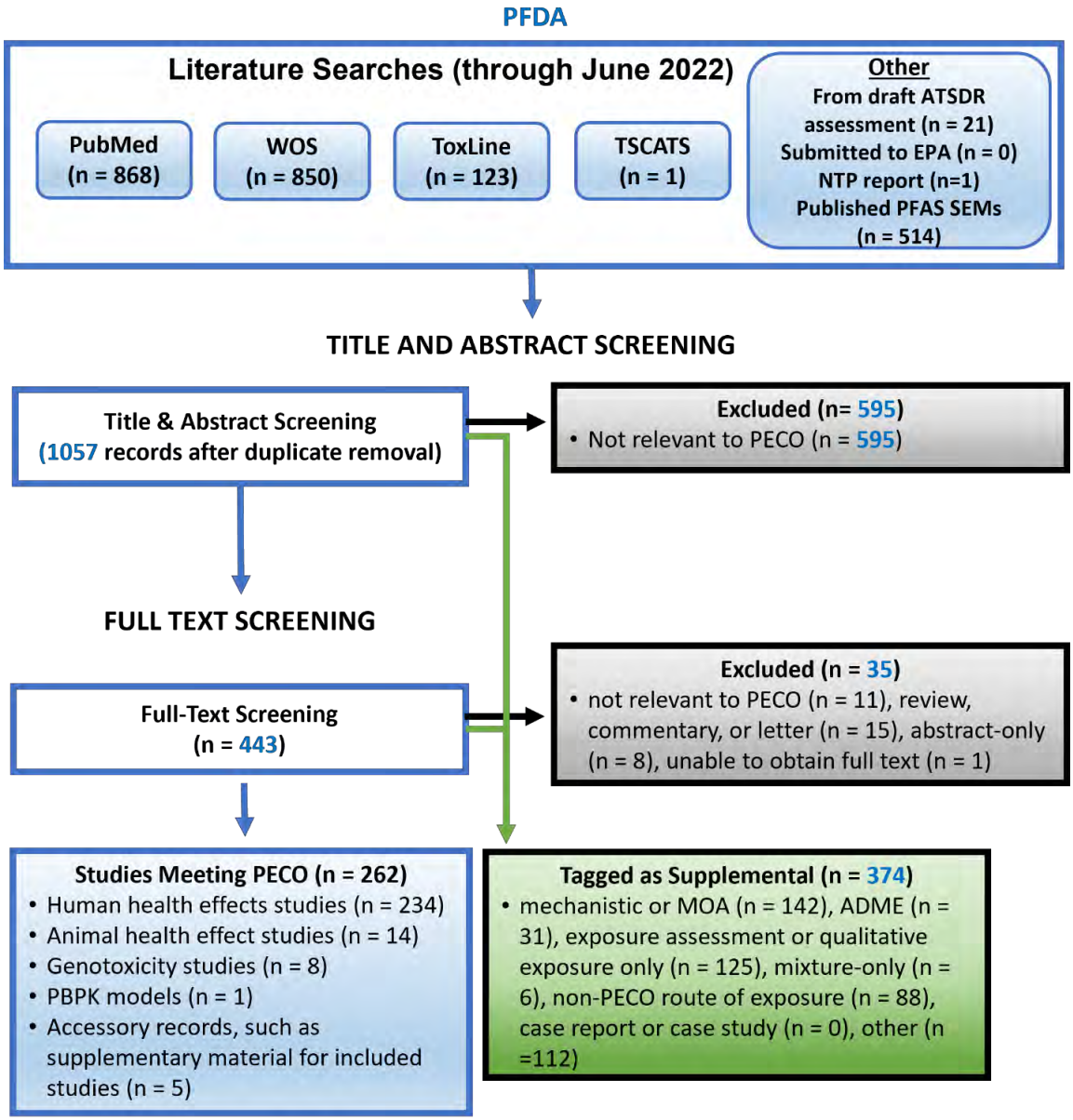


Figure 2-1. Literature search for perfluorodecanoic acid and related salts.

2.2. SUMMARY OF STUDIES MEETING PECO CRITERIA

Human and animal studies have evaluated potential effects to the liver, immune system, developing fetus, male and female reproductive systems, endocrine, cardiometabolic, neurodevelopmental, urinary, general toxicity and other organ systems (e.g., hematology) following exposure to PFDA. The evidence base for these outcomes is synthesized in Sections 3.2.1–3.2.11. A limited number of available studies in humans and animals informing of potential carcinogenic

effects with PFDA exposure are summarized in Section 3.3. The single identified PBPK model is discussed in Section 3.1.

Two hundred and thirty-four epidemiological studies were identified that report on the potential association between PFDA and non-cancer and cancer human health effects (list of studies filterable by health effect available at: https://hawcprd.epa.gov/summary/visual/assessment/100500072/Epi-studies-of-PFDA-health-effects/). The database of animal toxicity studies for PFDA consists of oral exposure studies (see Table 2.1), including five dietary exposure studies in rats exposed for 7–14 days (Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991); two drinking water studies in mice exposed for 12–49 days (Li et al., 2022; Wang et al., 2020), two 28-day gavage studies in rats and/or mice (Frawley et al., 2018; NTP, 2018), one 14-day oral study (presumed to be gavage) in mice (Lee and Kim, 2018) and one gestational exposure study in mice via gavage with two exposure windows (GD 10–13 and 6–15) (Harris and Birnbaum, 1989). In addition, three single exposure studies in animals via the oral route were identified with limited utility for the evaluation of repeat-dose toxicity and the derivation of oral reference dose (RfD) values (Kawabata et al., 2017; Brewster and Birnbaum, 1989; Harris et al., 1989).

Table 2-1. Animal toxicity studies examining health effects after PFDA administration

Author (year) Reference	Species, strain (sex)	Exposure route and duration	Dose range[a]
NTP (2018)	Rat, Harlan Sprague–Dawley (male and female)	Oral gavage; daily over 28 days	0, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/kg-d
Frawley et al. (2018)	Rat, Harlan Sprague-Dawley (female)	Oral gavage; daily over 28 days	0, 0.125, 0.25 and 0.5 mg/kg-d
Frawley et al. (2018)	Mouse, B6C3F1/N (female)	Oral gavage; weekly over 28 days	0.04464, 0.0893, 0.179, 0.36 and 0.71 mg/kg-d (reported as 0, 0.3125, 0.625, 1.25, 2.5 and 5 mg/kg-wk)
Takagi et al. (1991)	Rat, F344 (male)	Diet; daily over 14 days	0, 10 mg/kg-d (reported as 0 and 0.01%)
Lee and Kim (2018)	Mouse, ICR (male)	Uncharacterized (presumed to be oral gavage); days 9, 11, and 13 over 14 days	0 and 21.4 mg/kg-d (reported as 0 and 100 mg/kg)
Li et al. (2022)	Mouse, C57BL/6J (female)	Drinking water; daily for 14 days	0 and 25 mg/kg-d
Wang et al. (2020)	Mice, CD-1 (male)	Drinking water; daily over 12–49 days	0, 13 mg/kg-d (reported as 0 and 0.1 mM)
Permadi et al. (1993)	Mouse, C57BL/6 (male)	Diet; daily over 10 days	0, 37.8 mg/kg-d (reported as 0 and 0.02%)

Author (year) Reference	Species, strain (sex)	Exposure route and duration	Dose range[a]
Kawashima et al. (1995)	Rat, Wistar (male)	Diet; daily over 7 days	0, 1.15, 2.3, 4.6, and 9.22 mg/kg-d (reported as 0, 00125, 0.0025, 0.005, and 0.01%)
Yamamoto and Kawashima (1997)	Rat, Wistar (male)	Diet; daily over 7 days	0 and 4.6 mg/kg-d (reported as 0 and 0.005%)
Takagi et al. (1992)	Rat, Fisher F344 (male)	Diet; daily over 7 days	0 and 10 mg/kg-d (0 and 0.01%)
Harris and Birnbaum (1989)	Mouse, C57BL/6N (female)	Oral gavage; GD 10–13	0, 0.25, 0.5, 1, 2, 4, 8, 16, and 32 mg/kg-d
Harris and Birnbaum (1989)	Mouse, C57BL/6N (female)	Oral gavage; GD 6–15	0, 0.03, 0.1, 0.3, 1, 3, 6.4, and 12.8 mg/kg-d

Doses are presented as adjusted daily doses (ADD). Additional details on the ADD conversions can be found in the HAWC project page for PFDA.
GD = gestational day.

Graphical representations of outcome-specific study evaluations are presented and discussed within the hazard sections outlined above. Detailed rationales for each domain and overall confidence rating are available in Health Assessment Workspace Collaborative (HAWC).

3. PHARMACOKINETICS, EVIDENCE SYNTHESIS, AND INTEGRATION

3.1. PHARMACOKINETICS

Perfluorodecanoic acid (PFDA) and its salts have characteristics of absorption, distribution, metabolism, and excretion (ADME) comparable to other perfluoroalkyl acids (PFAAs) in that they are readily absorbed by gastrointestinal tract following oral exposure irrespective of sexes and species. Both animal and human data suggest that PFDA has a high affinity for protein binding and efficient renal reuptake. Therefore, PFDA tends to accumulate in organs to the extent similar to or greater than that of other PFAAs and has relatively slow clearance (Dzierlenga et al., 2019; Fujii et al., 2015; Zhang et al., 2013b). In general, PFDA accumulates primarily in liver, followed by kidney, blood, and other tissues. PFDA is specifically a perfluorocarboxylic acid (PFCA), which are a subset of PFAAs. Similar to other PFAAs, PFDA is also metabolically inert and therefore most of PFDA is eliminated unchanged in urine and feces.

Of note, growing mechanistic evidence (both animal and human) suggests that renal clearance becomes less efficient as the perfluorocarbon chain length increases (Dzierlenga et al., 2019; Kudo, 2015; Lau, 2015). The findings support previous reports indicating that fecal elimination may play an increasingly important role in elimination of long carbon chain-length of PFAAs like PFDA (C10) as compared to other shorted chain of PFAAs (C £ 8) (Vanden Heuvel et al., 1991). Collectively, these PFDA pharmacokinetic data support the conclusions of Kudo (2015) and Lau (2015) that PFDA has a much longer half-life than other shorter chained PFAAs (e.g., PFHxA). While female rats administered PFDA tended to have a higher dose-normalized area under the plasma concentration time curve (AUC) than males, (Dzierlenga et al., 2019) suggested that there was no sex difference in PFDA half-life. However, calculating the average clearance across studies, doses, and routes, the EPA obtains a value of 6.1 mL/kg-d in male rats and 4.3 mL/kg-day in female rats, i.e., 30% lower in female rats. The elimination half-life of PFDA is generally much longer in humans (4.5–12 years) than in rats (20–59 days) or mice (63–222 days). By comparison, Lau (2015) provides estimated half-lives of 2.3–3.8 years for PFOA in humans (modestly lower than PFDA), 5.4 years for PFOS (comparable to PFDA), but only 32 days for PFHxA. In rats PFOA has a half-life of 2–6 days, PFOS a half-life of 38–71 days and PFHxA 0.4–1.6 hours. So, the qualitative trend with chain-length and structure is similar, but there is an order of magnitude difference in elimination of PFOA vs. PFOS in rats but in humans the difference between PFOA and PFOS is no more than a factor of two.

3.1.1. Absorption

Bioavailability (or fractional absorption) is typically estimated by comparing the AUC of blood concentrations observed after an oral dose as compared to when the same dose is given IV, or with the AUCs normalized to the dose. If kinetics is linear and oral uptake is less than 100% the AUC after oral dosing will be less than that after IV dosing, and the fraction absorbed (Fabs) is estimated as AUC (oral)/AUC (IV).

In the most recent animal study by Dzierlenga et al. (2019), Hsd: Sprague Dawley (SD) rats were given PFDA or one of two other PFAA (perfluorohexanoic acid, PFHxA and perfluorooctanoic acid, PFOA) by intravenous (IV) injection at 2 mg/kg or oral gavage (2, 10, and 20 mg/kg). It was found that the time to peak concentration (Tmax) increases with the chain length of PFAAs and slightly with dose levels of oral administration for both sexes. For PFDA, Tmax (mean ± standard error of mean, hour) increases from 8.27 ± 0.63 to 10.0 ± 0.06 hour, and from 9.01 ± 0.80 to 10.8 ± 1.2 hour, with increased gavage doses (2–20 mg/kg) of PFDA for males and females, respectively. Peak concentration, Cmax (normalized with dose levels, mM/mmol/kg), also appeared to be somewhat higher with increasing oral doses in female rats, but not male rats. Oral bioavailability for PFDA was estimated to be 160–180% for both sexes. The nominal observation of >100% absorption may be the result of enhanced reabsorption by intestinal transporters (Dzierlenga et al., 2019). Other aspects of the results do not indicate nonlinearity; for example, AUC/dose did not change significantly among oral doses of 2, 10, and 20 mg/kg. But the peak concentration after the IV dose (2 mg/kg), measured just 5 minutes after dosing, was lower than the value estimated from the oral dose data, occurring 8–9 hours after the dose. Hence, there is no clear explanation for this observation of AUC/dose being so much higher after oral vs. IV dosing. Given the consistency of AUC/dose for the oral doses, it seems most likely that the error occurred for the IV dose.

Kim et al. (2019) estimated Fabs = 0.87 ± 0.25 and 0.65 ± 0.08 in female and male SD rats, respectively. On the other hand Dzierlenga et al. (2019) reported Fabs of 1.58–1.72 and 1.70–1.79 for male and female rats, respectively for 2–20 mg/kg doses, based on the serum AUC after oral vs. IV dosing. It is not clear how to interpret these data nor resolve the discrepancy between the two papers. In the Bayesian PK analysis of the rat data described in Appendix G 100% bioavailability (Fabs = 1) was assumed and the variation in AUC/dose is then interpreted as variation and effective uncertainty in the volume of distribution and clearance. Hence, the quantitative uncertainty is accounted for.

Fujii et al. (2015) measured PFDA PK in male and female FVB/NJcl mice dosed with 0.313 μmol/kg by IV administration and 3.13 μmol/kg by oral gavage. A shortcoming of the experimental design is that serum concentration data were only collected up to 24 hours, making it harder to estimate the PK parameters. However, based on the reported parameters the Tmax was 12 and 15 hours in male and female rats, respectively. Fujii et al. (2015) reported the ratio of dose-adjusted AUC after oral and IV exposures as 1.1 and 1.2 in male and female mice, respectively,

indicating complete absorption. That these values are slightly greater than 1 may not only be due to experimental variability, but also because clearance might have been slightly slower for the oral dose, which was 10 times higher than the IV dose. Since the AUC/dose of values of Fujii et al. (2015) are not significantly different for oral vs. IV dosing, the difference is presumed due to experimental variability and the results are interpreted as showing 100% bioavailability (Fabs = 1) in mice.

Although there is no direct evidence of oral absorption of PFDA in humans, it can be inferred from observations in epidemiological studies that identified positive associations between PFDA concentrations in human tissues (e.g., blood or placenta) and environmental levels (e.g., drinking water) (Stubleski et al., 2016). Given these results for rats and mice and the lack of controlled PK studies in humans, Fabs = 1 will be used for humans.

No data on absorption of PFDA through the respiratory tract or skin has been found. While oral ingestion is considered the primary route of exposure, the contribution from these other routes would need to be better evaluated in the scientific literature to determine their significance.

3.1.2. Distribution

General considerations

Upon absorption, PFDA moves rapidly through the body via the bloodstream to various organs and tissues, mainly liver, lung, and kidney and, to a lesser extent, brain, and bone (Dzierlenga et al., 2019; Vanden Heuvel et al., 1991). In general, PFDA tends to accumulate in organs to an extent greater than or similar to that of other PFAS. It has been suggested that the extent of the covalent binding of PFDA with biological matrices (e.g., serum proteins) in blood and tissues is critical to its distribution and bioaccumulation (Kudo, 2015; Vanden Heuvel et al., 1992). For instance, Kim et al. (2019) measured binding of PFDA to plasma proteins in vitro to incorporate this factor into a PBPK model and reported that more than 99.7% was bound to protein in rat and human plasma. These measured values were in line with animal experimental data reported by Ylinen and Auriola (1990) that 99% of PFDA was bound with the serum proteins in Wistar rats with a single intraperitoneal (IP) dose of 20 mg/kg PFDA. However, if distribution to tissues is assumed to be limited by the product of free fraction and tissue blood flow, the PK distribution phase is predicted to be much longer than observed. Hence the plasma binding must be labile, not strictly limiting its distribution or clearance.

Of note, the degree of protein binding with PFDA affects not only its distribution but also the elimination. Specifically, the PFAS–serum protein complex mediates glomerular filtration since only the unbound fraction is filtered (Kudo, 2015). PFAS can then be extensively resorbed as fluid carrying them passes down the renal tubules, with this resorption mediated by other PFAS-protein complexes, specifically by organic anion transporters (Oat) proteins (Kudo, 2015). For instance, Weaver et al. (2010) investigated the roles of rat renal Oat proteins in the deposition of perfluorinated carboxylates with different chain lengths of carbons (C2–C18). The transport of PFDA (C10) was measured from 10 to 300 mM with renal Oat proteins (Chinese hamster ovary cell

line and kidney RNA from Sprague-Dawley rats). Of five Oat proteins (Oat1, Oat2, Oat3, Urat1, and Oatp1a1), Oatp1a1 appears to be the major Oat protein responsible for the reabsorption of C8 through C10, with highest affinities for C9 and PFDA (C10). These data collectively suggest that chain length is a factor in the extent to which PFAAs are substrates of various basolateral and apical transporters in renal proximal tubule cells, which in turn impacts the extent of elimination. Moreover, since saturation of these transporters will lead to nonlinearity in elimination, one can expect that PFAAs which are significant substrates will have greater nonlinearity in their elimination (as a function of exposure level) compared to PFAAs which for which the transporters have lesser affinity. While this is a general expectation, the PK data of Dzierlenga et al. (2019) did not exhibit nonlinear elimination with single doses in the range of 2–20 mg/kg, and it is not known if transporter saturation would occur with higher doses or multiple doses (leading to accumulation of PFDA) in this dose range.

Animals (rats and mice)

Distribution in rats and mice was examined in multiple toxicological studies of PFDA. Vanden Heuvel et al. (1991) specifically evaluated [1-14C] PFDA pharmacokinetics in rats and observed distribution into all tissues examined, including liver, kidney, heart, and gonads. Tissue levels outside of the liver were less than 1% of the administered dose in male rats and less than 2% of the dose in female rats. Vanden Heuvel et al. (1992) then examined the covalent binding of PFDA to protein in male rat at 2 hour, 1 and 4 days after intraperitoneal dosing with 4.8 mg/kg [1–14C] PFDA and found that ~0.1% of the administered dose was bound in plasma and liver and ~0.25% was bound in testes (results independent of sample time). So, while a large fraction of PFDA in the body is found in the liver, only a small fraction of that is covalently bound. While only a small fraction of the total dose is covalently bound, the quantity could be enough to interfere with estimation of long-term clearance or half-life.

Other investigators measured distribution into multiple tissues, most commonly kidney, liver, and brain (Dzierlenga et al., 2019; Kim et al., 2019; Fujii et al., 2015). Although PFDA can be found in the brain, the accumulation of PFDA was generally lower in the brain than in other organs or tissues, while the highest levels were found in liver. For instance, Kawabata et al. (2017) observed that the hepatic concentration of PFDA (mg/g tissue) was approximately 60 times higher than that of the brain in Wistar rats given a single-oral dose of 50 mg PFDA/kg. This measurement was made 9 days after the dose was administered, which should be a sufficient time for distribution among the tissues to equilibrate but is short enough compared to overall clearance to represent a significant portion of the administered dose.

Volume of Distribution in Rats and Mice

Ohmori et al. (2003) estimated the volume of distribution (Vd) for PFDA in male and female Wistar rats (three each sex) after IV administration (48.64 mmol/kg BW) as 347.7 ± 15.2 and 441.1 ± 55.1 mL/kg, respectively, for male and female Wistar rats (three each sex). The Vd of PFDA

obtained by Ohmori et al. (2003) only varied slightly by sex though up to 2-fold larger than those of other PFAAs tested in the same experiment (PFHA, PFOA, or PFNA). This sex difference is in contrast with two most recent studies showing that Vd was larger in males than in females (Dzierlenga et al., 2019; Kim et al., 2019). For instance, Dzierlenga et al. (2019) investigated the disposition of PFDA in Hsd: SD rats administered 2 mg/kg PFDA by IV and found Vd for the central compartment (V1) was slightly larger in males (274 ± 28 mL/kg) than females (238 ± 35 L/kg) while the peripheral (V2) distribution was almost twice as large in males (355 ± 69 mL/kg) than in females (186 ± 57 mL/kg). Summing V1 and V2 for these results from Dzierlenga et al. (2019), the total Vd in males is estimated to be 50% higher than females. Dzierlenga et al. (2019) also obtained a larger Vd in males vs. females when PFDA was given by oral gavage, similar to their results from IV dosing.

Kim et al. (2019) reported total volumes of distribution (i.e., not normalized to BW) for their IV exposure: 0.1182 L and 0.0584 L for male and female rats, respectively. However, if one assumes a BW of 0.25 kg then the Vd obtained is consistent with the reported Cmax values, i.e., Vd = dose/Cmax. Given these absolute volumes of distribution and 0.25 kg BW, Vd values were estimated to be 472.7 and 233.77 mL/kg for male and female rats in the Kim et al. (2019), which are quite similar to the values reported by Dzierlenga et al. (2019) (see Table 3-1).

There are limited data on ADME properties of PFDA in mice. Fujii et al. (2015) evaluated the PK of PFDA in FVB/NJc mice aged 8–10 weeks using single IV dose (0.31 μmol/kg) and oral gavage (3.13 μmol/kg). Unlike rats, while the Vd (mean ± standard deviation, mL/kg) was slightly larger in males (250 ± 60) than females (200 ± 50) after IV administration, the difference in PFDA distribution was not significant. Of note, once entering the body via IV administration, most of PFDA were retained in the liver of mice (64–80% for males, 46–55% for females). The overall distribution profiles of gavage route were similar to those of IV route (Fujii et al., 2015).

The Vd values from the mouse and rat studies are summarized in Table 3-1 along with results for rats from a hierarchical Bayesian analysis from partial pooling of the data, described in Appendix G.

Table 3-1. Volume of distribution values reported for animal studies

Study	Strain	Route	Dose (mg/kg)	Volume of distribution (mL/kg)[a]
Male Rats				
Dzierlenga et al. (2019)	Hsd: SD	IV	2	629 ± 97 *770.6 (485.8–1077.3)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	2	586 ± 57 *579.3 (475.4–670.6)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	10	411 ± 46 *417.6 (357.8–475.7)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	20	456 ± 35 *459.3 (396.3–523.8)*
Kim et al. (2019)	SD	IV	1	472.7 ± 37.2[b] *317.9 (259.2–381.7)*
Kim et al. (2019)	SD	oral	1	*448.5 (376.6–524.5)c*
Ohmori et al. (2003)	Wistar	IV	25	*412.7 (331.9–520.0)*
Population mean (90% CI)				***431.1 (108.9–696.4)***
Female Rats				
Dzierlenga et al. (2019)	Hsd: SD	IV	2	424 ± 92 *350.4 (294.7–405.2)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	2	277 ± 35 *241.4 (203.1–278.9)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	10	264 ± 36 *227.6 (190.9–262.9)*
Dzierlenga et al. (2019)	Hsd: SD	Oral	20	270 ± 40 *223.9 (187.6–260.3)*
Kim et al. (2019)	SD	IV	1	233.7 ± 17.8[b] *252.9 (218.8–288.2)*
Kim et al. (2019)	SD	oral	1	450.9 (385.8–509.8)[c]
Ohmori et al. (2003)	Wistar	IV	25	441.1 ± 55.1 *448.9 (413.9–482.7)*
Population mean (90% CI)				***313.4 (193.2–438.1)***
Male Mice				
Fujii et al. (2015)	FVB/NJc1	IV	0.16	250 ± 60
Female Mice				
Fujii et al. (2015)	FVB/NJc1	IV	0.16	200 ± 50

Values in plain text are as reported for each study unless otherwise noted. Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix G.

Kim et al. (2019) reported Vd as 118.18 ± 9.31 and 58.42 ± 4.46 mL for male and female rats, respectively, after IV exposures. These were normalized to an assumed 0.25 kg BW, which is consistent with Vd calculated as dose/Cmax, given that Cmax is the initial concentration for IV dosing.

Kim et al. (2019) did not report Vd for oral doses.

Distribution of PFDA in mice and rats during pregnancy/gestation has not been evaluated.

Distribution in Humans

While PFDA is distributed throughout the body, tissue concentrations are expected to be roughly 50% of the concentration in blood plasma based on the animal data presented above. Based on this concentration ratio, most of the PFDA mass may be in various tissues since they constitute over 90% of the body. Despite the expected mass apportionment, it is appropriate that measurement of blood PFDA has been extensively applied to assess PFDA exposure for humans and will be used in this review to estimate the risk from that exposure.

A recent study evaluated levels of several PFAS, including PFDA, in human serum as a function of various measures of body composition as well as localized measurements of adipose content throughout the body generated by dual-energy X-ray absorptiometry (DXA) and whole-body magnetic resonance imaging (WB-MRI) (Lind et al., 2022). In women the study showed a negative correlation between serum PFDA concentration and many measures of body fat, as well as with the volume of areas of the body with high fat fractions, although much less so with the volume of these regions. For example, there is a negative correlation between serum PFDA and the volume of hips and inner thighs in women, but no correlation with the fat content of these regions (Lind et al., 2022). In men the study showed no association between serum PFDA concentration and measures of body composition. Given the minimal distribution of PFDA to adipose tissues seen in rats (Kim et al., 2019) one might expect essentially no effect of the volume of these tissues on serum levels, as was seen in men. However, one would predict a negative correlation between Vd and body fat, and in fact the results in women appear to be consistent with that prediction if glomerular filtration increases with body mass or surface area, as will be discussed in the excretion section. It is also possible that the correlation is due to variation in exposure related to body fat, where in the male population exposure (per kg BW) was constant with body fat but for some reason exposure decreased with body fat among the women. Matched estimates of exposure from dietary surveys or samples, or matched measures of urinary clearance (PFAS concentrations in urine) are ultimately needed to determine whether or not the correlations actually reflect PK variation.

Human Distribution During Pregnancy and Lactation PFDA can also be found in human breast milk, placenta, embryo/fetal tissues, and cord blood (Mamsen et al., 2019; Mamsen et al., 2017; Zhang et al., 2013a; Liu et al., 2011; Kärrman et al., 2009; Monroy et al., 2008). Mamsen et al. (2019) and Mamsen et al. (2017), examined fetal tissues after voluntary abortions (first trimester) or intrauterine fetal death (second and third trimester; (Mamsen et al., 2019)). More specifically, Mamsen et al. (2017) reported time-matched maternal serum and fetal tissue levels from fetuses between 36 and 65 days of age (i.e., between 5 and 10 weeks); the data appeared to show an increasing trend in tissue concentration with fetal age, but the trend was not statistically significant. When tissues were analyzed separately, PFDA concentration in placenta, liver and lung were likewise found to increase with trimester, but were not detected in heart, CNS, or adipose tissue (Mamsen et al., 2019). Also, the first trimester data were from women with a mean age of 26.5

years, while the second and third trimester were from women with a mean age of 32.5 years. Interestingly, first trimester maternal serum concentrations (mean 0.34 ng/mL) were somewhat higher than the second and third trimester concentrations (mean 0.26 and 0.27 ng/mL, respectively), though the difference was not statistically significant. The ratio of placenta concentration to first-trimester maternal serum indicates a strong time-trend in distribution to the placenta, but this trend was also not statistically significant and the ratio of fetal liver and lung to placenta did not show a consistent pattern with trimester (Mamsen et al., 2019). In summary, while some of the data are indicative of a time-dependence in the ratio of placental and fetal tissue to maternal serum levels, none of those results are statistically significant and other aspects of the data indicate that the ratio is constant. Therefore, it will be assumed that the relative volume of distribution (L/kg BW) is constant during pregnancy in the PK modeling, as this also simplifies that analysis.

To compare the distribution between tissues and maternal blood matrices among different studies, adjustment should be made to correct for the distribution among blood components. Specifically, Poothong et al. (2017) measured a mean ratio of 1.7 for serum: whole blood and 1.3 for plasma: whole blood concentrations of PFDA. These factors will be used to adjust the subsequent tissue: blood matrix ratios to tissue: plasma, when reported for whole blood or serum. If the ratio of serum: whole blood concentration is 1.7 and hematocrit (hct) is 45%, then the mass fraction of PFDA in plasma, given this ratio, would be Fp = 1.7 × (1-hct) = 93.5%. Using the reported plasma: whole blood ratio and the same calculation, one obtains Fp = 1.3 × (1-hct) = 71.5%. Partitioning of PFDA and other PFAAs between human plasma and blood cells were also investigated by Jin et al. (2016). The estimated mass fraction in plasma (human samples) increased among perfluoroalkyl carboxylates as the carbon chain length increased from C6 (mean 0.24) to C11 (0.87) with the mean of 0.82 for PFDA (C10), which corresponds to a plasma: whole blood concentration ratio of 0.82/(1-hct) = 1.5. Since this value is intermediate between the serum: whole blood and plasma: whole blood values reported by Poothong et al. (2017), it will be used to convert tissue partitioning data relative to whole-blood concentrations to serum-based concentrations below.

While the placenta shares circulation from the mother and fetus, it is the only tissue for which PFDA concentrations in adult humans can be compared to plasma to evaluate overall distribution. Mamsen et al. (2017) reported time-matched maternal serum and placenta tissue levels from fetuses between 37 and 68 days of age (i.e., between 5 and 10 weeks), and obtained mean placenta: maternal plasma ratio of 43%. The results for this ratio from Mamsen et al. (2019), shown graphically, indicate mean values of about 40 and 55% for the second and third trimester, respectively. The ratio of placenta to maternal serum (estimated from blood) at birth measured by Zhang et al. (2013a) was 34% (both mean and median ratio; n = 32). These results for the placenta are generally consistent with the volume of distribution (L/kg) measured in female rats, described above. Given that the average Vd in female rats obtained above (0.38 L/kg) is based on a larger set of studies, which show a fair amount of variability between them (indicating that results from a

single study may not be reliable), the Vd in humans will be assumed to be the same as in rats; with the results for male rats being used for men and results for female rats for women.

The observed ratio ranges from 25–55%, with most data between 30 and 40%. Mean concentrations in lung and intestines were slightly greater than placenta (shown graphically in Figure 2 of Mamsen et al. (2017)), while other tissues were below, and the reported mean ratio of fetal tissue to maternal plasma was 27%. This indicates that distribution into the fetus as a whole is 50–80% of the range in placenta (34–55%).

Studies of the volume of distribution in newborns are not available, but one can reasonably assume that it is similar to fetal tissues. Mamsen et al. (2017) specifically reported PFDA concentrations in first trimester fetal liver, heart, intestine, lung, connective tissue, spinal cord, ribs, and extremities. Results for individual tissues were only shown graphically, but most fetal tissues had a mean concentration lower than the placenta, with mean maternal plasma concentration of 0.28 ng/g, placenta of 0.09 mg/g (43% of plasma), and fetal tissue of 0.05 ng/g (27% of plasma). While Mamsen et al. (2019) had fewer data for PFDA in older fetuses, from their supplemental data mean levels in first trimester fetal livers were less than those in the placenta (Mamsen et al., 2017). As will be discussed in the section on PK modeling below, the impact on distribution of the pregnant mother with her fetus will not be large, but these results are informative of distribution within the fetus, which will be imputed to newborns.

Several studies evaluated the cord serum: maternal serum ratio in humans at childbirth, with the following median (mean) values reported or calculated from the reported median (mean) concentrations in each matrix:

- Liu et al. (2011): 0.42 (0.39);
- Needham et al. (2011): 0.29 (mean not reported);
- Zhang et al. (2013a): 0.28 (0.25);
- Han et al. (2018): 0.38 (0.38 GM ratio);
- Yang et al. (2016a): 0.25 (0.35);
- Yang et al. (2016b): 0.39 (0.43);
- Li et al. (2020a) (preterm): 0.23;
- Li et al. (2020a) (full-term): 0.35.

The average of the median values from these studies is 0.324, indicating that the placenta creates a significant barrier for PFDA between maternal and fetal blood. But beyond this overall average, (Li et al., 2020a) observed a significant increase in the cord/maternal serum ratio between preterm and full-term pregnancies, from a median ratio of 0.23 to 0.35. The authors evaluated the correlation of the cord/maternal serum ratio with multiple placental transporters and identified a significant, positive correlation with p-glycoprotein (MDR1) and multidrug resistance-associated protein 2 (MRP2). These positive correlations, significant for full-term but not preterm pregnancies, indicate that the placenta acts as a passive barrier to PFDA in early pregnancy and this function is partly defeated by the expression of MDR1 and MRP2 transporters late in pregnancy.

However, if the ratio of fetal serum to maternal serum is 0.324 (32.4%) and the ratio of fetal tissue to maternal serum is 27%, then the ratio of fetal tissue to fetal serum would be 27%/32.4% = 83%, a much higher level of distribution than observed in adult rats and estimated for the adult woman (38%) based on placental data described above.

Since the total body burden of PFDA in the human PK studies is unknown, it is not possible to directly estimate Vd in humans. For male and female rats, the estimated (geometric mean) Vd values are 448 and 287 mL/kg, respectively (see Table 3-1). As described above, the fetal tissue: maternal plasma ratio varied between 0.25 and 0.55, with Mamsen et al. (2017) reporting a mean fetal tissue: maternal plasma ratio of 0.27, which is 80% of the average Vd in female rats (assuming 1 L/kg body density). These data indicate that fetal tissue levels are close to maternal levels: if the maternal Vd was that of female rats (0.287 L/kg) and fetal: maternal serum was 0.27, that implies similar average concentration in the fetus as the mother, which is not indicated by the comparison of fetal tissue and placenta concentration. Therefore, it will be assumed that Vd for women (both prior to and during pregnancy) is equal to the geometric mean for female rats, 287 mL/kg. For consistency, the Vd for men will be assumed equal to the average for male rats (448 mL/kg).

One can then ask if the somewhat different distribution into the fetus would impact the overall distribution in the mother and fetus together (e.g., for PK modeling during pregnancy). If one presumes that distribution into the fetus is fast compared to the rate of fetal development, such that the concentration in maternal and fetal tissues remains at equilibrium and recognizes that the fetus is less than 5% of the combined maternal and fetal mass, then the impact of slightly lower distribution into the fetus on distribution in the mother and fetus will be minimal. Hence, human maternal Vd is likely to be unchanged during pregnancy. The available data do not indicate a difference greater than 10% or 20%.

Since PFDA binds strongly to serum proteins, one possible explanation for the apparently higher distribution between fetal serum and tissues is that the fetus has a much lower level of these proteins than an adult, allowing for a greater proportion of PFDA in fetal tissue vs. fetal serum. However, data to support this hypothesis, i.e., measurements of PFDA binding in cord blood, are not available. Pharmacokinetic modeling of PFOA dosimetry in humans by Goeden et al. (2019) suggests another hypothesis: that the greater amount of extracellular water in the tissues of fetuses and children (Friis-Hansen, 1961) leads to a greater distribution of PFAS into these tissues. The amount of extracellular water in newborns was estimated to be 2.4 times higher than adults (Friis-Hansen, 1961). Multiplying the volume of distribution from female rats (38%) by 2.4, one obtains 91%, which is much closer to the estimate of 81% obtained here. Hence, while the mechanism by which distribution in a fetus, which we assume also applies to newborns, might not be the difference in extracellular tissue water, the available quantitative data for extracellular water can provide a reasonable prediction for the difference between newborns and adults, as well as the transition between them (see Figure 3-1).

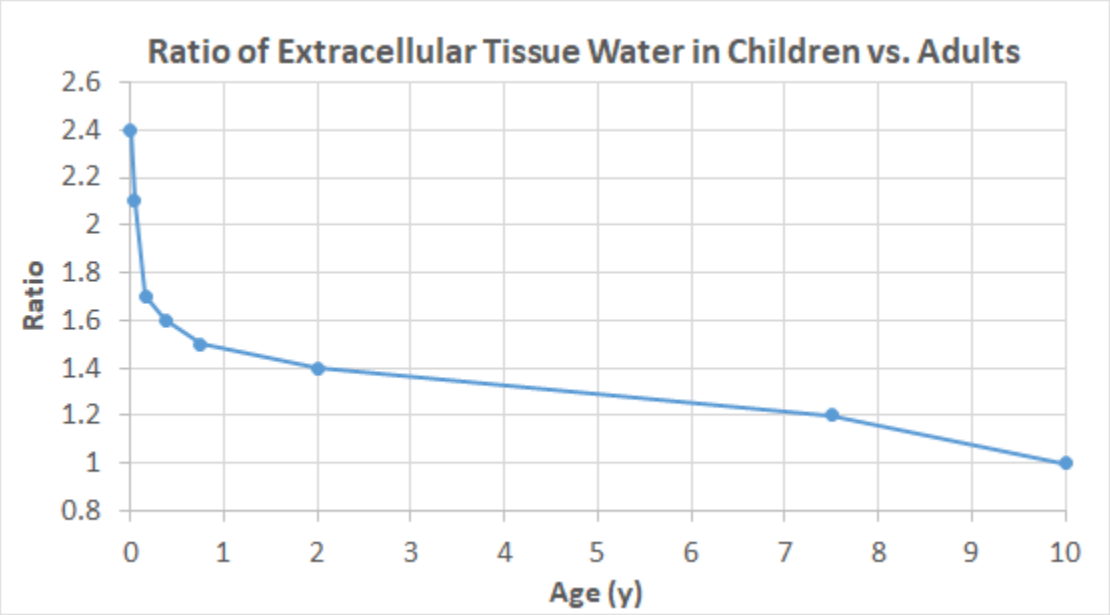


Figure 3-1. Ratio of extracellular water (% of body weight) in children vs. adults. Values (points) are calculated from results in **Friis-Hansen (1961)** and plotted at the mid-point for the corresponding age ranges evaluated.

The interpolation function shown in Figure 3-1 can be multiplied by the adult Vd (L/kg) to obtain the corresponding value for children under 10 years of age, as was done by Goeden et al. (2019). However, an opposing factor is the approximately 20% larger blood volume as a fraction of BW in young children compared to older children and adults (Darrow et al., 1928), given that a high fraction of PFHxS is bound to blood proteins. Hence, the extent and even the direction of any change in Vd with age are uncertain and will require further PK studies to address.

Liu et al. (2011) also investigated correlations between PFDA concentrations in matched maternal serum and breast milk samples collected from their subjects. The median value for the concentration ratio between milk and maternal serum was 0.03:1, hence indicating a rather limited level of lactational transfer to infants.

Effect of Liver Disease on Human Distribution

In a cross-sectional study by Yeung et al. (2013), the authors investigated the role of liver disease in the deposition of PFDA by analyzing the distribution of PFDA in serum and liver using samples from patients with hepatocellular carcinoma (HCC) and cirrhosis due to chronic hepatitis C viral infection, (HCV); while the mean and median liver: serum ratios were higher in HCC (0.65 and 0.66) patients than HCV (0.41 and 0.33, respectively), the difference was not significantly different. While the ratio of liver-to-serum PFDA concentration were not evaluated in control subjects of this study, the comparison of absolute liver concentrations of other PFAS in healthy vs. diseased samples indicated that pathological changes in diseased livers can alter the liver: serum PFAS distribution.

3.1.3. Metabolism

Vanden Heuvel et al. (1991) examined the metabolism of PFDA in male and female Wistar rats administrated with a single intraperitoneal (IP) dose of [1-14C] PFDA (9.4 µmol/kg, 5 mg/kg). The results showed that only parent compound of PFDA was found in urine or feces, suggesting that there was no appreciable metabolism of PFDA. The findings are expected since PFDA is a long-chain (C10) PFAAs with chemical stability similar to that of other shorter length PFAA chemicals (e.g., perfluorohexane sulfonic acid, PFHxS, C6). Although there have been no studies of PFDA biotransformation following inhalation or dermal exposure, metabolism by these administration routes is similarly not expected.

3.1.4. Excretion

In general excretion is one component of overall elimination of substances in the body, the other being metabolism. Total elimination is often evaluated by observing the decline in concentration of a compound in the blood or other tissues. Since PFDA does not undergo appreciable metabolism, as discussed just above, the elimination data discussed below are interpreted as measures of total excretion.

Excretion in Animals (rats and mice)

As observed for other PFAS, sex-specific elimination of PFDA was observed in rats. For example, after IV administration (2 mg/kg PFDA), the dose-normalized serum area-under-the-concentration curve (AUC/dose) was significantly higher in female rats (3,065 mM h/mmol/kg) than male rats (1,875 mM h/mmol/kg) (Dzierlenga et al., 2019). Similar results were obtained for oral exposures of 2–20 mg/kg, with AUC/dose in female rats being 5,200–5,500 mM h/mmol/kg vs. 2,960–3,320 mM h/mmol/kg in male rats (Dzierlenga et al., 2019). These observations collectively suggest that elimination is slower in female rats than males, perhaps because renal reuptake of PFDA is more efficient in female than male rats.

As noted earlier, the fecal excretion becomes increasingly important in elimination of long carbon chain-length of PFAAs like PFDA (C10) as compared to shorter chain PFAS. For instance, Kudo et al. (2001) attempted to evaluate the elimination of PFDA in Wistar rats (both sexes) with intraperitoneally administration of PFDA using a single dose of 20 mg/kg. It was found that PFDA was slowly excreted in urine, with only 0.2% of the dose being eliminated within 120 h. More of the administered PFSA (~ 4%) was found in feces, indicating fecal excretion was a major route of the elimination of PFDA for both sexes. Fecal excretion remained as the major route when rats were intravenously injected with a dose of 25 mg/kg. Similarly, Vanden Heuvel et al. (1991) evaluated the elimination of PFDA after 5 mg/kg intraperitoneal doses to male and female rats. Fecal elimination accounted for 51% and 24% of the administered dose to the males and females, respectively, over 28 days, while urinary excretion was less than 5% of the dose. These results are partly contradicted by the data of Kim et al. (2019), who observed slightly over 3% total excretion

in urine and feces after 120 hours, but that urine accounted for 25% of this excretion in male rats (and 38% at 150 days) while urine was over or close to 50% of excretion in females.

Dzierlenga et al. (2019) also found that the total clearance (CL_{tot}) of PFDA was extremely low compared to other short-chain of PFAA compounds (e.g., PFHxA and PFOA) in both male and female Hsd:Sprague-Dawley rats. These results were in line with previous findings of Vanden Heuvel et al. (1991) and Ohmori et al. (2003), and those of Kim et al. (2019), that PFAAs with shorter carbon chain length tended to show higher CL_{tot}.

Reported values of CL_{tot} for rats are listed in Table 3-2. While the respective ranges of study specific reported CL_{tot} values for male and female rats indicate a degree of inter-laboratory variability in the method of determination, the studies are all considered to be of adequate quality and therefore there is no reason to preclude any one of them from an overall analysis. Therefore, a hierarchical Bayesian analysis from partial pooling of all these data, described in Appendix G, was performed in order to obtain overall population mean values and credible intervals for male and female rats, listed in Table 3-2. These values (intervals) for CL_{tot} are considered to be robust estimates of average clearance in rats (and uncertainty therein).

Table 3-2. PFDA total clearance in rats and mice

Citation	Dose (mg/kg)	Route	CL_{tot} * (mL/d/kg)	n
Male Rats				
Ohmori et al. (2003)	25	IV	5.2 ± 1.3 *5.32 (3.66 – 6.77)*	3
Kim et al. (2019)	1	IV	3.04 ± 0.40[a] *1.61 (1.08 – 2.1)*	5
Kim et al. (2019)	1	Oral	*2.94 (2.34 – 3.52)*	5
Dzierlenga et al. (2019)	2	IV	12.82 ± 0.74 *7.45 (5.91 – 8.89)*	3[b]
	2	Oral	7.44 ± 0.31 *5.04 (4.36 – 5.75)*	3[b]
	10	Oral	7.94 ± 0.31 *5.83 (5.32 – 6.33)*	3[b]
	20	Oral	8.11 ± 0.22 *5.71 (5.19 – 6.26)*	3[b]
Population mean (90% credible interval)	--		***4.14 (0.68 – 7.02)***	
Female Rats				
Ohmori et al. (2003)	25	IV	5.3 ± 0.2 *5.31 (4.44 – 6.29)*	3
Kim et al. (2019)	1	IV	3.24 ± 0.24[a] *2.07 (1.84 – 2.31)*	5

Kim et al. (2019)	1	Oral	*2.61 (2.13 – 3.09)*	5
Dzierlenga et al. (2019)	2	IV	7.85 ± 0.58 *7.4 (6.67 – 8.19)*	3[b]
	2	Oral	4.61 ± 0.22 *3.78 (3.41 – 4.16)*	3[b]
	10	Oral	4.37 ± 0.24 *3.57 (3.22 – 3.93)*	3[b]
	20	Oral	4.61 ± 0.24 *3.77 (3.39 – 4.14)*	3[b]
Population mean (90% credible interval)	--		***4.06 (2.05 – 6.05)***	
Male Mice				
Fujii et al. (2015)	0.16	IV	3.9[c]	9
Female Mice				
Fujii et al. (2015)	0.16	IV	2.2[c]	9

* Values in plain text are as reported for each study unless otherwise noted. Values in italics are the mean (90% credible interval) from the Bayesian analysis described in Appendix G

[a]Reported absolute CL (mL/d) was divided by 0.25 kg; value is consistent with dose/AUCinf¬ reported.

[b]Dzierlenga et al. (2019) indicates 3 rats/time-point used

[c]Total of urinary and fecal clearance; see text below for details.

While Vanden Heuvel et al. (1991) also evaluated the elimination of PFDA in rats, they did not report clearance values nor AUC values that could be used to calculate clearance. The half-lives estimated from the decline in total body burden (based on 14C activity) were 23 and 43 days in males and females, respectively, while the half-lives based on blood concentrations were 22 and 29 days, respectively (Vanden Heuvel et al., 1991). These female half-lives are comparable to the beta-phase half-lives reported for female rats by Dzierlenga et al. (2019) (18–44 days), though somewhat lower than reported for female rats by Kim et al. (2019) (50–75 days). The half-life estimates of Vanden Heuvel et al. (1991) for male rats are between the alpha-phase (1.7–2.1 days) and beta-phase values (80–110 days) reported by Kim et al. (2019), but much less than those reported by Dzierlenga et al. (2019) (215–300 days beta- or single-phase half-life). This range of half-life values reflects the fact that half-life estimates are sensitive to noise in the experimental data and study design, with Vanden Heuvel et al. (1991) having only measured elimination for 28 days, while Dzierlenga et al. (2019) measured plasma concentrations to 105 days and Kim et al. (2019) to 150 days. Hence the results of Vanden Heuvel et al. (1991) appear to be generally consistent with the other studies described here but will not be used in quantitative evaluation of clearance.

Only Fujii et al. (2015) evaluated the urinary and fecal clearance of PFDA in FVB/NJcl mice using single IV dose (0.16 mg/kg) and oral gavage (1.6 μmol/kg). PFDA appeared to have the smallest total (feces and urine) clearance as compared to short chained PFAAs (C ≤ 8) (Fujii et al.,

2015). Mouse urinary and fecal clearance were determined by dividing the total amounts excreted in the urine and feces during a 24 hour period by the area under the curve (AUC) of the serum concentration of each PFCA between 0 to 24 hours. Fecal elimination appeared to the primary elimination route regardless of exposure routes (IV and oral gavage). For IV administration, there were no marked differences in total clearance between sexes: 2.2 (1.4 and 0.8 mL/day/kg fecal and urinary clearance, respectively) and 2.8 mL/day/kg (1.8 and 1.0 mL/day/kg fecal and urinary clearance, respectively) for male and female mice, respectively. In comparison, the total clearances for gavage-administered of PFDA were 3.9 mL/day/kg for male (3.6 and 0.3 mL/day/kg fecal and urinary clearance, respectively) and 2.2 mL/day/kg for females (1.9 and 0.3 mL/day/kg fecal and urinary clearance, respectively) (Fujii et al., 2015). Since the toxicological studies being evaluated used oral exposure, the oral PK results are considered most relevant and sex-specific PK parameters are therefore suggested for calculating HEDs from corresponding points of departure in male and female mice. The beta-phase half-lives obtained for male and female mice after oral gavage are 1.4 day and 4.1 day, respectively (calculates as $\ln(2)/\lambda 2$ from Table 1 of Fujii et al. (2015)). However, since clearance was only observed for 24 h in the Fujii study, these half-life estimates of half-life are considered uncertain and are not used for HED calculation. Instead, the CLtot values in Table 3-2, which are determined from the amount of PFDA excreted in urine and feces, will be used. While it would be preferable to have PK data from at least one other study in mice, the results of Fujii et al. (2015) are considered adequate for evaluating the relative clearance in mice vs. humans.

Excretion in Humans

Fujii et al. (2015) also estimated the elimination of PFDA in humans using 24-hour urine samples collected from healthy volunteers, bile from patients who underwent biliary drainage, and matched blood from both the healthy volunteers and patients. The clearance rate to urine and bile from these data involves a straightforward calculation of the ratio of the daily amount excreted by the route to the matched blood sample in a subject. However, the fecal clearance rate is based on an estimate of 98% resorption from the intestine (i.e., enterohepatic recirculation), that they obtained by comparing their results for PFOA to direct observation of PFOA half-lives in humans by Olsen et al. (2007): 98% intestinal resorption is required to match the total (urinary and biliary) excretion otherwise estimated for PFOA with the previously measured PFOA half-life. It may be reasonable to assume that these two compounds are resorbed in the intestines to a similar extent, but this assumption is made in combination with use of biliary excretion data from five patients (three female and two males), three suffering from choledocholithiasis, one from cholecystolithiasis, and one from carcinoma of the head and pancreas. It is possible that anchoring the estimated fecal clearance to the data from Olsen et al. (2007) in healthy subjects corrects for possible effects of biliary disease, but these results should be considered with some caution.

The PFFDA urinary, biliary, fecal, and total clearances (sum of urinary and fecal clearance) estimated by Fujii et al. (2015) for humans were: 0.015 ± 0.01, 2.51 ± 2.1, 0.050 ± 0.04, and 0.066 ±

0.05 (mean ± standard deviation, mL/kg/day). The difference between biliary clearance (not included in the total) and fecal clearance is presumed to be the result of resorption after biliary elimination. There may also be uncertainty due to the fact that Fujii et al. (2015) only measured PFDA levels for 24 hours and there was considerable variability in the results, as reflected by large standard deviation (e.g., 2.51 ± 2.1 mL/day/kg for biliary clearance). On the other hand, collection of 24-hour urine data provides a much better estimate of clearance by that route than extrapolating from a single spot-sample.

An alternate approach to estimating human fecal clearance would be to assume that the ratio of fecal/urinary clearance is similar in humans as in rats. Kim et al. (2019) observed a mean fecal excretion 1.63 times higher than urinary excretion in male rats, but only 0.742 times urinary excretion in female rats. Both of these ratios are considerably lower than the ratio of 3.3 estimated by Fujii et al. (2015). Given the uncertainty described above for the estimated fecal clearance of Fujii et al. (2015), these sex-specific ratios will be applied to the estimated human urinary clearance from Fujii et al. (2015) (0.015 mL/kg/day) to obtain total estimated urinary plus fecal clearance rates of 0.039 mL/kg/day in men and 0.026 mL/kg/day in women.

In general, the total clearance profiles (urinary and fecal) of Fujii et al. (2015) were comparable between humans and mice: total clearance in humans decreased as a function of chain length for C7–C9, then increased only slightly as the length increased further to C13, while mice showed a clear decrease from C7–C10 followed by a clear increase with chain length from C10–C3. In humans, the pattern in total clearance for C7 and higher was due to a shifting balance as fecal clearance increased with chain length, but urinary clearance decreased.

A recent evaluation of women with children 2–5 years of age by Kim et al. (2020b) found that PFDA is decreased in women who have breast-fed by a factor of 1.3% (95% CI: 0.5, 2.1%) per month of breast-feeding, indicating that this is a significant route of elimination for such mothers, and correspondingly a source of exposure for their children. This rate of elimination is comparable to that estimated for younger women, below, indicating that elimination roughly doubles during breast-feeding. The specific bioassays being extrapolated from animals to humans only involved exposure to young adult animals or during an initial pregnancy, when lactational excretion would not be a factor. However, it could be significant for the estimation of dosimetry in human children, useful for the interpretation of epidemiological data. The elimination that occurs during breastfeeding would reduce the body burden in a mother who then becomes pregnant again, hence the risk to her subsequent children. While reduction due to breast-feeding would not be predicted for women who formula-feed their children, some reduction in maternal PFDA would also be expected due to distribution to the fetus, along with the placenta, umbilical cord and amniotic fluid that are lost at childbirth, independent of how the child is subsequently fed.

Zhang et al. (2013b) estimated the urinary clearance of PFDA from matched urine and blood or serum samples from 86 healthy volunteers. The resulting median clearance rate in young females (age ≤ 50 years, n = 20), 0.047 mL/kg/day, is three times higher than the urinary clearance

estimated by Fujii et al. (2015). The reason for the discrepancy between the results of Zhang et al. (2013b) and Fujii et al. (2015) is unclear, but a possible factor is that Zhang et al. (2013b) used single urinary voids ("spot samples") to estimate clearance while Fujii et al. (2015) collected 24-hour urine samples, which avoids assumptions required to extrapolate from a spot sample to total daily excretion. Given that Fujii et al. (2015) used a more reliable method and provides a more health-protective value, the total human clearance estimated from their results will be used for human equivalent dose estimates.

Another factor to be considered is clearance through menstrual blood and serum loss. As there is no known mechanism for resorption of PFDA from menstrual blood and serum (unlike urinary and biliary/fecal pathways). Therefore, it is reasonable to assume that any fluid lost by this process would carry with it the PFDA it contained.

Zhang et al. (2013b) calculated a rate for menstrual clearance assumed to apply for all PFAS based on a study of PFOA and PFOS that estimated menstrual blood loss using measurements of the blood quantity excreted (Harada et al., 2005). This estimate was not specific to PFOA or PFOS and might also be applied to PFDA. However, Harada et al. (2005) cite Hallberg et al. (1966) as the source for a menstrual blood loss of 70 mL per cycle, but according to Hallberg, "the upper normal limit of the menstrual blood loss is situated between 60–80 mL." Thus, 70 mL/cycle appears to be closer to an upper bound for healthy women. On the other hand, Verner and Longnecker (2015) reviewed Hallberg et al. (1966) who evaluated both blood loss and total fluid loss from menstruation and concluded that the fluid lost in addition to blood was likely to be serum, with the corresponding serum binding proteins and associated PFAS. Including this serum loss and assuming 12.5 menstrual cycles per year, Verner and Longnecker (2015) estimated an average yearly total serum loss of 868 mL. Assuming a standard human body weight of 80 kg, the corresponding average rate of clearance is 868 mL/ (365 days)/(80 kg) = 0.030 mL/kg-day.

Lorber et al. (2015) examined the effects of ongoing blood loss through menstruation or through frequent blood withdrawal as a medical treatment. Male patients with frequent blood withdrawal had serum concentrations 40–50% less than males from the general population for the chemicals observed in the study (PFOA, PFNA, PFDA, PFHxS, and PFOS). Female patients also had a lower serum concentration than females from the general public. Though the trend of lower PFAS serum concentration in patients compared to the general public was consistent, there was not a clear trend in relation to the number of recent blood draws or in the recency of the last blood draw. This study's analysis of the impact of menstrual blood loss was purely a modeling exercise, which was performed for PFOA and PFOS. The authors estimated a monthly blood loss of 35 mL (which is similar to the median loss reported by Hallberg et al. (1966)), 50% of which was serum, resulting in a clearance of 17.5 mL/month, or 0.0073 mL/kg-day in an 80 kg woman. This value is also chemical-independent and could be applied to PFHxS instead of the menstrual clearance estimated by Verner and Longnecker (2015).

In summary, the total estimated urinary plus fecal clearance based on Fujii et al. (2015) (urinary) and Kim et al. (2019) (fecal/urinary) is 0.039 mL/kg-day in men and 0.026 mL/kg-day in women. The estimated urinary clearance from Fujii et al. (2015) is considered particularly reliable because 24-hour urine samples were used to determine the rate. Adding the menstrual clearance of 0.030 mL/kg-day based on the results of Verner and Longnecker (2015) yields total CL = 0.056 mL/kg-day for women between 12.4 (menarche) and 50 years of age, except during pregnancy and until menstruation resumes postpartum. These values are considered appropriate for use in animal-human dose extrapolation and hence will be used in the calculation of data-derived extrapolation factors (DDEFs) (see Section 3.1.7).

3.1.5. Summary of pharmacokinetic parameters

Summary rat, mouse, and human pharmacokinetic parameters (clearance, volume of distribution, and fraction absorbed (Fabs)) from the preceding analyses are provided in Table 3-3, along with overall half-lives calculated from the clearance and volume of distribution.

Table 3-3. Rat, mouse, and human pharmacokinetic parameters.

Sex and species	Clearance (mL/kg-d)	Volume of distribution (mL/kg)	T1/2[a] (d)	References
Male rats	4.14	431.1	72	Kim et al. (2019) Dzierlenga et al. (2019) Ohmori et al. (2003)
Female rats	4.06	313.4	54	
Rats (M + F)[b]	4.10	372.3	63	
Male mice	3.9	250	44	Fujii et al. (2015)
Female mice	2.2	200	63	
Mice (M + F)[b]	3.1	225	50	
Men	0.039	431.1 (men)[c]	7,662 (21 yr)	Fujii et al. (2015) Kim et al. (2019) Verner and Longnecker (2015) (This document.)
Women < 12.4 or > 50 years	0.026	313.4[d]	8,355 (23 yr)	
Women 12.4–50 years old[d]	0.056	313.4[d]	3,879 (10.6 yr)	

[a] T1/2 = (volume of distribution [mL/kg]) × ln (2) / (clearance [mL/kg-d]).
[b]Average of separate male and female values.
[c]Vd in women assumed equal the value for female rats, Vd in men assumed equal to male rats.
[d]Includes 0.03 mL/kg/d for menstrual clearance based on Verner and Longnecker (2015).

Some mechanistic insight can be gained by comparing the clearance values shown in Table 3-3 with species-specific glomerular filtration rate (GFR), with and without adjustment for serum protein binding. Davies and Morris (1993) summarized GFR for multiple species. Using 0.25 kg as the species average BW for the rat, the GFR/BW for rats is 7.55 L/kg-day, which is 1,800 and 1,900

times higher than the population mean clearance in male and female rats, respectively. Considering the time-period of (Davies and Morris, 1993), it seems appropriate to use their value for average human BW, 70 kg, which results in an estimated GFR/BW of 2.57 L/kg/d in humans, which is 66,000 times greater than the estimated clearance for human males. Thus, GFR itself is not a limiting factor for PFDA clearance in rats or humans.

Binding to serum proteins plays a likely role in these very large differences. As discussed above in the context of distribution, PFDA binds to albumin with high affinity, which mediates glomerular filtration since only the unbound fraction is filtered (Kudo, 2015), in addition to any role played by renal transporters. Kim et al. (2019) measured reported PFDA free fractions (f_{free}) of 0.00118 and 0.000112 in male and female rat plasma. Using these values, GFR× f_{free} = 8.9 and 0.85 mL/kg-day in male and female rats. This alternative estimate of clearance for male rats is close to the empirical population mean in Table 3-3 (6.8 mL/kg-day), which could be interpreted as implying that there is moderate renal resorption. However, for female rats GFR× f_{free} is 4.8-fold lower than the empirical clearance of 4.06 mL/kg-day. Section 3.1.6 provides discussion of the fact that the PBPK model of Kim et al. (2019), which assumes that tissue distribution is similarly limited by the free fraction, under-predicts the short-term distribution of PFDA in rats. Hence, while we expect that serum protein binding limits renal excretion (and tissue distribution) to some extent, the reduction appears to be less than predicted by assuming that clearance is strictly limited to the equilibrium free fraction. Alternately, there could simply be an error in the measured free fraction.

Kim et al. (2019) also measured reported average PFDA f_{free} values of 0.00157 and 0.00123 in human males and females, respectively, which leads to GFR× f_{free} = 4 and 3 mL/kg-day for men and women, which are still 100 and 56 times greater than the respective estimated total clearance values (which include fecal and menstrual elimination). Thus, it appears likely that there is significant renal resorption of PFDA in humans, which acts above and beyond the limitation predicted based on measured serum protein binding.

According to EPA's $BW^{0.75}$ guidelines (U.S. EPA, 2011) use of chemical-specific data for dosimetric extrapolation such as described above is preferable to the default method of $BW^{0.75}$ scaling. However, for the purpose of comparison, using the standard species BWs of 0.25 kg in rats and 80 kg in humans, the clearance in humans is predicted to be 4.2 times lower than rats. Given clearance rates of 6.8 and 4.7 mL/kg-day in male and female rats, one would then predict clearance rates of 1.6 mL/kg-day in men and 1.1 mL/kg-day in women, which are respectively approximately 40 and 20 times higher than the respective clearance values listed in Table 3-3 from human PK data for men and women of reproductive age. Thus, based on the PFDA-specific PK data, use of $BW^{0.75}$ could lead to an over-prediction of human elimination, hence an over-prediction of human equivalent doses (HEDs) of 20–40-fold.

3.1.6. Evaluation of PBPK and PK Modeling

The PFAS protocol (Supplemental Information document, Appendix A) recommends the use of PBPK models as the preferred approach for dosimetry extrapolation from animals to humans,

while allowing for the use of data-informed extrapolations (such as the ratio of serum clearance values) for PFAS that lack a scientifically sound and/or sufficiently validated PBPK model. If chemical-specific information is not available, the protocol then recommends that doses be scaled allometrically using body weight $(BW)^{3/4}$ methods. Selection from amongst this hierarchy of decisions considers both the inherent and chemical-specific uncertainty (e.g., data availability) for each approach option. This hierarchy of recommended approaches for cross-species dosimetry extrapolation is consistent with EPA's guidelines on using allometric scaling for the derivation of oral reference doses (U.S. EPA, 2011). This hierarchy preferentially prioritizes adjustments that result in reduced uncertainty in the dosimetric adjustments (i.e., preferring chemical-specific values to underpin adjustments vs. use of default approaches).

A PBPK model is available for PFDA in rats and humans Kim et al. (2019). The computational code for this model was obtained from the model authors and evaluated for consistency with the written description in the published paper, the PK data for PFDA, known physiology, and the accepted practices of PBPK modeling. Unfortunately, several flaws were found in the model. One flaw, an error in the balance of blood flow through the liver, had only a moderate impact on model predictions. A much larger issue is that the model had only been calibrated to fit the oral PK data for rats and the set of model parameters selected by the model authors to match those data included an oral bioavailability (BA) lower than is otherwise supported by the empirical PK data. For example, the fraction absorbed by the male rat was effectively set to 25% in the model when the empirical PK analysis showed 65 ± 8% bioavailability. Further, when the model was used to simulate the intravenous PK data, which are data to which a PK model should be calibrated, the parameters were found to be completely inconsistent with these data. Figure 3-2 compares results obtained with a replication of the PBPK model, which exactly matches the published PBPK model results for oral dosimetry, to the data and empirical PK fit for a 1 mg/kg IV dose to male rats.

The over-prediction (approximately three to four times higher than these key pharmacokinetic data for male rats) of the IV data by the Kim et al. (2019) model indicates that distribution into the body is significantly under-predicted by the model, which was offset in the simulations of oral dosimetry data by using an unrealistically low oral bioavailability. Initial efforts to re-fit the model to the data did not produce acceptable fits to both the IV and oral dose PK data and involved changing model assumptions in a way that would require separate experimental validation before use. It was therefore determined that the published model structure and underlying assumptions did not allow a sufficiently sound calibration of the model to the PK data, given the currently available data.

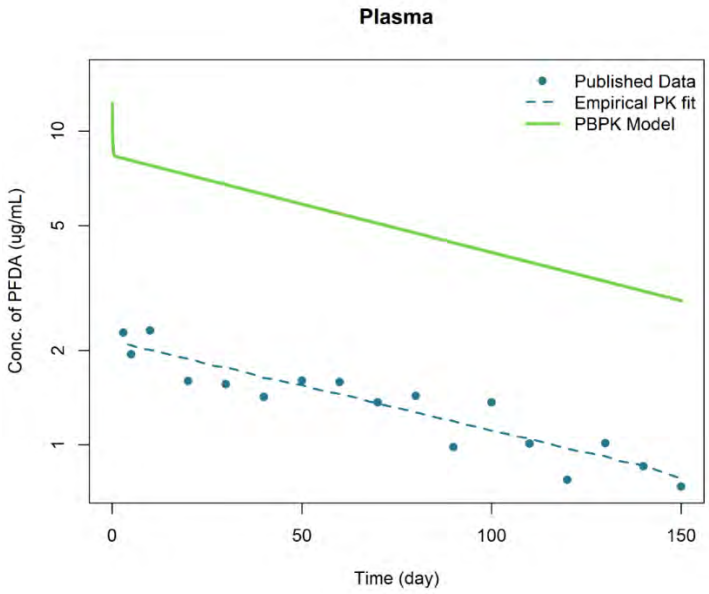


Figure 3-2. Comparison of PFDA PBPK model predictions to IV dosimetry data (circles) of Kim et al. (2019) for a 1 mg/kg dose.

"Empirical PK fit" is the result of an empirical PK analysis shown by Kim et al. (2019) (digitized). EPA's replication of the PBPK model exactly reproduces the PBPK model results of Kim et al. (2019) for oral dosimetry hence is considered an accurate reproduction of the model. The discrepancy between the PBPK model prediction for a 1 mg/kg dose and the data demonstrates that the published model structure and parameters are very inconsistent with the empirical data, hence that there is a significant flaw in the model.

The U.S. EPA also evaluated the use of a one-compartment PK compartment to explicitly describe the time-dependent dosimetry of PFDA. Specifically, the population mean CL_{tot} and Vd from Table 3-3 for male rats were incorporated into a one-compartment PK model and evaluated against independent PK data, specifically end-of-study serum levels from the NTP (2018) bioassay. Details of the model and evaluation are provided in Appendix G. Based on those results, EPA considers use of a one-compartment PK model to predict time- and dose-dependent changes in PFDA serum concentration as being too unreliable, even with PK parameters that EPA otherwise considers to be sound. While the species- and sex- dependent CL_{tot} values in Table 3-3 are believed to provide reasonably sound measures of average serum concentration vs. dose in rats, mice and humans and hence a better basis for HED calculation than use of default BW3/4 scaling, the greater level of precision that would be implied by integrating these parameters in a PK model which nominally can predict the exact rate of accumulation and clearance over time is not supported.

Another factor in considering use of a PK model is the potential to extrapolate across life-stages. However, as described in Distribution in Humans above, there are only limited data on PFDA distribution during gestation, the interpretation of which is not entirely clear. There are no PK data in young children or young animals to evaluate differences in that life-stage, nor data on clearance during pregnancy vs. non-pregnant adults. Even if the model was judged to be adequate for low-

dose extrapolation of dosimetry in adult animals, use of the PK model for some endpoints and not others would create inconsistency in the extrapolation approach. Therefore, recognizing the range of uncertainties and the poor performance of the model in predicting the NTP bioassay data, a simple PK model such as that described in Appendix G will not be used for dosimetric extrapolation despite its potential promise.

3.1.7. Approach for pharmacokinetic extrapolation of PFDA among rats, mice, and humans

Empirical PK data from all published studies, including Kim et al. (2019), were evaluated and summarized above to obtain values for the volume of distribution (Vd, mL/kg) and total clearance (CLtot, mL/kg-day) in male and female rats and mice, women of child-bearing age (<50 years of age) and men and older women (see Table 3-3). However, evaluation of a published PBPK model (Kim et al., 2019) and a one-compartment PK model showed significant errors in the PBPK model, and that the simpler PK approach also did not reliably predict PFDA serum concentrations measured at the end of the NTP bioassay (also see Appendix G). An alternative to use of PK (or PBPK) models for dosimetric extrapolation is use of data-derived extrapolation factors (DDEFs). As stated in EPA's guidance for DDEFs (U.S. EPA, 2014), use of these factors "maximize the use of available data and improve the scientific support for a risk assessment." As discussed above in the section on Excretion, the estimated population average values of CL_{tot} for male and female rats, female mice and male and female humans are considered sufficiently sound for use in such extrapolation and use of the alternative (default) approach, $BW^{3/4}$ scaling, would lead to significant errors in HED calculations. Therefore, application of DDEFs calculated from the clearance values listed in Table 3-3 is considered the next preferred option in the absence of a reliable PK (or PBPK) model.

Specifically, the ratio of sex-specific human clearance to clearance in the animal species and sex used to identify a specific point-of-departure (POD) will be used to estimate HEDs for points of departure (PODs) identified from bioassays performed with those animal species. For example, to extrapolate from a POD from the NTP bioassay for an endpoint in male rats to human males,

$$HED = POD \times (F_{abs,rat,m}/F_{abs,H}) \times CL_{H,m}/CL_{rat,m},$$

where $F_{abs,H}$ is the fraction absorbed in humans and $CL_{H,m}$ is the clearance in human males, while $F_{abs,rat,m}$ is the fraction absorbed in male rats and $CL_{rat,m}$ is the clearance in male rats. The DDEF is then $(F_{abs,rat,m}/F_{abs,H}) \times CL_H/CL_{rat,m}$. As discussed in Section 3.1.1, F_{abs} is assumed to be 1 in rats due to the range of results found, with the estimated clearance values based on this assumption. $F_{abs,H}$ is likewise assumed to be 1 but is shown here for generality.

For gestational effects the clearance in the female animal (dam) is assumed to determine dosimetry to the fetus. While a higher clearance can be estimated for women of reproductive age by including menstrual blood loss, menstruation does not occur during pregnancy and may not resume until after weaning of the child. Female babies also clearly do not menstruate. Further, as described

in Section 3.1.6, there is uncertainty in the clearance estimate for humans and there may be differences in PK among human life-stages that cannot be quantified because of a lack of empirical PK data during gestation, lactation, and childhood. While effects in adults do not involve extrapolation across life-stages, the degree of accumulation of PFDA in rats during a 28-day bioassay could be less than the accumulation during a comparable portion (4%) of the human lifespan. Therefore, HEDs for developmental and immune effects have been calculated using the health-protective CL_H from Table 3-3 for women below age 12.4 and over age 50, which is based on the average renal clearance for a mixed population of men and older women from Fujii et al. (2015); while assuming fecal clearance is 74.2% of renal clearance based on female rat data Kim et al. (2019), 0.026 mL/kg-day = 2.6×10^{-5} L/kg-day, to assure an adequate level of protection. In particular, the level of fecal clearance observed in male rats by Kim et al. (2019) was 163% of urinary clearance and the fecal clearance estimated by Fujii et al. (2015) for humans was 333% of urinary clearance, but the estimate by Fujii et al. (2015) was considered highly uncertain because of the multiple assumptions involved and use of the female rat fecal/urinary ratio is health-protective compared to both the male rat ratio and the uncertain human estimate.

Liver effects observed in adult female rats are assumed to be relevant to older women, hence the same CLH (0.026 mL/kg-day) will be used to extrapolate those. Liver and reproductive effects observed in adult male rats will be extrapolated using the CLH for men, 0.039 mL/kg-day. Finally, reproductive effects observed in adult female rats will be extrapolated using the CLH for women of reproductive age, 0.056 mL/kg-day.

The key assumption in calculating a DDEF for a given endpoint evaluated are then that for effects observed in adult male and female rats or mice, the CL for the corresponding rat or mouse sex and human sex (and relevant life-stage for women) from Table 3-3 are used to calculate the DDEF. The following table then shows the resulting DDEFs.

Table 3-4. DDEF calculations

Sex and species of observation (lifestage [endpoint])	CLA (mL/kg-d)	CLH (mL/kg-d)	DDEF[a]
Male rats (adult)	4.14	0.039	9.42×10^{-3}
Female rats (adult [liver])	4.06	0.026	6.40×10^{-3}
Female rats (adult [reproductive])	4.06	0.056	1.38×10^{-2}
Mouse (developmental)	2.2	0.026	1.18×10^{-2}

[a] DDEF = (CLH/CLA) × (Fabs,A/Fabs,H), with Fabs,A and Fabs,H assumed to be 1. Rat and mouse CL values from Table 3-3. No data exist showing that CL in juveniles is different from adults.

When an internal dose POD, specifically a serum concentration, is obtained from human epidemiological studies (Budtz-Jørgensen and Grandjean, 2018a; Grandjean et al., 2012), the HED will likewise be calculated as:

$$HED = POD_{int} \times CL_{H,}$$

using the health-protective estimate for human clearance, CLH = 0.026 mL/kg-day = 2.6×10^{-5} L/kg-day, i.e., the value estimated for pregnant and breast-feeding women and their female children.

Uncertainty in HED calculations for PFDA

The population mean male rat clearance value has a 90% credible interval from 2.6-fold below to 1.6-fold above its value (see Table 3-2). However, application of a DDEF assumes that a steady-state concentration is reached, equal to dose/CL. When the end-of-study PFDA levels observed by NTP (2018) are compared to the corresponding dose/CL using the mean CL estimated for male rats the resulting dose/CL values are 2.7 to 1.4 times higher than the data. Hence the uncertainty in use of the male rat CL in the current analysis is considered to be less than a factor of 3.

Likewise, the 90% credible interval for female rat CL only ranged from 2-fold below to 1.4-fold above the population mean (see Table 3-2) but the estimated steady-state levels (dose/CL values) are 2–3-fold higher than the end-of-study values measured by NTP (2018), so the uncertainty from use of the mean CL for adult female rats is also considered to be less than 3-fold.

While these uncertainties in the male and female rat CL values could lead to over-prediction of the HEDs, the clearance value used for humans was based on the results of Fujii et al. (2015), rather than the results of Zhang et al. (2013b) who obtained a rate of urinary clearance in young women approximately three times higher than the (mixed population) estimate of Zhang et al. (2013b). A more modest correction for fecal absorption (using the ratio of fecal/urinary elimination observed in rats after IV dosing) was applied vs. the rate estimated by Fujii et al. (2015), which was roughly 3-fold higher than the rate used. An additional term for menstrual blood loss is only applied for the extrapolation of reproductive effects observed in female rats. Hence, the population average human clearance is unlikely to be lower than the values used for HED calculation and these choices for human clearance is expected to offset the uncertainty in rat dosimetry, at least to some extent. The overall uncertainty in the animal-human extrapolation is therefore judged to be less than a factor of 3, which is considered reasonable for a pharmacokinetic analysis.

Uncertainties in the extrapolation to developmental exposure and dosimetry in children remain, given that developmental PK studies have not been conducted in rats and mice and there are only limited developmental PK data for humans. (As described in Distribution in Humans, the available data for distribution in human fetuses indicate that it is similar to distribution in adult female rats, so there is no indication of a marked lifestage difference in the volume of distribution.) There are likewise no data on clearance or excretion in early lifestages in comparison to adult animals or humans, so there is uncertainty in the extent to which such differences may exist. However, since the available data do not indicate significant lifestage variation in clearance or volume of distribution, the uncertainties from extrapolation across lifestages are judged unlikely to

be greater than is accounted for by application of the standard human interindividual uncertainty factor (UFH), of which a factor of three is typically attributed to pharmacokinetic uncertainty.

While the PK model parameter estimates seek to make the best use of the available chemical- and species-specific data, there are also many uncertainties noted above, in particular for humans. Therefore, we also evaluated the use of default $BW^{3/4}$ scaling of total clearance (CL·BW), i.e., if CLhuman = CLrat × (BWhuman/BWrat) - 0.25. The resulting clearance values for men and women (scaled from male and female rats, respectively) are 40 and 20 times higher than the values estimated from human data (Summary of pharmacokinetic parameters). Hence, estimates of human equivalent doses using $BW^{3/4}$ scaling of clearance would be significantly less health protective than the proposed DDEFs (see Table 3-4). While it is plausible that human clearance is actually that high, given the limited human PK data, lack of exposure control and quantification, and other uncertainties discussed above, the clearance values obtained from chemical-specific data are preferred and used in the derivations below since they are based on direct observation of human excretion.

3.2. NONCANCER HEALTH EFFECTS

For each potential health effect discussed below, the synthesis describes the evidence base of available studies meeting the PECO criteria, as well as the supplemental studies that most directly inform questions relating to coherence, MOA, biological plausibility, or human relevance during evidence integration.

For this section, evidence to inform organ/system-specific effects of PFDA in animals following developmental exposure are discussed in the individual organ/system-specific sections (e.g., liver effects in adult animals after gestational exposure are discussed in the liver effects section). Given that spontaneous abortion and preterm birth are informative of both female reproductive and developmental toxicity, these endpoints are also discussed in the sections for Developmental and Female reproductive effects. General toxicity effects, including body weights and survival, were summarized to aid in interpretation of other potential health effects considering the association between PFDA exposure and induction of wasting syndrome (rapid and marked reductions in body weight and food consumption) in animals (refer to Section 3.2.10 for more details).

3.2.1. HEPATIC EFFECTS

Methodological considerations

Serum enzymes and other clinical markers of hepatocellular and biliary function were evaluated across human and animal studies. For the animal studies, the results were interpreted together with histopathology and liver weight measures to aid in the assessment of potential adverse liver effects in response to PFDA exposure. Elevated serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are useful indicators of

hepatocellular damage that results in the release of these enzymes into the blood, with ALT considered more specific and sensitive (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Alkaline phosphatase (ALP) is localized to the bile canalicular membrane, and therefore, more indicative of hepatobiliary damage. Increases in circulating ALP, γ-glutamyltransferase (GGT; another canalicular enzyme) and bile components (bilirubin and bile salts/acids) and are associated with obstruction of hepatic bile flow and damage to biliary epithelial cells (Hall et al., 2012; EMEA, 2008; Boone et al., 2005). Blood proteins such as albumin, globulin, and total protein (amount of albumin and globulin) are routinely evaluated in clinical chemistry. Albumin is synthesized in the liver and then excreted into the bloodstream, where it is bound by fatty acids, cations, bilirubin, thyroxine (T4), and other compounds. Globulins, a collection of blood proteins larger than albumin, is made by both the liver and immune system. Decreased levels of these blood proteins can be good indicators of protein loss due to kidney disease or impaired synthesis as a result of liver damage (Whalan, 2015).

Human studies

Serum biomarkers

Eight epidemiology studies report on the relationship between PFDA exposure and liver serum biomarkers. As discussed above, ALT and AST are considered reliable markers of hepatocellular function/injury, while levels of ALP (Boone et al., 2005), bilirubin, and γ-GGT are routinely used to evaluate hepatobiliary toxicity (Hall et al., 2012; EMEA, 2008; Boone et al., 2005).

Of the remaining seven studies, all were *medium* confidence, including five studies in adults (four cross-sectional and one cohort) and one cohort (analyzed cross-sectionally) of children, and one cross-sectional study examined all ages (3–79 years) (see Figure 3-3). In addition, one study, a cross-sectional study of pregnant women, was considered uninformative due to lack of consideration of potential confounding (Jiang et al., 2014). All studies measured liver enzymes using standard laboratory approaches.

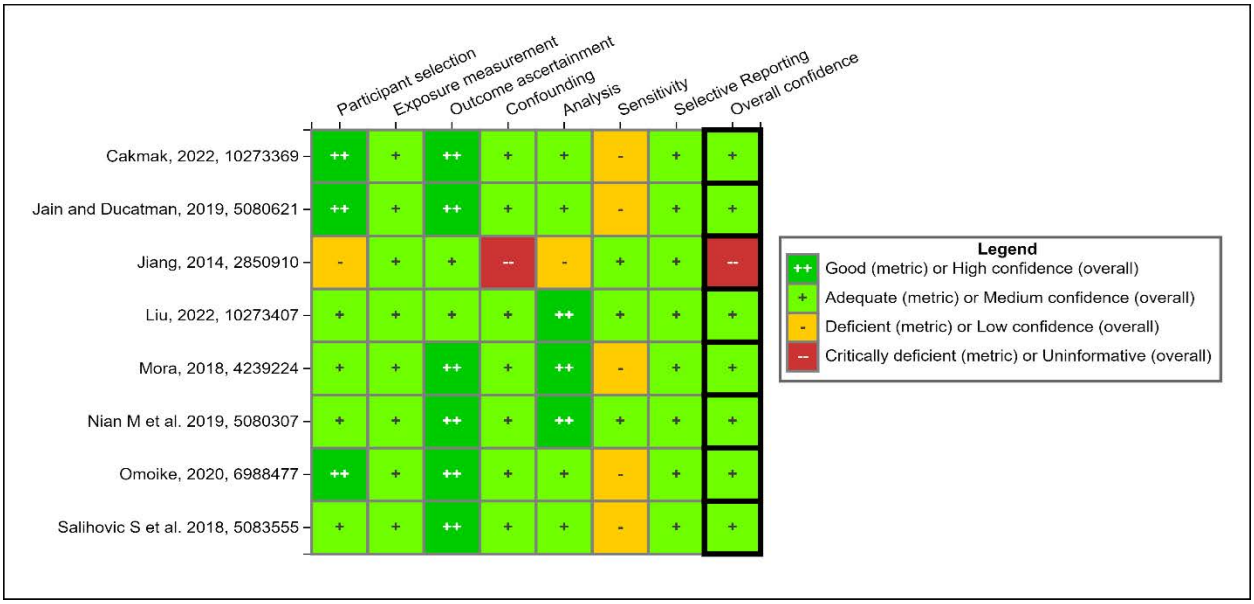


Figure 3-3. Hepatic effects, human study evaluation heatmap. Refer to HAWC for details on the study evaluation review: **HAWC Human Hepatic Effects**.

The results for the seven medium confidence studies are summarized in Table 3-5. While most studies reported statistically significant associations with some clinical markers, the direction of association varied across studies for individual markers and across markers within studies, and the clinical relevance of the changes is unclear. The studies in adults were somewhat inconsistent with respect to the direction and size of the effect estimates, though differences in the populations and analyses complicate their comparison. Five of the studies examined general population adults with cross-sectional measurement of exposure and outcome (Cakmak et al., 2022; Liu et al., 2022; Omoike et al., 2020; Jain and Ducatman, 2019b; Nian et al., 2019) (the latter also includes children). Nian et al. (2019) observed positive associations with ALT, AST, GGT, and total bilirubin but an inverse association with ALP. Jain and Ducatman (2019b) observed positive associations with ALT and total bilirubin but inverse associations with AST and ALP. Liu et al. (2022) reported positive associations with ALT, AST, GGT, and total bilirubin with exposure response gradients observed across quartiles, but an inverse association with ALP. Cakmak et al. (2022) reported positive associations with ALT, AST, ALP, GGT, and total bilirubin, but only GGT was statistically significant. In contrast, Salihovic et al. (2018) examined changes in liver function with changes in PFDA exposure over 10 years in elderly adults. They observed positive associations with ALT, ALP, and GGT, but an inverse association with total bilirubin. The inconsistency across markers in the same study and within markers across studies increases the uncertainty in the evidence, though it is conceivable that the differences across markers could be explained by different mechanisms. Since the individual markers reflect involvement of multiple types of hepatic cells, elevations in all of them would not be expected, but there is no biological explanation for the reduced levels that were

observed. Differences observed in Salihovic et al. (2018) could be explained by the different population, or more likely, the examination of change in outcome over time vs. cross-sectional analysis, but there is not enough data available to confirm this. Study sensitivity may also play a role in the inconsistency. Nian et al. (2019) and Liu et al. (2022) had the largest exposure contrast (IQR 0.9–1.0 ng/mL) and the strongest adverse associations. In the only available study in children, the only marker examined (ALT) was found to be lower with higher exposure in girls.

Overall, there is reasonably consistent evidence of a positive association between exposure to PFDA and ALT in adults, including positive associations in four of five available studies (three statistically significant) and an exposure-response gradient observed in the one study that examined quartiles of exposure. The larger associations in studies with adequate sensitivity (due to larger exposure contrast) also increases certainty in the association. Evidence for other biomarkers of hepatic function, particularly ALP and total bilirubin, are less consistent. The biological relevance of the small observed changes in any of these liver enzymes is unclear. No studies of more clinical or apical endpoints are available to provide coherence with these findings.

Table 3-5. Associations between PFDA and serum biomarkers of hepatic function in *medium* confidence epidemiology studies

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	Markers of hepatocellular injury		Markers of hepatobiliary injury		
				ALT	AST	ALP	GGT	Total bilirubin
Adults								
Jain and Ducatman (2019b)	Cross-sectional (NHANES 2011–2014); U.S.; 2883 adults	Serum 0.2	β (p-value) per log-unit change	Nonobese 0.003 (0.9) Obese 0.01 (0.5)	Nonobese −0.009 (0.6) Obese −0.01 (0.5)	**Nonobese −0.03 (0.01) *** Obese −0.006 (0.7)	Nonobese −0.003 (0.9) Obese 0.003 (0.9)	**Nonobese 0.05 (0.02) *** Obese 0.02 (0.3)
Omoike et al. (2020)	Cross-sectional (NHANES 2005-12); U.S. 6652 adults	Serum 0.3 (20^{th}-80^{th}: 0.1-0.5)	% change (95% CI) per 1% increase	NR	NR	NR	NR	0.01 (-0.0, 0.03)
Salihovic et al. (2018)	Cohort (2001–14); Sweden; 1002 elderly adults	Plasma at baseline (70 yrs) 0.3 (0.2–0.4)	β (95% CI) for changes in liver function with change in ln-PFDA over 10 years (mixed random effects)	**0.02 (0.01,0.04)***	NR	**0.2 (0.1, 0.2) ***	0.06 (0.0,0.1)	**−2.3 (−2.7, −1.9) ***
Liu et al. (2022)	Cross-sectional (2018-19); China; 1303 adults	Serum 0.9 (0.5-1.4)	% difference (95% CI) for quartiles vs Q1	**Q2: 3.46 (1.84, 5.09)* Q3: 8.03 (4.33, 11.86)* Q4:**	**Q2: 1.16 (0.13, 2.21)* Q3: 4.63 (2.27, 7.05)* Q4:**	Q2: -0.75 (-1.63, 0.13) **Q3: -2.09 (-4.00, -0.14)* Q4:**	**Q2: 3.31 (1.41, 5.24)* Q3: 8.97 (4.57, 13.55)* Q4: 19.37 (8.37, 31.48)***	**Q2: 2.67 (1.50, 3.86)* Q3: 6.23 (3.56, 8.98)* Q4: 12.03 (5.53, 18.94)***

Reference	Population	Median exposure (IQR) or as specified	Effect estimate	Markers of hepatocellular injury		Markers of hepatobiliary injury		
				ALT	AST	ALP	GGT	Total bilirubin
				Q4: 15.51 (6.44, 25.35)*	**11.75 (5.91, 17.91)***	-4.40 (-8.72, 0.13)		
Nian et al. (2019)	Cross-sectional (2015–16); China; 1605 adults	Serum 0.9 (0.5–1.5)	% change (95% CI) per ln-unit change	**3.1 (0.1, 6.1) ***	1.0 (−0.9, 3.0)	**−3.8 (−5.4, −2.2) ***	2.2 (−0.9, 5.3)	**4.3 (2.1, 6.6) ***
Adults and Children								
Cakmak et al. (2022)	Cross-sectional (2007-2017); Canada; 6109	Plasma 0.2	% change (95% CI) per 1 GM increase	3.0 (-0.1, 6.2)	2.2 (-0.6, 5.0)	1.0 (-3.3, 5.6)	**15.5 (2.2, 30.4)***	1.6 (-7.8, 11.9)
Children								
Mora et al. (2018)	Cross-sectional analysis of cohort (1999–2002), U.S.; 682 children (7–8 yrs)	Plasma 0.3 (0.2–0.5)	β (95% CI) with IQR increase	−0.3 (−1.2, 0.5) Boys: 0.1 (−1.3, 1.4) Girls: −0.9 (−1.8, −0.1) *	NR	NR	NR	NR

***$p < 0.05$**.

IQR: interquartile range (i.e., 25th–75th percentile); NR = not reported.

Animal studies

The toxicity database for PFDA liver effects in experimental animals consists of three 28-day gavage studies (Frawley et al., 2018; NTP, 2018), five short-term studies (≤14 days) via the diet (Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991), one short-term study via drinking water (Wang et al., 2020) and one developmental study via gavage (Harris and Birnbaum, 1989). The studies included several strains of rats (S-D, Wistar and Fischer 344) and mice (C57BL/6N, B6C3F1/N and CD-1[ICR]) and measured endpoints considered informative for evaluation of liver toxicity, such as histopathology, serum biomarkers of effects, and organ weights.

Histopathology

Liver histopathology was examined across five short-term oral exposure studies: two *high* confidence studies in rats exposed via gavage (Frawley et al., 2018; NTP, 2018), a low confidence study in mice exposed via the diet (Kawashima et al., 1995) and two *low* confidence studies in mice exposed via drinking water (Wang et al., 2020). The primary issue contributing to the *low* confidence rating for the Kawashima et al. (1995), Li et al. (2022), Wang et al. (2020) studies was the incomplete reporting of histopathology data (no information on incidence or severity) (see Figure 3-4). Additional deficiencies were identified in the study evaluation domains for allocation (non-reporting of randomization), nonreporting of blinding practices, and chemical administration and characterization in Kawashima et al. (1995).

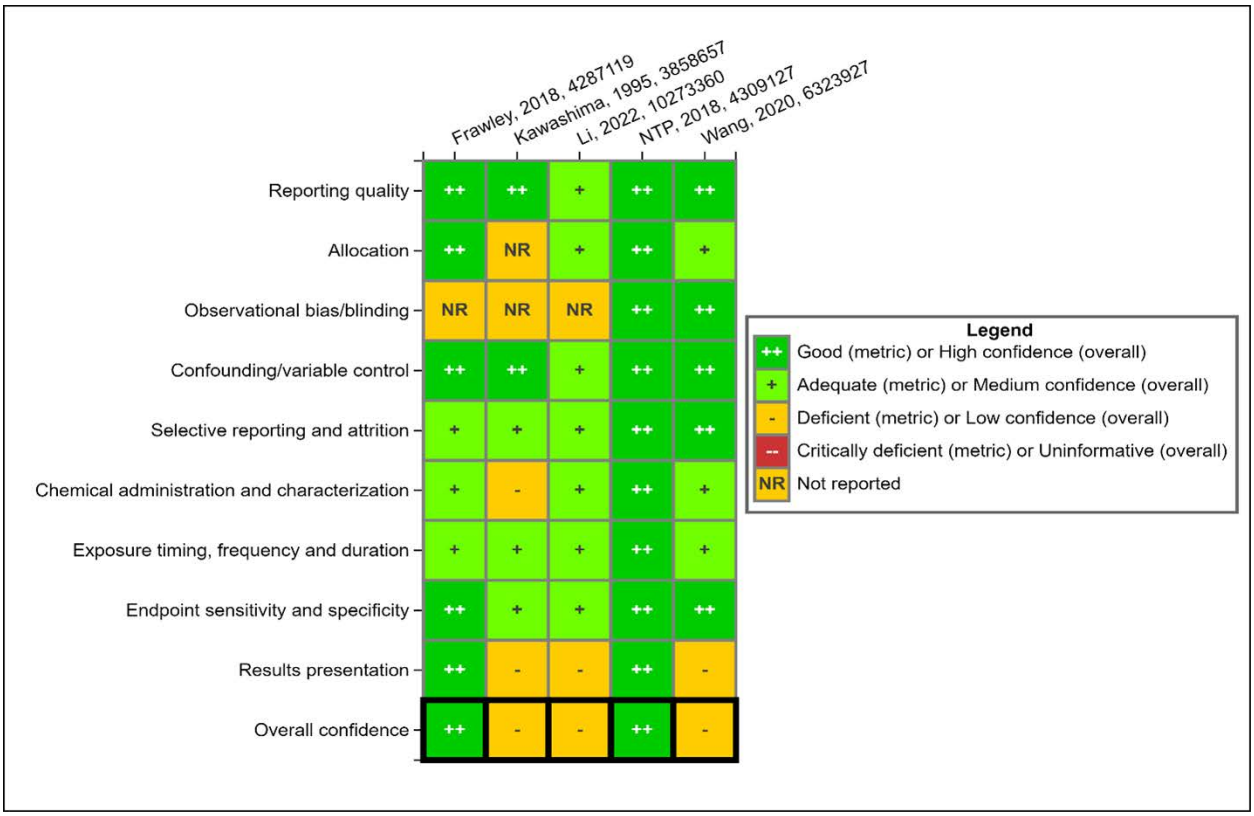


Figure 3-4. Evaluation results for animal studies assessing effects of PFDA exposure on liver histopathology. Refer to HAWC for details on the study evaluation review.

Hepatocyte lesions were identified in male and female S-D rats at exposure doses of 0.5–2.5 mg/kg-day across the two high confidence studies with 28-day exposure and these lesions were not present in control animals (Frawley et al., 2018; NTP, 2018) (see Table 3-6 and Figure 3-5). Cytoplasmic alterations that consisted of accumulation of eosinophilic granules within the cytoplasm of centrilobular hepatocytes were observed in nearly all rats at doses of 0.625–2.5 mg/kg-day in the study by NTP (2018). Cytoplasmic vacuolation that that was largely centrilobular in distribution and characterized by accumulation of microvacuoles within the cytoplasm was also reported in males and females at 1.25 and 2.5 mg/kg-day (10–100% incidence rate) (NTP, 2018). Hepatocyte hypertrophy (i.e., increase in the size of primarily centrilobular hepatocytes) was significantly elevated in these animals at similar doses (80–100% incidence) (NTP, 2018). The severity of these lesions increased with dose, ranging from minimal to marked in males and minimal to moderate in females. Minimal hepatocyte necrosis was increased in rats across studies and sexes (Frawley et al., 2018; NTP, 2018) with incidence rates ranging from 10–40%; a statistically significant trend was reported in females at doses ≥1.25 mg/kg-day in one study (NTP, 2018). Frawley et al. (2018) characterized the changes as centrilobular, single cell hepatocyte necrosis occurring in female rats (males were not tested in the study). Hepatocyte necrosis in male and female rats was described in the (NTP, 2018) report as "a few widely scattered, variably sized,

randomly distributed foci of necrotic hepatocytes within the hepatic parenchyma mixed with variable numbers of mononuclear inflammatory cells."

PFDA treatment had no appreciable effect on cellular infiltration in the liver in either male or female rats up to a dose of 2.5 mg/kg-day after 28-day exposure (NTP, 2018) (see Figure 3-5). The low confidence studies also observed hepatocyte changes in animals at higher PFDA doses (4.6–25 mg/kg-day); however, data were only summarized qualitatively and, therefore, are not displayed in Figure 3-5 (Li et al., 2022; Wang et al., 2020; Kawashima et al., 1995). Kawashima et al. (1995) described increases in lipid droplets, cell size (hypertrophy), peroxisome proliferation and vacuolated nuclei in male Wistar rats in the two high-dose groups after 7-day exposure via the diet (4.6 and 9.22 mg/kg-day). Similarly, increased hypertrophy and lipid accumulation was reported in the liver of female C57BL/6J mice after exposure to 25 mg/kg-day for 14 days via drinking water. Additionally, liver necrosis, steatosis, edema, and degeneration were found in male CD-1 mice exposed to a PFDA dose of 13 mg/kg-day via drinking water for 12 days (Wang et al., 2020). Although there is no information on incidence or severity, the findings from the Kawashima et al. (1995), Li et al. (2022) and Wang et al. (2020) studies are coherent with observations from the high confidence 28-day studies (e.g., vacuolation, hypertrophy and necrosis).

Altogether, PFDA induced a spectrum of morphological changes in rodent hepatocytes that included cytoplasmic alterations and vacuolization, hypertrophy, and some evidence of structural degenerative lesions (minimal necrosis accompanied in some cases by evidence of possible inflammation) after short-term exposure. Furthermore, a general pattern of increased severity (within and across lesions) was apparent with increasing dose.

Table 3-6. Incidence and severity of hepatocyte lesions in S-D rats exposed to PFDA in 28-day gavage studies

Animal group	Dose (mg/kg-d)								
	0	**0.125**	**0.156**	**0.25**	**0.312**	**0.5**	**0.625**	**1.25**	**2.5**
Cytoplasmic alterations									
NTP (2018) – Female (n= 10 in all groups)	0		0		0		**8 (minimal)**	**10 (minimal)**	**10 (mild)**
NTP (2018) – Male (n= 10 in all groups)	0		0		0		**10 (minimal)**	**10 (marked)**	**10 (marked)**
Cytoplasmic vacuolization									
NTP (2018) – Female (n= 10 in all groups)	0		0		0		0	1 (minimal)	**10 (moderate)**
NTP (2018) – Male (n= 10 in all groups)	0		0		0		0	**9 (mild)**	**10 (moderate)**
Hypertrophy									
NTP (2018) – Female (n= 10 in all groups)	0		0		0		0	**8 (minimal)**	**10 (moderate)**
NTP (2018) – Male (n= 10 in all groups)	0		0		0		2 (mild)	**10 (moderate)**	**10 (moderate)**
Necrosis									
Frawley et al. (2018) – Female (n= 8 in all groups)	0	0		0		3 (minimal)			
NTP (2018) – Female (n= 10 in all groups)	0		0		0		0	1 (minimal)	**4 (minimal)**
NTP (2018) – Male (n= 10 in all groups)	0		1 (minimal)		0 (minimal)		1 (minimal)	3 (minimal)	1 (minimal)

Bold values indicate instances where statistical significance ($p < 0.05$) compared to controls was reported by study authors; shaded cells represent doses not included in the individual studies. For example, the dose of 0.125 mg/kg-d was not used in the (NTP, 2018) study. Severity was normalized to a four-point scale by the study authors as follows: minimal, mild, moderate, and marked.

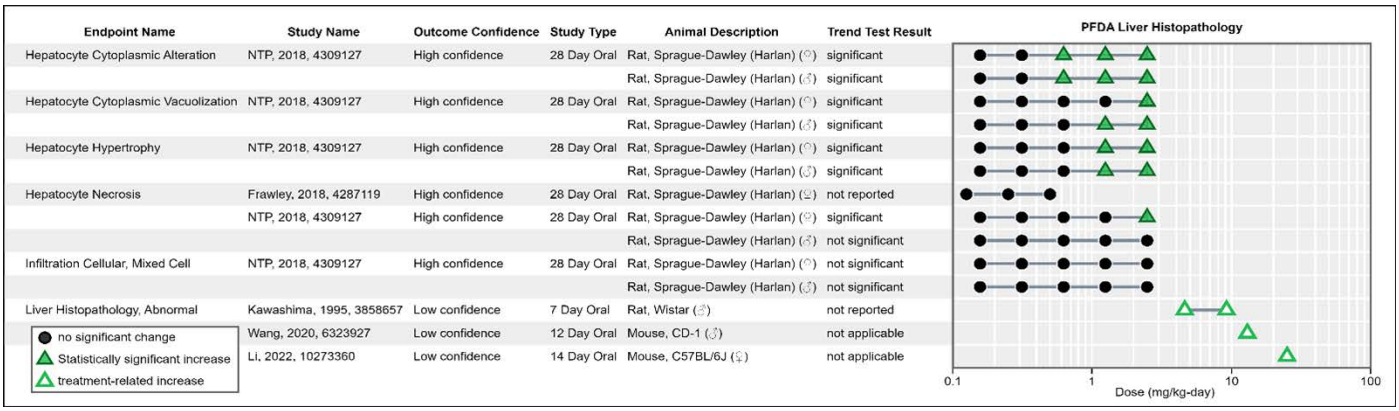


Figure 3-5. Effects on liver histopathology following exposure to PFDA in short-term oral studies in animals. (results can be viewed by clicking the HAWC link).

Serum biomarkers

Effects on serum biomarkers of liver function, including serum enzymes (ALT, AST, ASP), biliary components (bile salts and bilirubin) and blood proteins (albumin, globulin, and total protein) were evaluated in rodents across two short-term oral exposure studies (Wang et al., 2020; NTP, 2018). The studies were considered high confidence with no notable concerns with respect to risk of bias or sensitivity. Outcome-specific study evaluations are displayed in Figure 3-6.

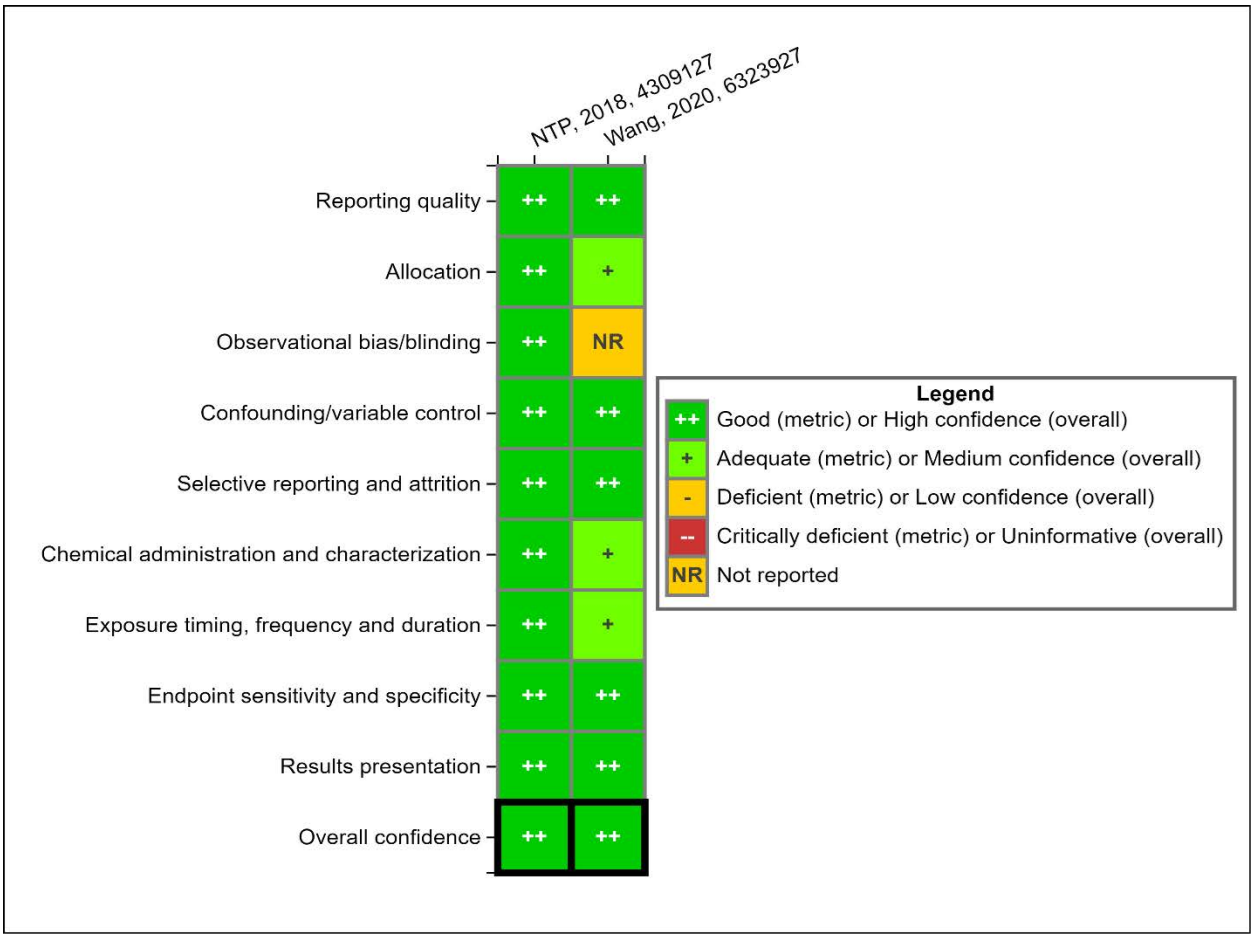


Figure 3-6. Evaluation results for animal studies assessing effects of PFDA exposure on liver serum biomarkers. Refer to HAWC for details on the study evaluation review.

Increases in ALT and AST, two markers of hepatocellular damage, were consistently reported in S-D rats with 28-day gavage exposures and CD-1 mice exposed for 12 days via the diet (Wang et al., 2020; NTP, 2018) (see Table 3-7 and Figure 3-7). Increased ALT was reported in male and female rats, although only effects in females showed a significant trend with 44% and 20% changes from controls occurring at 1.25 and 2.5 mg/kg-day, respectively. AST levels increased in a dose-dependent manner in both sexes, reaching statistical significance at all exposure doses in males (13–42% compared to controls across 0.156–2.5 mg/kg-day) and at 1.25 and 2.5 mg/kg-day in females (31% and 80% compared to controls, respectively). In mice exposed to a higher PFDA dose (13 mg/kg-day), these enzymes were similarly elevated, increasing by 338% and 649% relative to controls for ALT and AST, respectively (Wang et al., 2020).

Table 3-7. Percent change relative to controls in hepatocellular serum markers in short-term animal studies after PFDA exposure

Animal group	Dose (mg/kg-d)					
	0.156	0.312	0.625	1.25	2.5	13
Alanine aminotransferase (ALT)						
28 d gavage; female S-D rats (NTP, 2018)	−3	3	13	**44**	**20**	
28 d gavage; male S-D rats (NTP, 2018)	21	**45**	**46**	28	7	
12 d drinking water; male CD-1 mice (Wang et al., 2020)						**338**
Aspartate aminotransferase (AST)						
28 d gavage; female S-D rats (NTP, 2018)	−3	−8	1	**31**	**80**	
28 d gavage; male S-D rats (NTP, 2018)	**13**	**18**	**25**	**34**	**42**	
12 d drinking water; male CD-1 mice (Wang et al., 2020)						**649**

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

Markers of hepatobiliary function including ALP, bile salts/acids and bilirubin (total, direct and indirect) were also altered in S-D rats after a 28-day exposure (NTP, 2018) (see Table 3-8 and Figure 3-7). ALP levels increased significantly at doses ≥0.312 mg/kg-day in both males and females (22–106% compared to controls). Levels of bile salts/acids and bilirubin (total, direct and indirect) were elevated in male and female rats at doses ≥1.25 mg/kg-day; the effects showed a significant trend and were large in magnitude (205–1,207% and 28–733% compared to controls for bile salts/acids and bilirubin, respectively).

Table 3-8. Percent change relative to controls in hepatobiliary serum markers in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Alkaline phosphatase (ALP)					
Female S-D rats	14	**34**	**35**	**106**	**92**
Male S-D rats	9	**22**	**41**	**90**	**41**
Bile salts/acids					
Female S-D rats	−6	55	34	**205**	**658**

Animal group	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Male S-D rats	−53	−39	37	**440**	**1207**
Total bilirubin					
Female S-D rats	−6	−9	−10	**28**	**356**
Male S-D rats	4	5	13	**46**	**350**
Direct bilirubin					
Female S-D rats	0	4	4	**104**	**700**
Male S-D rats	−22	−4	−7	**78**	**733**
Indirect bilirubin					
Female S-D rats	−7	−11	−14	**10**	**275**
Male S-D rats	11	7	19	**37**	**255**

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

Albumin, globulin, and total protein were examined in S-D rats after 28-day exposure (NTP, 2018) (see Table 3-9 and Figure 3-7). Dose-related decreases in albumin were reported in males, decreasing by 8% and 20% at 1.25 and 2.5 mg/kg-day, respectively. In females, statistically significant increases in albumin levels were reported at 0.312 (11%) and 0.625 (13%) mg/kg-day but there was no dose-response gradient. A significant trend for globulin levels was found in both males and females, with decreases of 9–42% at ≥0.156 mg/kg-day. The albumin and globulin findings corresponded well with a decrease in total protein and increase in albumin/globulin (A/G) ratio in animals. Statistically significant increases in the A/G ratio (13–47%) occurred in males and females at all exposure doses (0.156–2.5 mg/kg-day) and total protein decreased significantly (4–28%) in males at similar doses. In females, total protein decreased by 2% and 12% at the highest doses (1.25 and 2.5 mg/kg-day, respectively), but a significant trend was not established.

Table 3-9. Percent change relative to controls in serum proteins in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Albumin					
Female S-D rats	7	**11**	**13**	7	−10
Male S-D rats	1	3	0	**−8**	**−21**
Globulin					
Female S-D rats	−9	**−18**	**−18**	**−21**	**−14**
Male S-D rats	**−13**	**−19**	**−27**	**−36**	**−42**
Albumin/Globulin ratio					
Female S-D rats	**17**	**36**	**36**	**36**	**13**
Male S-D rats	**15**	**27**	**40**	**47**	**36**
Total Protein					
Female S-D rats	3	3	3	−2	−12
Male S-D rats	**−4**	**−5**	**−10**	**−17**	**−28**

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

In summary, coherent effects across serum enzymes, biliary system components and blood proteins that are consistent with altered liver function were reported in rats and mice after short-term PFDA exposure. In mice, the serum enzyme changes were accompanied by a 40% reduction in body weights at the high PFDA dose tested (13 mg/kg-day) (Wang et al., 2020). Although the 28-day rat study reported significant body weight reductions at ≥1.25 mg/kg-day, dose-related changes in some serum biomarkers of hepatic injury occurred at doses lower (0.156–0.625 mg/kg-day) than those associated with marked systemic toxicity (NTP, 2018).

Figure 3-7. Effects on serum liver biomarkers following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Organ weight

The studies evaluating liver weight changes in animals consist of four high confidence studies (see Figure 3-8): two 28-day gavage studies using female B6C3F1/N mice or male and female S-D rats (Frawley et al., 2018; NTP, 2018), one 14-day study in female C57BL/6J mice exposure via drinking water (Li et al., 2022) and one developmental study measuring effects in female P0 C57BL/6N mice exposed via gavage during gestational days (GD) 6–15 or 10–13 (Harris and Birnbaum, 1989). The 28-day rat study by Frawley et al. (2018) included three cohorts exposed to similar experimental conditions. There are also four medium confidence studies that administrated PFDA via the diet for 7–14 days in male Wistar rats (Kawashima et al., 1995), male Fischer F344 rats (Takagi et al., 1992, 1991) or male C57BL/6N mice (Permadi et al., 1993). Overall confidence in these shorter duration studies was reduced to medium based primarily on uncertainties surrounding the characterization of the test compound (no analytical verification methods) and administered doses (lack of information on food consumption for estimating dietary exposure doses) (see Figure 3-8). A low confidence study with incomplete reporting on liver weight data (no information on sample size) is also available in Wistar rats exposed via the diet for 7 days (Yamamoto and Kawashima, 1997) (see Figure 3-8).

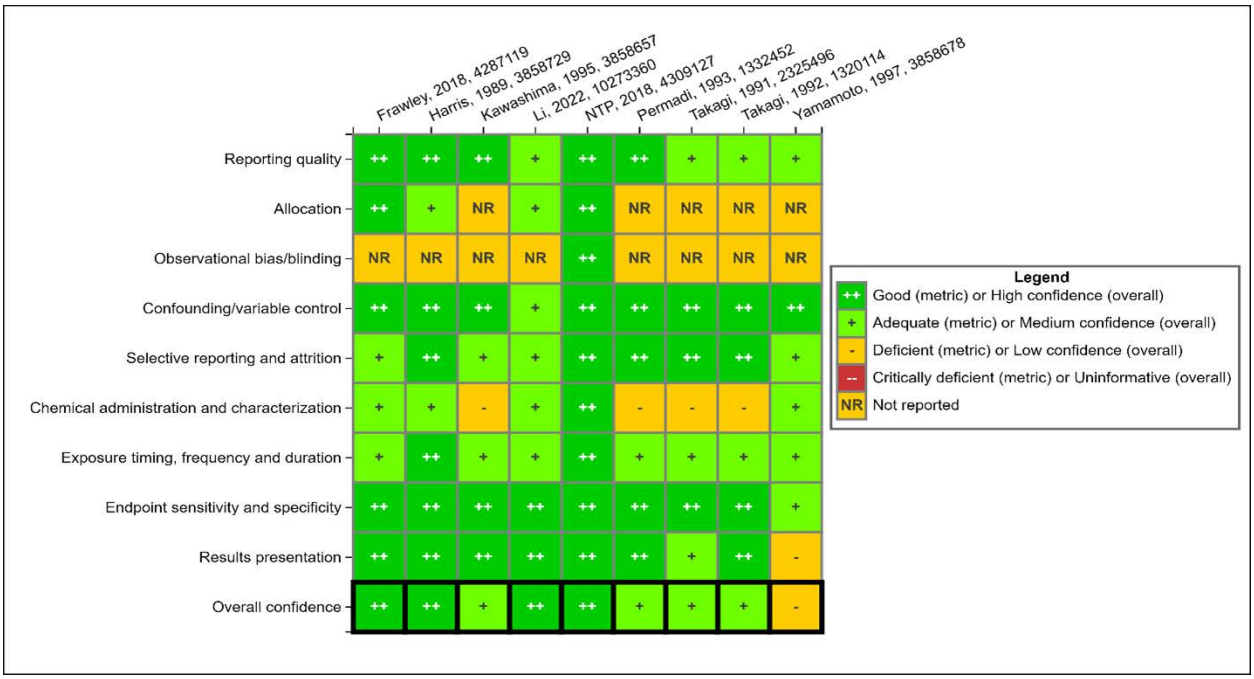


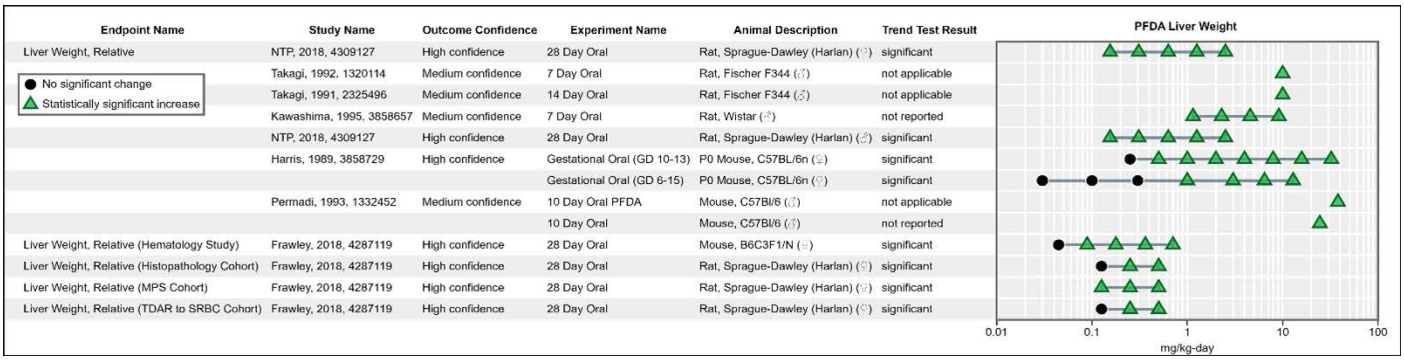
Figure 3-8. Evaluation results for animal studies assessing effects of PFDA exposure on liver weight. Refer to HAWC for details on the study evaluation review.

Increased liver weight was consistently reported across all studies, species, strains, and sexes (see Table 3-10 and Figure 3-9). Relative liver weight is often preferred over absolute liver weight as it accounts for variations in body weight that may mask organ weight changes (Bailey et al., 2004). Statistically significant increases in relative liver weights were reported in rats and mice at ≥0.089 mg/kg-day across the short-term studies, while reductions in terminal body weight occurred in these animals at higher doses (≥1.25 mg/kg-day) (see Section 3.2.10 on General toxicity effects for more details). In general, the changes in relative liver weights demonstrated a dose and time dependency. For example, dose-related increases in relative liver weights of 17–56% compared to controls were reported in male Wistar/Fisher rats at doses 1.15–10 mg/kg-day after 7–14 days across three studies with dietary exposure (females were not examined) (Kawashima et al., 1995; Takagi et al., 1992, 1991). In female P0 C57BL/6N mice exposed during gestion (GD 10–13 and 6–15), relative liver weights increased by 12–127% compared to controls at doses of 1–16 mg/kg-day (Harris and Birnbaum, 1989). At a longer exposure duration (28 days), similar magnitudes of relative liver weight increases were observed in female B6C3F1/N mice and male/female S-D rats but at lower PFDA doses (16–81% at 0.089–0.71 mg/kg-day and 10–102% at 0.125–2.5 mg/kg-day, respectively). Further, in the studies that evaluated liver weight and other relevant liver toxicity endpoints, the increases in liver weight corresponded with the reported observations of hepatocellular histopathology (Frawley et al., 2018; NTP, 2018) and alterations in serum biomarkers of hepatocellular/biliary function (NTP, 2018).

Table 3-10. Percent change relative to controls in liver weight (relative to body weight) due to PFDA exposure in short-term oral toxicity studies

Animal group	Dose (mg/kg-d)											
	0.03–0.045	0.089	0.1–0.179	0.25–0.36	0.5–0.71	1.0–1.25	2.0–3	4–4.6	6.4–8	9.22–12.8	16-25	32–37.8
7 d; male Wistar rats (Kawashima et al., 1995)						**17**	**28**	**42**		**27**		
7 d; male Fisher F344 rats (Takagi et al., 1992)										**56**		
14 d; male Fisher F344 rats (Takagi et al., 1991)										**56**		
28 d; male S-D rats (NTP, 2018)			**11**	**20**	**28**	**54**	**91**					
28 d; female S-D rats (NTP, 2018)			**12**	**20**	**32**	**52**	**102**					
28 d; female S-D rats – Histopathology cohort (Frawley et al., 2018)			1	**8**	**16**							
28 d; female S-D rats – MPS cohort (Frawley et al., 2018)			**10**	**13**	**23**							
28 d; female S-D rats – TDAR to SRBC cohort (Frawley et al., 2018)			2	**19**	**35**							
GD 10–13; pregnant P0 female C57BL/6N mice (Harris and Birnbaum, 1989)				−4	**3**	**12**	**15**	**45**	**72**		**93**	**106**
GD 6–15; pregnant P0 female C57BL/6N mice (Harris and Birnbaum, 1989)	0		3	1		**18**	**54**		**106**	**127**		
10 d; male C57BL/6N mice (Permadi et al., 1993)												**100**
14 d; Female C57BL/6J mice (Li et al., 2022)											**219**	
28 d; female B6C3F1/N mice (Frawley et al., 2018)	4	**16**	**27**	**51**	**81**							

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



Endpoint Name	Study Name	Outcome Confidence	Experiment Name	Animal Description	Trend Test Result
Liver Weight, Relative	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Takagi, 1992, 1320114	Medium confidence	7 Day Oral	Rat, Fischer F344 (♂)	not applicable
	Takagi, 1991, 2325496	Medium confidence	14 Day Oral	Rat, Fischer F344 (♂)	not applicable
	Kawashima, 1995, 3858657	Medium confidence	7 Day Oral	Rat, Wistar (♂)	not reported
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
	Harris, 1989, 3858729	High confidence	Gestational Oral (GD 10-13)	P0 Mouse, C57BL/6n (♀)	significant
			Gestational Oral (GD 6-15)	P0 Mouse, C57BL/6n (♀)	significant
	Permadi, 1993, 1332452	Medium confidence	10 Day Oral PFDA	Mouse, C57Bl/6 (♂)	not applicable
			10 Day Oral	Mouse, C57Bl/6 (♂)	not reported
Liver Weight, Relative (Hematology Study)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	significant
Liver Weight, Relative (Histopathology Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative (MPS Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Liver Weight, Relative (TDAR to SRBC Cohort)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant

Figure 3-9. Effects on relative liver weight following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Mechanistic studies and supplemental information

The liver effects in response to oral exposure to PFDA in short-term animal studies consist of increased serum biomarkers of liver function, increased liver weight and increased incidence of hepatocellular lesions (e.g., cytoplastic alterations, vacuolation, and to a lesser extent necrosis). Increased liver weight and hepatocellular hypertrophy can be associated with changes that are adaptive in nature (Hall et al., 2012), and not necessarily indicative of adverse effects unless observed in concordance with other clinical, pathological markers of overt liver toxicity (see PFAS Protocol; Appendix A). As discussed in the protocol, Hall et al. (2012) was focused on framing liver effects in the context of progression to liver tumors so additional information was considered when evaluating noncancer liver effects for PFDA exposure. The additional information consists of multiple in vitro and in vivo mechanistic studies in rodents (including peroxisome proliferator activated receptor alpha (PPARα)-null mice) and limited studies in human-relevant models (mostly in vitro systems but also studies in animal models with reduced PPARα sensitivity) as well as evidence from other PFAS that help elucidate possible modes of action of PFDA liver toxicity.

Summary of mechanistic studies for PFDA

Mechanistic evidence relevant to potential PFDA-induced liver effects was collected from the peer-reviewed literature and from in vitro high-throughput screening (HTS) assays accessed through the EPA's CompTox Chemicals Dashboard (https://comptox.epa.gov/dashboard)((U.S. EPA, 2021a); data were retrieved on November 2022). Given the relatively abundant evidence base compared to most other PFAS, the available in vitro and in vivo mechanistic studies on PFDA were considered in the context of what is known about the mode-of-action (MOA) for hepatic effects elicited by related PFAS, including PFOA and PFOS, the most well-studied PFAS. MOA information for PFOS and PFOA was based primarily on published reviews. As discussed in the systematic review protocol (Appendix A), an Adverse Outcome Pathway (AOP)-type approach was employed to organize and discuss the evidence according to the following levels of biological organization: molecular interactions, cellular effects, organ effects, and organism effects. A summary of the

mechanistic and supplemental evidence related to the potential mechanisms of hepatotoxicity for PFDA is provided below. A detailed description of the methodology and results of the analysis undertaken herein can be found in Appendices D and E.

Mechanistic evidence from in vivo and in vitro rodent cell models indicates that PFDA can activate (potentially directly) several xenobiotic-sensing nuclear receptors and other cell signaling pathways, namely PPARα, constitutive androstane receptor (CAR)/pregnant × receptor (PXR), nuclear factor erythroid 2 related factor 2 (Nrf2), tumor necrosis factor alpha (TNFα), nuclear factor kappa B pathway (NFκB) and c-Jun-N-terminal kinase (JNK)/activating transcription factor 2 (ATF-2) (see Appendix D.3.1 on Molecular initiating events for more details). PFDA exposure was also associated with alterations in the hepatic expression and activity of XME enzymes, ROS production and markers of oxidative damage (DNA oxidation and lipid peroxidation), disruption of mitochondrial functions, induction of inflammatory responses, cellular damage/stress and abnormal liver metabolic functions related to bile acid, glucose, and lipid metabolism in animals (see Appendix D.3.2 on Cellular effects for more details). These molecular and cellular mechanisms are associated with chemical-induced liver disorders such as steatohepatitis and fibrosis (Angrish et al., 2016; Cao et al., 2016; Joshi-Barve et al., 2015; Wahlang et al., 2013) and provide support for the biological plausibility of the observed liver effects in rats and mice after short-term PFDA exposure (see synthesis of Animal studies in this Section for more details).

The available mechanistic information in human models is limited to a few in vitro studies in the peer-reviewed literature and ToxCast and Tox21 HTS assay results (https://comptox.epa.gov/dashboard). The available evidence suggests some concordance with responses evaluated in animal models. PFDA could modulate the activity of a number of human nuclear receptor pathways potentially relevant to its mechanism(s) of hepatotoxicity. For example, PFDA activated PPARα in primary and immortalized human liver cell lines (Rosenmai et al., 2018; Buhrke et al., 2013; Rosen et al., 2013) and Table E-2 for in vitro HTS assay results) and exhibited direct binding towards the human PPARα in vitro (Ishibashi et al., 2019). However, there was reduced sensitivity in the binding and transcriptional activity towards the human PPARα compared to the mouse, Baikal seal and polar bear isoforms in some studies (Ishibashi et al., 2019; Routti et al., 2019; Wolf et al., 2012; Wolf et al., 2008). Reduced PPARα sensitivity in human versus rodent models (i.e., rats and mice) has been previously demonstrated in studies with other perfluorinated compounds (Corton et al., 2018; Wolf et al., 2012; Wolf et al., 2008). Moreover, PFDA activated nuclear receptors other than PPARα in human liver cell lines (i.e., PPARγ, PXR and FXR) and displayed high potency towards the human FXR in a receptor-ligand binding assays (Buhrke et al. (2013), Rosen et al. (2013), Zhang et al. (2017) and Table E-2 for in vitro HTS assay results). At the cellular level, PFDA elevated ROS production and induced markers of cellular stress and cytotoxicity in human hepatoma HepG2 cells (see Appendix D.3.2 on Cellular effects for more details).

PPARα activation is described as one of the mechanisms through which perfluorinated compounds induce liver toxicity in animals (ATSDR, 2018b; U.S. EPA, 2016a, b). PPARα appears to be important for disruption of bile acid homeostasis and downstream effects related to bile acid synthesis and transport mechanisms, as well as, signaling pathways associated with cellular stress and anti-inflammatory responses in PFDA-exposed mice (Luo et al., 2017). However, other responses appear to occur, at least in part, independently of PPARα. Rosen et al. (2013) reported transcriptional induction of PPARα-dependent and -independent genes in primary human hepatocytes exposed to PFDA. Lim et al. (2021) showed that PFDA-mediated transcriptional regulation of transporters involved in metabolism and xenobiotic biotransformation in HepaRG cells was more consistent with activation of the ROS-sensitive transcription factor Nrf2 as opposed to PPARα or CAR. Increased liver weight and activation of Nrf2 were reported after PFDA treatment in both WT and PPARα-null mice (Luo et al., 2017; Maher et al., 2008). PFDA-mediated induction of hepatic Mrp transporters involved in cholestasis was attenuated in mice devoid of Nrf2 or Kupffer cell function (Maher et al., 2008). A study that evaluated PFDA animal models known to be generally resistant to PPARα activation (i.e., Guinea pigs and/or Syrian hamsters) displayed histological responses indicative of hepatocellular stress, mitochondrial damage, hepatic lipid accumulation and liver enlargement with PFDA exposure (Van Rafelghem et al., 1987b). Noteworthy, hepatic lipid accumulation was characterized as more pronounced in Guinea pigs and Syrian hamsters compared to rats and mice and the opposite was found for peroxisome proliferation (Van Rafelghem et al., 1987b). Finally, the NTP (2018) study that reported PFDA-induced liver effects in rats exposed for 28 days, also evaluated the effects of the potent PPARα inducer, Wyeth-14,643, on the liver. Similar to PFDA, Wyeth-14,643 caused increases in liver weights, hepatocyte hypertrophy and changes in serum liver biomarkers (e.g., increased ALT, ALP and AST) in rats; however, unlike PFDA, Wyeth-14,643 exposure was not associated with any structural degenerative changes (i.e., hepatocyte necrosis).

Overall, the mechanistic evidence supports the biological plausibility of liver effects observed in animal bioassays. Further, the available data indicate a likely role for both PPARα-dependent and -independent mechanisms in the hepatotoxicity of PFDA in animals. Existing evidence from in vitro studies and animal models considered more relevant to humans with respect to PPARα sensitivity suggest that some responses may be conserved across species (including activation of relevant nuclear receptor pathways [PPARα/γ, PXR and FXR] and outcomes related to hepatocellular stress, mitochondrial damage, lipid accumulation and liver enlargement). Taken together, these data provide some support for the potential human relevance of the observed hepatic effects in animals. Some uncertainties remain based on differences in experimental design and/or confounding effects with cytotoxicity in in vitro test systems, as well as limited information available from in vivo models to characterize the putative involvement of PPARα and other cell signaling pathways in the mechanisms of hepatotoxicity of PFDA in animals and humans (see Appendix D.3 and E.1 for more details).

Evidence from related PFAS

Based on the limitations in the mechanistic evidence for PFDA described above, studies investigating the effects of structurally related long-chain PFAS (perfluoroalkyl sulfonic acids containing ≥ 6 carbons or PFCAs with ≥ 7 carbons) are summarized herein, focusing on studies conducted in null and humanized animal models identified from literature searches conducted for other ongoing EPA PFAS IRIS assessments (i.e., PFHxS and PFNA) or in final EPA human health assessments (i.e., PFOA and PFOS). Data in these models available for short-chain PFAS (e.g., PFBA) are not summarized herein, as they were considered less relevant to PFDA exposure than those data available for long chain PFAS, although extrapolations from other PFAS are all inherently uncertain.

Gene expression profiling in response to exposure to several long-chain PFAS has been evaluated in wild-type and PPARα-null mice and the results indicate a role for both PPARα-dependent and independent pathways in the liver effects of these compounds. Gene expression changes induced by PFOA, PFHxS and PFNA in wild-type mouse livers were largely attributable to PPARα; however, a subset of transcriptional changes related to lipid metabolism, inflammation and xenobiotic metabolism occurred in PPAR-α null mice that reflect potential activation of additional nuclear receptors such as CAR and PPARγ (Rosen et al., 2017; Rosen et al., 2010; Rosen et al., 2008).

Consistent with transcriptional regulation, the data support that these long-chain PFAS induced tissue-level responses which are likely to be mediated by PPARα- dependent and independent mechanisms. Increases in liver weight and hepatocyte hypertrophy and/or proliferation were reported in PPARα wild-type and null mice exposed to PFOA (Das et al., 2017; Wolf et al., 2008). Similarly, hepatomegaly (characterized by increased liver weight and cell size and decreased DNA content) and hepatic lipid accumulation (indicating or leading to steatosis) were observed with PFHxS or PFNA exposure in wild-type mice and mice devoid of PPARα function (Das et al., 2017). In contrast, these liver effects were only induced in wild-type animals treated with the prototype PPARα agonist, Wyeth 14,643. Nakagawa et al. (2012) showed elevated levels of hepatic triglycerides in wild-type, PPARα-null and humanized PPARα (hPPARα) mouse strains exposed to ammonium perfluorooctanoate, but macrovesicular and/or microvesicular steatosis in PPARα-null and hPPARα mice only. Additionally, PFOS and PFHxS decreased triglyceride and cholesterol levels in plasma and increased triglycerides in the liver of APOE*3-Leiden CETP mice, which exhibit attenuated clearance of apoB-containing lipoproteins and human-like lipoprotein metabolism on a Western diet (Bijland et al., 2011). Likewise, PFDA exposure was associated with marked increases in hepatic lipid content (including triglyceride levels) and accumulation in rats and mice (Kudo and Kawashima, 2003; Adinehzadeh and Reo, 1998; Kawashima et al., 1995; Sterchele et al., 1994; Brewster and Birnbaum, 1989; Harrison et al., 1988; Van Rafelghem et al., 1988b; Van Rafelghem et al., 1987a), as well as, Guinea pigs and Syrian hamsters (Van Rafelghem et al., 1987b), which like humans, appear to be less responsive to PPARα activation.

The precise mechanism(s) of how these long chain PFAS induced hepatic lipid accumulation and the potential association of this accumulation with progression to steatosis remain unclear. Das et al. (2017) showed that PFOS, PFHxS, and PFNA, which are known to induce significant hepatic lipid accumulation in animals, alter the expression of genes involved in fatty acid synthesis and oxidation in mouse livers, and that these transcriptional changes are partly independent of PPARα (Das et al., 2017). The authors hypothesized that perfluorinated compounds disrupt the balance of fatty acid synthesis and oxidation in favor of accumulation, which leads to steatosis. In contrast, exposure to potent PPARα activators such as Wyeth 14,643, is not associated with steatosis-like changes because, these compounds likely favor fatty acid oxidation over synthesis/accumulation (Das et al., 2017).

Collectively, studies in PPARα null and humanized animal models for structurally related long chain PFAS are consistent with the plausible PPARα-dependent and independent mode of action for PFDA liver toxicity and add further support to the potential human relevance of the observed liver effects in animals. Further, the evidence suggests that these perfluorinated compounds have the potential to induce steatosis, a well-known chemical-induced response that can progress to steatohepatitis, fibrosis, and impaired liver function (Al-Eryani et al., 2015).

Considerations for potentially adaptive versus adverse responses

Increases in liver weight and hepatocyte hypertrophy were observed in rodents with PFDA administration in short-term oral studies (see Figure 3-5 and 3-9 above). Enlargement of the liver and/or individual hepatocytes is a common chemical-induced response that can involve lipid accumulation (e.g., micro- or macro-vesicular steatosis), organellar growth and proliferation (e.g., peroxisomes, endoplasmic reticulum), increased intracellular protein levels (e.g., Phase I and II enzymes), and altered regulation of gene expression (e.g., stress response, nuclear receptors) (reviewed by Batt and Ferrari (1995)). Hepatocyte hypertrophy related to organelle growth and proliferation in response to activation of xenobiotic-sensing receptors (primarily PPARα) is often considered an adaptive response (Hall et al., 2012). However, hepatocyte swelling is also associated with cell death processes, oncosis or oncotic necrosis (Kleiner et al., 2012), which occurred in several liver diseases or conditions, such as ischemia-reperfusion injury, drug-induced liver toxicity, and partial hepatectomy (Kass, 2006; Jaeschke and Lemasters, 2003). Furthermore, mechanistic evidence for PFDA and other long-chain PFAS suggests that in addition to PPARα induction, these compounds activate non-PPARα-related mechanism of liver toxicity (see Appendix D.3 and E.1 for more details on the synthesis of PFDA-induced mechanisms of hepatotoxicity).

Hall et al. (2012) indicated that concordant histopathological evidence of degenerative or necrotic changes (e.g., hepatocyte necrosis, fibrosis, inflammation, steatosis, biliary degeneration, necrosis of resident cells within the liver) can be used to support the argument that liver weight/hepatocyte enlargement are adverse (Hall et al., 2012). In addition to increases in liver weight and/or hepatocyte hypertrophy, PFDA caused cytoplasmic alterations and vacuolization as well as, necrosis in rat hepatocytes across two high confidence 28-day gavage studies (Frawley et

al., 2018; NTP, 2018). Cytoplasmic alterations of minimal to marked severity were observed in nearly all male and female rats at ≥0.625 mg/kg-day (NTP, 2018). Cytoplasmic vacuolization of minimal/mild to moderate severity occurred in males and females at ≥1.25 mg/kg-day (NTP, 2018). Minimal necrosis was reported in females in the two 28-day studies with statistically significant increases at the highest dose, 2.5 mg/kg-day (Frawley et al., 2018; NTP, 2018). Male rats were only tested in one study, showing increased incidence of hepatocyte necrosis, but the effect was not dose-dependent (NTP, 2018). The lesions show a clear pattern of increased hepatocyte damage/injury with dose, ranging from cytoplasmic changes to hypertrophy to necrosis (NTP, 2018). The necrotic lesions were accompanied in some cases by evidence of an initial inflammatory response (NTP, 2018) and, although these changes were characterized as minimal, the findings indicate some degree of structural degeneration considered adverse and that may progress to more severe liver pathologies with increasing dose or exposure duration. Consistent with these observations, steatosis, necrosis, edema, and degeneration were reported in mice at 13 mg/kg-day and extensive lipid accumulation was reported in rats at 9.22 mg/kg-day in low confidence short-term studies with PFDA administered orally (Wang et al., 2020; Kawashima et al., 1995). Acute i.p. studies provide additional support for the accumulation of lipids in the liver with PFDA exposure (see synthesis of Metabolic Effects in Appendix D.3.2), which is a key event leading to hepatic steatosis (Angrish et al., 2016). As discussed above, steatosis is a common liver response in animals that is associated with exposure to perfluorinated compounds such as PFOA, PFHxS or PFNA. Sustained steatosis can progress to steatohepatitis and other adverse liver diseases such as fibrosis and cirrhosis (Angrish et al., 2016).

Alterations in serum liver biomarkers were also present in rats that exhibited increases in liver weight, hepatocyte hypertrophy and other histological lesions (i.e., necrosis) after 28-day gavage exposure to PFDA (NTP, 2018). According to Hall et al. (2012), clinical markers of liver damage and function can provide evidence in support of the adversity of concomitant increases in liver weight/hepatocyte hypertrophy. These authors suggested that a weight-of-evidence approach should be applied when evaluating clinical marker data, considering dose-dependent and biologically significant changes in at least two of the following parameters: 2- to 3-fold increase in ALT; change in biomarkers of hepatobiliary damage (e.g., AST, ALP and γ-glutamyltranspeptidase [γGT]); a change in biomarkers of liver dysfunction (e.g., albumin, bilirubin, bile acids/salts and coagulation factors). PFDA increased ALT levels in female rats at ≥1.25 mg/kg-day (NTP, 2018); similar changes were observed in male rats, but the effects did not show a significant trend. Although the increases in circulating ALT levels in females were relatively small (20–44% or 1.2- to 1.4-fold), concordant changes in other clinical biomarkers occurred in these animals. Dose-dependent increases in ALT and ALP were found in male and female rats at ≥0.156 mg/kg-day. Similarly, levels of bile salts/acids and bilirubin were elevated in rats of both sexes at ≥1.25 mg/kg-day, exhibiting marked changes (205–1,207% or 3.1- to 13.1-fold for bile acids/salts and 28–733% or 1.3- to 8.3-fold for bilirubin).

Overall, application of the recommendations from Hall et al. (2012) clearly supports the conclusion that PFDA exposure has multiple and coherent effects on liver histopathology, serum biomarkers and liver weights in exposed animals (primarily rats) that meet the criteria for adversity.

Evidence integration

There is *slight* evidence of an association between PFDA exposure and hepatic effects in humans based on associations with liver biomarkers in the blood in eight studies (see Table 3-5). Positive associations between exposure to PFDA and ALT were observed in four of five studies of adults. However, there is inconsistency in the direction of association within other specific clinical markers and lack of coherence across clinical markers that reduces the strength of the evidence.

The evidence for PFDA-induced liver effects from short-term animal studies via the oral route is considered *moderate* based on coherent effects across multiple endpoints relevant to the assessment of liver toxicity (serum biomarkers, histopathology, and organ weight) (see Figures 3-5, 3-7 and 3-9). Increases in serum biomarkers of hepatocellular/hepatobiliary injury (ALT, AST, ALP, bile salts/acids and bilirubin) (NTP, 2018) and liver weights were reported in male and female S-D rats at ≥0.156 mg/kg-day after 28-day gavage exposure (Frawley et al., 2018; NTP, 2018). In general, the responses were consistent in directionality across sexes and dose groups, exhibiting a clear dose-response gradient. Furthermore, the evidence for increased liver weights was consistent across several species (rats and mice), strains (S-D, Wistar, Fischer F344, C57BL/6N, C57BL/6J and B6C3F1/N) and exposure designs (gavage and dietary) (see synthesis of Organ weight in this Section for more details). At higher doses (≥0.5 mg/kg-day), a consistent pattern of hepatocellular lesions was observed in S-D rats that included cytoplastic alterations and vacuolization, hypertrophy, and necrosis (Frawley et al., 2018; NTP, 2018). The pattern of hepatocellular changes showed a progression in severity within and across lesions with an increase in exposure dose, which adds certainty to the interpretation of the evidence. In combination with the histopathological findings, alterations in serum biomarkers and liver weights support the development of adverse liver effects in rats after continuous PFDA exposure (see section on Considerations for potentially adaptive versus adverse responses above). The evidence base is limited in that there is an absence of studies via relevant exposure routes with durations longer than 28 days (i.e., no subchronic and chronic exposure studies) examining potential hepatic effects of PFDA exposure.

Analysis of mechanistic and supplementary data from in vivo and in vitro rodent models provide experimental (e.g., liver weight changes after i.p. exposure) and biological support for the phenotypic effects reported in the short-term oral studies summarized above. Exposure to PFDA was associated with the activation of several molecular signaling pathways and altered cellular functions hypothesized to be involved in the MOA for liver toxicity of related perfluorinated compounds (see Summary on mechanistic and supplementary studies for PFDA and Appendices B

and C for more details). Additionally, the evidence for PFDA-mediated liver effects implicates both PPARα-dependent and -independent mechanisms.

The activation of PPARα in the MOA for non-cancer liver effects in rodents has implications to human health assessment based on perceived differences in PPARα response between rats/mice versus humans. PFDA can activate the human PPARα in vitro but it exhibits less sensitivity towards the human isoform in comparison to other mammalian species. PFDA also interacts with other nuclear receptors and cell signaling pathways relevant to its potential mechanism of hepatotoxicity in both human and animal models. Furthermore, some hepatic responses in animals occurred, at least in part, independent of PPARα or were found to be activated in human in vitro assays or animal models that are more relevant to humans with respect to PPARα sensitivity (see Summary on mechanistic studies for PFDA and Appendices D.3 and E.1 for more details). These observations are consistent with studies in PPARα null and humanized animals for other long-chain PFAS such as PFOA, PFHxS and PFNA that suggest non-PPARα mechanisms of liver toxicity (see Evidence for other PFAS for more details). Given that the precise role of PPARα in the non-cancer liver effects of PFDA remains largely unknown and the possible involvement of PPARα-dependent and independent pathways, the effects observed in animals are considered potentially relevant to humans (Soldatow et al., 2013).

Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause hepatotoxicity in humans given sufficient exposure conditions[9] (see Table 3-11). This conclusion is based primarily on coherent liver effects in rats (and, to a lesser extent, mice) exposed to doses ≥0.156 mg/kg-day for 28 days. The available mechanistic information overall provides support for the biological plausibility of the phenotypic effects observed in exposed animals as well as the activation of relevant molecular and cellular pathways across human and animal models in support of the human relevance of the animal findings.

[9] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-11. Evidence profile table for PFDA exposure and liver effects

<table>
<tr><th colspan="5">Evidence stream summary and interpretation</th><th>Evidence integration summary judgment</th></tr>
<tr><td colspan="5">Evidence from studies of exposed humans (see Section 3.2.1: Human studies)</td><td rowspan="4">⊕⊕⊙
Evidence indicates (likely)

Primary basis:
Two high confidence studies in rats at ≥0.156 mg/kg-d after short-term exposure

Human relevance:
Effects in rats are considered relevant to humans (see Section 3.2.2: Mechanistic Evidence and Supplemental Information)

Cross-stream coherence:
Alterations in serum liver biomarkers were reported in animals and in a few epidemiological studies, although the latter observations are uncertain.

Susceptible populations and lifestages: None identified, although individuals with pre-existing liver disease could potentially be at greater risk

Other inferences: the MOA for PFDA-induced liver effects is unknown, although the available evidence indicates the involvement of PPARα-dependent and -independent mechanisms</td></tr>
<tr><td>Studies and confidence</td><td>Summary and key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Evidence stream judgment</td></tr>
<tr><td><u>Serum Biomarkers</u>
7 medium confidence studies (5 in adults, 1 in adults and children, 1 in children)</td><td>• 6/7 studies in adults reported positive associations between some clinical liver function markers and PFDA exposure
• 4/5 studies of ALT in adults reported positive associations</td><td>• Exposure-response gradient observed in one study that examined it
• Consistency for ALT</td><td>• Lack of expected coherence across related clinical markers
• Unclear biological significance of small changes in ALT</td><td>⊕⊙⊙
Slight

Evidence of a positive associations between PFDA exposure and ALT in adults, but there is a large degree of uncertainty due to inconsistency among other clinical markers and lack of clear adversity</td></tr>
<tr><td colspan="5">Evidence from in vivo animal studies via the oral route (see Section 3.2.1: Animal studies)</td></tr>
<tr><td>Studies and confidence</td><td>Summary and key findings</td><td>Factors that increase certainty</td><td>Factors that decrease certainty</td><td>Evidence stream judgment</td></tr>
<tr><td><u>Histopathology</u>

2 high and 3 low confidence studies in adult rats or mice

• 7-day dietary
• 12- and 14-day drinking water
• 28-day gavage (2×)</td><td>• Hepatocellular lesions (ranging from cytoplasmic alterations to necrosis) at ≥0.5 mg/kg-d across high confidence studies
• Other liver lesions (i.e., lipid accumulation and edema) were found in low confidence studies at higher</td><td>• Consistency across two high confidence studies
• Coherent pattern of hepatocellular lesions across all studies
• Increased severity (within and across lesions) with increasing exposure</td><td>• No factors noted</td><td>⊕⊕⊙
Moderate

Consistent and coherent changes in serum biomarkers, histopathology, and liver weights, with the strongest evidence in rats at ≥0.156 mg/kg-d although data are limited to short-term studies. Taken together, the coherent changes across markers of hepatic injury were judged</td></tr>
</table>

Evidence stream summary and interpretation					Evidence integration summary judgment
	doses (≥4.6 mg/kg-day)			as adverse (see "Considerations for potentially adaptive versus adverse responses")	
<u>Serum biomarkers</u> 2 *high* confidence studies in adult rats or mice • 12-day drinking water • 28-day gavage	• Increased serum markers of liver and hepatobiliary toxicity at ≥0.156 mg/kg-d	• *Consistency* across *high* confidence studies • *Coherence* across serum markers • *Dose-response* gradient for most effects	• No factors noted		
<u>Organ weight</u> 4 *high*, 4 *medium* and 1 *low* confidence studies in adult rats and mice. • 7–14-day dietary (5×) • 14 day drinking water (1×) • 28-day gavage (2×) • Gestational gavage (1×)	• Increased relative liver weights at ≥0.089 mg/kg-d	• *Consistency* across all studies, including multiple species and both sexes • Dose-response gradient • *Coherence* with serum markers and histopathology	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated** **Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Molecular initiating events— PPARα and other cell signaling pathways	*Key findings and interpretation:* • Evidence of activation of PPARα, CAR/PXR, Nrf2, TNFα, NFκB and JUNK/ATF-2 in rodent hepatic in vivo and/or in vitro models.			Evidence of PPARα-dependent and -independent pathways in studies in rodents and	

Evidence stream summary and interpretation			Evidence integration summary judgment
	• Some evidence of activation of PPARα/γ, PXR and FXR in human liver cells and/or cell-free binding assays • The human FXR was a sensitive target for PFDA in vitro. • Reduced sensitivity towards the human PPARα compared to Baikal seal, polar bear and mouse PPARα isoforms in vitro. *Limitations:* Lack of humanized in vivo models. Some inconsistencies in the vitro results may be due to differences in experimental model and/or design or confounding issues with cytotoxicity.	human in vitro models that support the biological plausibility of PFDA-induced liver effects.	
Cellular effects	*Key findings and interpretation:* • Alterations in hepatic XMEs, oxidative stress, cell and mitochondrial damage, inflammation, and liver metabolic functions in rodents. • PPARα appears to be important for disrupting bile acid homeostasis in mice and associated downstream effects. • Activation of Nrf2 in wildtype and KO PPARα mice and observations of hepatocellular stress, mitochondrial damage and lipid accumulation in animal models known to be less responsive to PPARα activation (i.e., Guinea pigs and/or Syrian hamsters) support involvement of PPARα-independent mechanisms. • PFDA increased ROS production and markers of cellular stress/cytotoxicity in human hepatoma HepG2 cells. *Limitations:* Few studies examining the role of PPARα and other cell signaling pathways and no evidence in humanized vivo models. Inconsistencies in the in vivo results are likely attributable to differences in experimental model and/or design features.		
Organ-level effects	*Key findings and interpretation:* • Increased liver weights in rats, mice (both WT and PPARα-KO animals) and in a rodent species known to be resistant to PPARα activation (i.e., Syrian hamsters). *Limitations:* Lack of evidence examining other organ-level effects, including histological evidence.		

1

3.2.2. IMMUNE EFFECTS

Methodological considerations

Immune-related health effects evaluated from human and animal studies are grouped according to immunotoxicity guidance from the World Health Organization/International Programme on Chemical Safety (WHO/IPCS) and considered for evidence of major categories of immunotoxicity: (1) immunosuppression, (2) immunostimulation, (3) sensitization and allergic response, or (4) autoimmunity and autoimmune disease (IPCS, 2012). Evidence for potential immune effects is considered within these four categories because of common and related mechanisms. Within each category, health effects data are organized and discussed from most to least relevant for drawing hazard conclusions about immunotoxicity (IPCS, 2012). For human data, clinical studies on disease or immune function assays are considered most relevant, then general/observational immune assays (lymphocyte phenotyping or cytokines), and finally endpoints such as hematology (i.e., blood leukocyte counts) are considered least relevant. Similarly, animal data are presented from most to least relevant for immunotoxicity assessment as described by WHO/IPCS as follows: host resistance, immune function assays, general/observational immune assays, blood leukocyte counts and immune organ histopathology and weights (IPCS, 2012). The available human and animal evidence provide relevant information for the assessment of immunosuppression and sensitization or allergic response. However, the available evidence is lacking or inappropriate to specifically address the potential for immunostimulation and autoimmunity following PFDA exposure; therefore, these categories of potential immunotoxicity are not discussed further.

Human studies

Epidemiology studies examining immune effects of PFDA exposure include studies on antibody response, infectious diseases, and hypersensitivity-related outcomes, which includes asthma, allergies, and atopic dermatitis. Outcomes related to immunosuppression were considered within two subcategories: antibody response and infectious disease incidence. Several different outcomes were included in the sensitization and allergic response category. The health effects evidence from human studies is summarized below for each category.

Antibody response outcomes

The production of antigen-specific antibodies in response to an immune challenge (e.g., vaccination in humans or injection with an antigen [e.g., sheep red blood cells] in rodents) is a well-accepted measure of immune function included in risk assessment guidelines and animal testing requirements for immunotoxicity (ICH Expert Working Group, 2005; U.S. EPA, 1998; IPCS, 1996). Antibodies are proteins circulating in blood and other body fluids that bind to antigens and thereby identify them for destruction or removal. The production, release, and increase in

circulating levels of antigen-specific antibodies are important for protection against infectious agents and preventing or reducing severity of influenza, respiratory infection, colds, and other diseases as part of the humoral immune response. Reduced antibody production is an indication of immunosuppression and may result in increased susceptibility to infectious diseases generally (i.e., not limited to those specifically studied).

There are five studies (six publications) that examined PFDA exposure and antibody responses following vaccination for diphtheria or tetanus in children and adults; study evaluations are summarized in Figure 3-10 and Table 3-12. These included three independent prospective birth cohorts in the Faroe Islands, all medium confidence, one with enrollment in 1997-2000 and subsequent follow-up to age 7 (Grandjean et al., 2012) and age 13 (Grandjean et al., 2017a), one with enrollment in 2007–2009 and follow-up to age 5 (Grandjean et al., 2017b), and one with enrollment in 1986–1987 and follow-up to age 28 (Shih et al., 2021). Shih et al. (2021) also examined antibody response to Hepatitis types A and B vaccination. These three cohorts are all separate study populations born in the Faroe Islands and enrolled at different times and thus considered independent of each other. The analyses, in Grandjean et al. (2017b) combined new data from the cohort born in 2007–2009 with new follow-up data from the cohort born in 1997–2000 (Grandjean et al., 2012), which are labeled in the results table. There was also a cross-sectional study of children in Greenland (Timmermann et al., 2021). These studies were generally well conducted, but exposure contrast was a concern in most of them, with median exposure levels around 0.3 ng/mL and interquartile ranges around 0.2 ng/mL (exposure contrast was slightly better in (Timmermann et al., 2021)). Potential for confounding across PFAS was considered in individual studies evaluations as well as across studies in evidence synthesis (see below). In addition to these developmental exposure studies, there was one study in healthy adult volunteers in Denmark considered low confidence because of limited information provided on recruitment of study subjects, lack of consideration of confounders, and a small study population (12 individuals) leading to concerns with potential selection bias, confounding and low sensitivity (Kielsen et al., 2016).

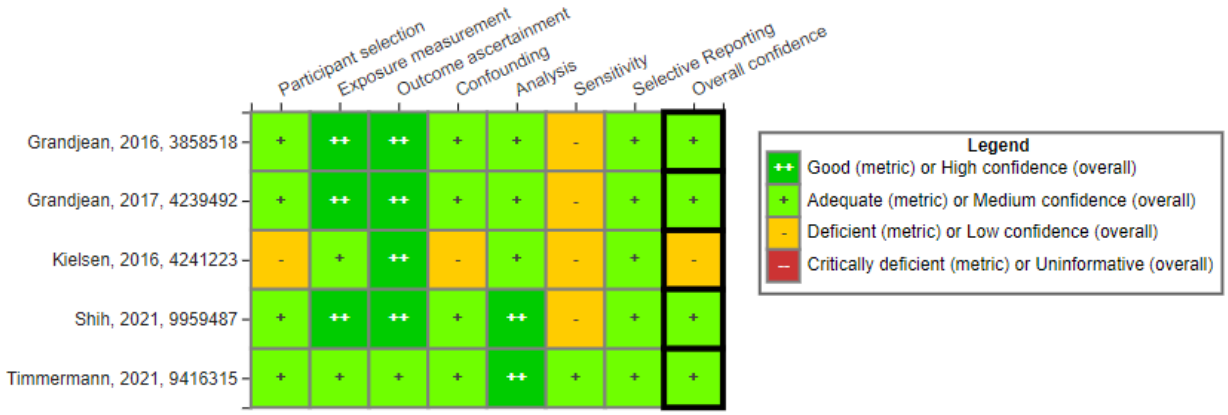


Figure 3-10. Summary of evaluation of epidemiology studies of PFDA and antibody response to vaccination. Refer to HAWC for details on the study evaluation review: HAWC Human Vaccine Response Effects.

Domain and overall confidence ratings may vary by outcome; outcome-specific ratings and rationales are available in HAWC and described in the relevant sections below. Multiple publications of the same study: Grandjean et al. (2017a) also represents Grandjean et al. (2012).

The two prospective birth cohorts in the Faroe Islands with antibody levels measured during childhood reported inverse associations between higher concentrations of serum PFDA and lower anti-vaccine antibody levels for diphtheria and tetanus (see Table 3-12). Although results were not always statistically significant, the general trend towards lower antibody levels was apparent. Antibody levels were measured in individuals of several age groups (and therefore different lengths of time since their initial vaccination or booster vaccination) and compared to serum PFDA concentrations also measured at different ages. Although results were not always statistically significant, inverse associations were observed in most (but not all) of these comparisons. No biological rationale is understood as to whether one time period is more predictive of an overall immune response and given the long half-life of PFDA (approximately 4.5–12 years), there is reasonably high correlations across time periods (Grandjean et al., 2017a). Antibodies to diphtheria decreased with increasing PFDA concentrations in 11 of the 13 exposure and outcome measurement timing combinations assessed. One of the two results that did not support the trend was a statistically significant increase in diphtheria antibodies in children at 5 years of age (before receiving the 5-year booster) associated with increases in PFDA concentrations at 18 months of age. This increase appears to be a response in this specific exposure and outcome timing combination in the 2007–2009 cohort as there was an increase with all PFAS measured at 18 months and outcome measured at 5 years of age in the 2007–2009 cohort. However, the 1997–2000 cohort from the same population and all other exposure and outcome timing combinations, including in the 2007–2009 cohort when exposure was measured at birth, resulted in a decrease of diphtheria antibodies (Grandjean et al., 2017b). There is no clear explanation for the discrepant findings for this specific exposure and outcome timing combination

in the 2007–2009 cohort. The only other result that did not show a decrease in diphtheria antibodies was among 7-year-olds based on maternal PFDA concentration Grandjean et al. (2012). However, because a decrease in diphtheria antibodies was observed within 7-year-olds when PFDA concentrations were measured at age 5, the lack of effect may be explained by differences in the long-term influence of the maternal exposure measurement.

Similar to the diphtheria results, tetanus antibodies had a decreasing trend with increasing PFDA concentrations with few exceptions (10 of the 13 combinations indicated decreased antibody levels). One of the exceptions is a statistically significant increase in tetanus antibodies in 7-year-olds with increasing maternal PFDA concentrations (similar to the discrepancy observed for diphtheria for a similar exposure-outcome combination). Tetanus antibody levels at 13 years of age were also increased with increasing PFDA concentrations measured in the children at ages 7 and 13 years of age (Grandjean et al., 2017a). This may indicate that by 13 years of age, the effect of maternal and childhood exposure is less relevant to tetanus antibody levels.

The other two studies of developmental exposure and antibody response to vaccination reported less consistent findings. The cross-sectional results in Timmermann et al. (2021) differed in direction of association based on the covariate set selected (with or without adjustment for area of residence). The exposure measurement in these analyses may not have represented an etiologically relevant window; cross-sectional analyses in the Faroe Islands studies at similar ages also found weaker associations than analyses for some other exposure windows. However, a subset of the study population did have maternal samples available, and those results were also inconsistent by vaccine. On the other hand, this study was the only one to examine the odds ratio for not being protected against diphtheria (antibody concentrations <0.1 IU/mL), which has clear clinical significance, and they reported an OR of 5.08 (95% 1.32, 19.51) among children with known vaccination records (adjusted for area of residence, consistent with continuous antibody results). Shih et al. (2021), which examined antibody levels at age 28 with exposure measures at multiple time points, reported inconsistent associations across exposure windows and vaccines. Results also differed by sex, but without a consistent direction (i.e., stronger associations were sometimes observed in women and sometimes men). Results were similarly inconsistent for antibodies to Hepatitis A and B (not shown). Similar to the results in 13 year-olds in the other Faroe Islands cohorts, this may indicate that by age 28, the effect of developmental exposure is less relevant. Lastly, one low confidence study examined exposure to PFDA in adulthood and found inverse associations with antibodies to both diphtheria and tetanus (statistically significant for diphtheria) (Kielsen et al., 2016).

It is plausible that the observed associations with PFDA exposure could be explained by confounding across the PFAS. Exposure levels to other PFAS in the Faroe Islands populations were considerably higher (PFOS 17 ng/mL, PFOA 4 ng/mL, PFNA 1 ng/mL, PFDA 0.3 ng/mL at age 5 years in Grandjean et al. (2012), and there was a high correlation between PFDA and PFNA (r = 0.78) and moderate correlations with PFOS and PFOA (r = 0.39 and 0.35, respectively). The authors

assessed the possibility of confounding in a follow-up paper (Budtz-Jørgensen and Grandjean, 2018a) that reanalyzed data from both Grandjean et al. (2012) and (Grandjean et al., 2017b) for benchmark analysis. In this re-analysis, estimates were adjusted for PFOS and PFOA. There were variable attenuation of the observed effect estimates across the different analyses (though some of the adjusted estimates were not estimable, likely due to collinearity), and PFNA was not adjusted for in these models. However, associations with PFDA were stronger than for PFNA, and adjustment by PFOS and PFOA did not eliminate the association, so confounding by co-occurring PFAS is unlikely to fully explain the associations. Overall, while it is not possible to rule out confounding across PFAS, the available evidence suggests that it is unlikely to explain the observed effects. Other sources of potential confounding, including possible co-exposures such as PCBs, were controlled appropriately.

Overall, in the two birth cohorts examining effect in children in the Faroe Islands, of the 26 paired antibody-to-PFDA exposure evaluations of diphtheria and tetanus antibody responses, 21 of them support a decrease in antibodies with increasing PFDA concentration (see Table 3-12). Although the results were not always statistically significant, the decreases were generally large, with decreases in antibody concentration ranging from 2–25% per doubling of PFDA concentration. The variability in some of the results could be related to differences in etiological relevance of exposure measurement timing, differences in timing of the boosters since this was uncontrolled by the study (children were vaccinated according to the official Danish/Faroese vaccination program), as well as differences in timing of antibody measurements in relation to the last booster and PFDA exposure measurement. In addition, a cross-sectional study of children in Greenland reported a large odds ratio for lack of protection against diphtheria following vaccination (Timmermann et al., 2021), and similar decreases in both diphtheria and tetanus antibodies were also observed in a very small study in adults (n = 12) from Denmark based on a reduced change in antibodies after a booster shot (Kielsen et al., 2016). These associations were observed despite poor sensitivity resulting from narrow exposure contrasts in all three studies, which increases confidence in the association. There is some remaining uncertainty resulting from variability in the response by age of exposure and outcome measures as well as vaccination (initial and boosters) in the Faroe Islands childhood cohorts, and due to potential for confounding across PFAS. There is also uncertainty due to inconsistent results in Timmermann et al. (2021) as well as a birth cohort with follow-up to young adulthood in the Faroe Islands (Shih et al., 2021). However, the findings in children in the Faroe Islands are based on both outcome measurement in childhood and prospective exposure measurement, and the inconsistency may conceivably be attributed to these differences.

Table 3-12. Summary of PFDA exposure and selected data on antibody response in humans

Reference, N, confidence	PFDA exposure timing and	Outcome measure timing	Diphtheria vaccine (% Change in antibodies with increase in PFDA[b])	Tetanus vaccine

	concentration in ng/mL[a]			(% Change in antibodies with increase in PFDA[b])
Grandjean et al. (2012), Faroe Islands, N = 380–537, medium Grandjean et al. (2017a) Faroe Islands, 1997–2000 cohort	Maternal; mean (IQR): 0.3 (0.2–0.4)	Children (age 5), prebooster	**−21.7 (−35.7, −4.8)**	−2.5 (−18.5, 16.8)
		Children (age 5), postbooster	**−18.8 (−30.5, −5.0)**	−6.1 (−23.5, 15.3)
		Children (age 7)	0.7 (−18.2, 24.0)	16.4 (−6.7, 45.2)
	Children (age 5); mean (IQR): 0.3 (0.2–0.4)	Children (age 5), prebooster	−16.0 (−29.6, 0.3)	−13.6 (−26.3, 1.4)
		Children (age 5), postbooster	−8.7 (−20.6, 5.0)	**−19.9 (−33.1, −3.9)**
		Children (age 7)	−14.4 (−28.4, 2.4)	**−22.3 (−35.8, −5.8)**
	Children (age 13); mean (IQR): 0.3 (0.2–0.4)	Children (age 13)	−3.7 (−22.0, 18.9)	18.7 (−11.8, 59.8)
Grandjean et al. (2017b), Faroe Islands, N = 349, medium 2007–2009 cohort (unless specified)	At birth, not reported	Children (age 5), prebooster	−3.54 (−23.19, 21.15)	−8.40 (−26.27, 13.79)
	Infant (18 months); median (25th–75th percentile): 0.3 (0.2–0.4)	Children (age 5), prebooster	2007–2009 cohort **25.52 (2.00, 54.48)** 1997–2000 cohort −22.87 (−60.92, 52.24)	2007–2009 cohort −5.78 (−23.56, 16.13) 1997–2000 cohort −14.47 (−56.88, 69.66)
	Children (age 5); median (25th–75th percentile): 0.3 (0.2–0.5) ng/mL	Children (age 5), prebooster	−8.99 (−23.63, 8.46)	−1.76 (−16.73, 15.91)
Shih et al. (2021), Faroe Islands, N = 281, medium	Cord blood; median (IQR) 0.07 (0.06)	Adults (age 28)	Total: 7.29 (-11.2, 29.6) Women: -1.39 (-24.8, 29.2) Men: 16.16 (-10.6, 51.0)	Total: -12.9 (-25.0, 1.2) Women: -17.0 (-33.0, 3.0) Men: -8.8 (-26.0, 12.4)
	Children (age 7); 0.22 (0.16)		**Total: 37.89 (1.8, 86.8)** Women: 30.99 (-16.5, 105.4) Men: 43.8 (-4.4, 116.3)	Total: 3.2 (-18.5, 30.7) Women: -2.6 (-31.3, 38.0) Men: 8.3 (-21.1, 48.7)
	Children (age 14); 0.28 (0.17)		Total: -7.2 (-35.1, 32.7) Women: 39.4 (-28.7, 172.8) Men: -20.5 (-47.4, 20.3)	Total: -22.6 (-42.9, 4.9) Women: -41.0 (-66.6, 4.1) Men: -14.3 (-39.7, 21.9)
	Adults (age 22); 0.39 (0.26)		**Total: 34.9 (4.9, 73.6)*** **Women: 39.0 (2.2, 89.0)*** Men: 27.2 (-17.6, 96.3)	Total: -5.2 (-22.9, 16.4) Women: -4.0 (-25.4, 23.5) Men: -7.6 (-35.1, 31.6)
	Adults (age 28); 0.34 (0.25)		Total: 19.6 (-1.2, 44.9) Women: 24.7 (-2.9, 60.0) Men: 12.8 (-16.2, 51.8)	Total: -5.3 (-18.7, 10.3) Women: -1.9 (-19.6, 19.8) Men: -9.9 (-28.8, 14.2)
Timmerman n et al. (2021),	Children (age 7–12)	Children (age 7–12)	Adjusted for time since vaccine booster, breastfeeding duration 126 (32, 289)	Adjusted for time since vaccine booster, breastfeeding duration 74 (12, 169)

Greenland, N = 314, medium			Additionally adjusted for area of residence -39 (-70, 27)	Additionally adjusted for area of residence -29 (-61, 28)
	Maternal (N = 57)	Children (age 7–12)	-39 (-84, 133)	95 (-45, 591)
Kielsen et al. (2016), Denmark N = 12, low	Adult (10 days post vaccination); median (IQR): 0.3 (0.2–0.3) ng/mL	Adult – change from 4 days to 10 days post vaccination	**−18.18 (−29.52, −5.00)**	−8.31 (−18.10, 2.66)

[a]Exposure timing is organized into groups based on maternal exposure, childhood exposure (including from birth through age 13), and adult exposure.
[b]Linear regression (β or % change in antibody per 2-fold increase of PFDA). Numbers in parentheses are 95% confidence intervals.
Bold font indicates ***p* < 0.05**.

Infectious disease

Direct measures of infectious disease incidence or severity such as respiratory tract infections, pneumonia or otitis media are useful for evaluating potential immunotoxicity in humans. Increases in incidence or severity of infectious disease can be a direct consequence of impaired immune function whether the specific functional deficit has been identified or not. Given the clear adversity of most infectious diseases, they are generally considered good measures for how immunosuppression can affect individuals and communities. Physician diagnosis is the most specific way to assess infectious diseases, but these are usually only available for severe diseases and are less likely for diseases where treatment is not sought. Self-reported incidence or severity of disease may be less reliable but may be the only way to assess diseases such as the common cold or gastroenteritis which while less adverse, are more common and can thus provide information about immunosuppression and susceptibility to more severe infections. In general, symptoms of infection alone are not considered reliable measures of disease because of their lack of specificity. Antibody levels in response to infection are also included in this section (differentiated from antibody levels in response to vaccination, described above); the utility of these measures depends on the study design and population due to various factors such as potential confounding and prevalence of infection.

Six studies examined PFDA exposure and infectious disease outcomes in children and one study examined disease severity in adults (see Figure 3-11). Three of these focused on the number of episodes of infectious disease. One was a medium confidence prospective birth cohort study in Japan which looked at the association of PFDA exposure with total infectious disease (including otitis media, pneumonia, RS virus, and varicella) from birth to age 4 (Goudarzi et al., 2017), with outcomes ascertained using a questionnaire identifying physician diagnosed disease incidents. A second medium confidence birth cohort in China identified cases of common cold or bronchitis/pneumonia reported by parents with verification with medical records (Wang et al., 2022). A low confidence cohort with PFDA exposure measured in childhood examined number of episodes of parent-reported lower respiratory tract infections and common colds based on parent

reports using an unvalidated questionnaire (Kvalem et al., 2020). Another prospective birth cohort in examined days of infectious disease symptoms (fever, diarrhea, coughing, nasal discharge, vomiting) with follow-up at 1–4 years (Dalsager et al., 2016). This study was considered low confidence due to the non-specific nature of the symptoms reported, which may not represent infectious disease. In the same birth cohort in Denmark, but with a larger sample size, hospitalizations due to infectious disease were identified from a national registry (Dalsager et al., 2021a). These two studies were evaluated separately due to their different samples and outcomes measurement methods but should not be considered fully independent samples. Also in children, one study examined antibody response to hand, foot, and mouth disease (HFMD) infection. This birth cohort in China (Zeng et al., 2019b) measured antibody levels in infants at birth and 3 months of age, a time-period expected to reflect passive immunity from maternal antibodies. This study is low confidence because the outcome is broad and difficult to interpret and there are concerns for confounding by timing of HFMD infection as well as other limitations. Lastly, one study examined severity of COVID-19 illness in Denmark using biobank samples and national registry data (Grandjean et al., 2020). There was concern for selection bias in this study due to the expectation that biobank samples were more likely to be available for individuals with chronic health concerns. In addition, severity of COVID-19 is not a direct measure of immune suppression as other factors may contribute to illness severity.

The results for this set of studies are summarized in Table 3-13. Results were overall inconsistent. Positive associations (though mostly not statistically significant) between PFDA exposure and specific infectious diseases were observed in some studies (diarrhea, common cold, and lower respiratory infection in Wang et al. (2022), lower respiratory infections in Kvalem et al. (2020), upper respiratory tract infections in Dalsager et al. (2021a), fever in Dalsager et al. (2016)), but inverse associations were observed in other studies. Where two studies were available for a given infectious disease, the results were generally not in the same direction. The single study of HFMD antibodies reported lower levels of protective antibody concentrations with higher PFDA exposure and higher odds of having antibody levels below a clinically protective level (Zeng et al., 2019b). Exposure contrast was limited across studies which makes it difficult to interpret the null findings. Associations were slightly stronger in Wang et al. (2022), the only medium confidence study with adequate sensitivity (due to slightly higher exposure levels and contrast), but this likely does not fully explain the inconsistency in direction of association across studies.

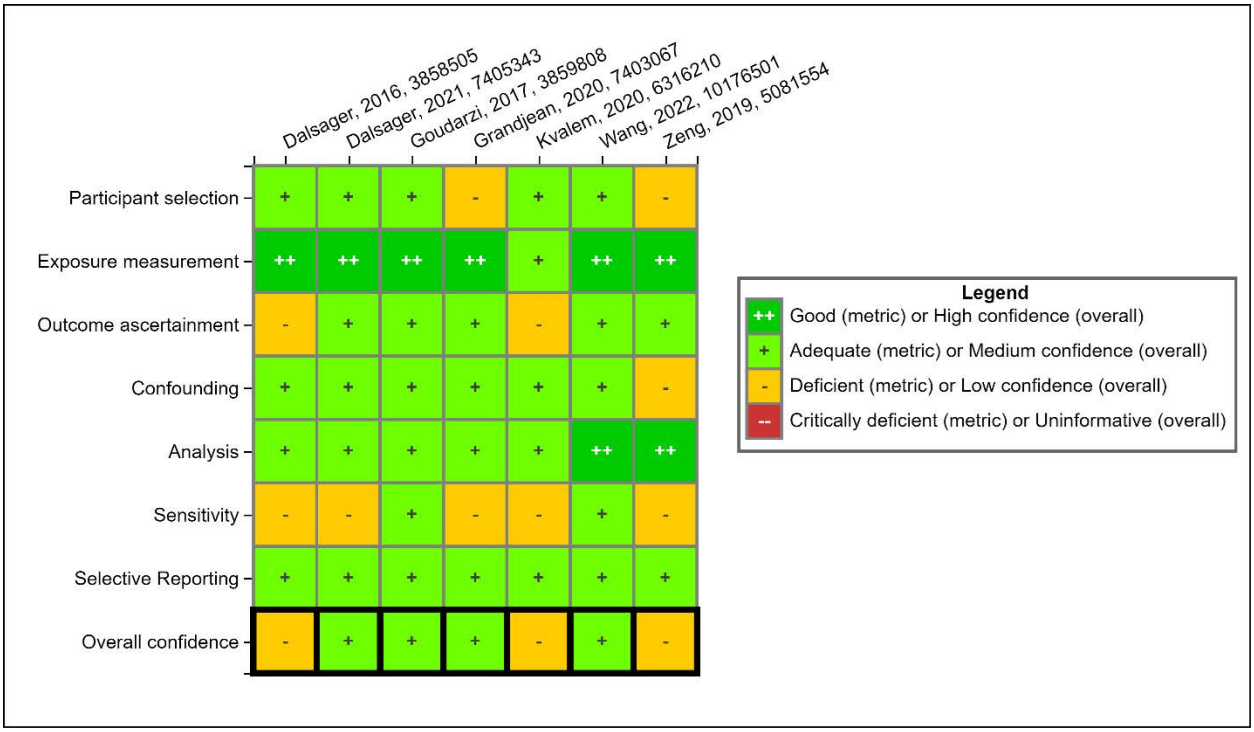


Figure 3-11. Summary of evaluation of epidemiology studies of PFDA and infectious disease. Refer to HAWC for details on the study evaluation review: HAWC Human Infectious Disease Effects.

Table 3-13. Studies on PFDA and infectious disease in humans

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA Results
Total infectious disease[a]	Goudarzi et al. (2017) medium	Maternal; median (IQR): 0.3 (0.2–0.4) ng/mL	From birth to age 4	OR (95% CI): Q1: Ref Q2 1.00 (0.73, 1.35) Q3 0.89 (0.66, 1.21) Q4 0.80 (0.59, 1.08)
	Dalsager et al. (2021a), medium	Maternal; median: 0.3	From birth to age 4	HR (95% CI) 1.06 (0.93, 1.22)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA Results
Lower respiratory tract infection[b]	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1	OR (95% CI) for event during first year of life per log10 increase: 1.84 (0.36, 9.49) IRR (95% CI) for count of events per log10 increase: 0.85 (0.26, 2.79)
	Dalsager et al. (2021a), medium	Maternal; median (IQR): 0.6 (0.4–0.8)	From birth to age 4	HR (95% CI) 1.06 (0.85, 1.32)
	Kvalem et al. (2020) medium	Child age 10; median (IQR): 1.3 (0.9)	Age 10-16	RR (95% CI) per IQR increase 1.09 (0.86, 1.39)
			Age 16 (last 12 months)	1.34 (0.84, 2.14)
Diarrhea	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1-3	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 0.91 (0.53, 1.56) High: 0.91 (0.52, 1.57)
	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1	OR (95% CI) for event during first year of life per log10 increase: 3.36 (0.90, 12.63) IRR (95% CI) for count of events per log10 increase: **2.16 (1.23, 3.79)***

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA Results
	Dalsager et al. (2021a), medium	Maternal; median (IQR): 0.6 (0.4–0.8)	From birth to age 4	HR (95% CI) for GI 0.81 (0.46, 1.43)
Common cold (No. episodes/ frequency)	Wang et al. (2022), medium	Maternal; median (IQR): 0.6 (0.4–0.8)	Age 1	OR (95% CI) for event during first year of life per log10 increase: 1.66 (0.48, 5.75) IRR (95% CI) for count of events per log10 increase: 1.05 (0.65, 1.68)
	Dalsager et al. (2021a), medium	Maternal; median (IQR): 0.6 (0.4-0.8)	From birth to age 4	HR (95%) for upper respiratory tract infection 1.16 (0.95, 1.42)
	Kvalem et al. (2020), medium	Child age 10; median (IQR): 1.3 (0.9)	Age 10-16	OR (95% CI) per IQR increase: Reference 1–2 colds 3-5 colds: 1.69 (0.46, 6.18) >5: 1.36 (0.39, 4.80)
			Age 16 (last 12 months)	Reference 0 colds 1–2 colds: 0.78 (0.55, 1.09) **≥3: 0.56 (0.37, 0.84)***
Cough	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1-3	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 0.63 (0.37, 1.07) High: 0.85 (0.50, 1.46)

Disease	Reference, confidence	Exposure measurement timing and concentration	Disease assessment timing	PFDA Results
Fever	Dalsager et al. (2016) low	Maternal; median (range): 0.3 (0.02–1.0) ng/mL	Age 1-3	OR (95% CI) for proportion of days with symptoms Low exposure: Ref Medium: 1.07 (0.63, 1.81) High: 1.45 (0.85, 2.49)
Hand Foot and Mouth Disease Virus Antibodies	Zeng et al. (2019b), low	Cord; median (IQR): 0.1 (0.01–0.2)	Birth and Age 3 mo	OR (95% CI) for HFMD antibody concentration below clinically protective level Cord blood: 1.19 (0.82, 1.71) **3 mo: 2.22 (1.42, 3.47)***
COVID-19 severity	Grandjean et al. (2020), medium	Biobank prior to illness; median (IQR): 0.1 (0.1–0.2)	Adulthood	OR (95% CI) for 1 unit increase Increased severity based on hospitalization, admission to intensive care and/or death 0.53 (0.10, 2.84)

Bolded values are statistically significant. ***p < 0.05**.
[a]Includes Otitis media, pneumonia, RS virus, Varicella.
[b]Lower respiratory tract infections include bronchitis, bronchiolitis, and pneumonia.

Sensitization and allergic response

Another major category of immune response is the evaluation of sensitization-related- or allergic responses that are a result of aggravated immune reactions (e.g., allergies or allergic asthma) to foreign agents (IPCS, 2012). A chemical may be either a direct sensitizer (i.e., promote a specific IgE-mediated immune response to the chemical itself) or may promote or exacerbate a hypersensitivity-related outcome without evoking a direct response. Hypersensitivity responses occur in two phases. The first phase, sensitization, is without symptoms, and it is during this step that a specific interaction is developed with the sensitizing agent so that the immune system is prepared to react to the next exposure. Once an individual or animal has been sensitized, contact with that same (or, in some cases, a similar) agent leads to the second phase, elicitation, and symptoms of allergic disease. While these responses are mediated by circulating factors such as T-cells, IgE, and inflammatory cytokines, there are many health effects associated with hypersensitivity and allergic response. Functional measures of sensitivity and allergic response consist of measurements of health effects such as allergies or asthma, and skin prick test responses. Observational tests such as measures of total IgE levels measure indicators of sensitivity and allergic responses but are not a direct measurement of the response. The section is organized by the different types of measurements, starting with functional measures as the most informative.

Seven cohorts (10 publications) examined hypersensitivity outcomes in children. Study evaluations are summarized in Figure 3-12 and Table 3-14. Study sensitivity was a concern across most of the studies, due to narrow exposure contrasts which makes interpretation of the null findings difficult.

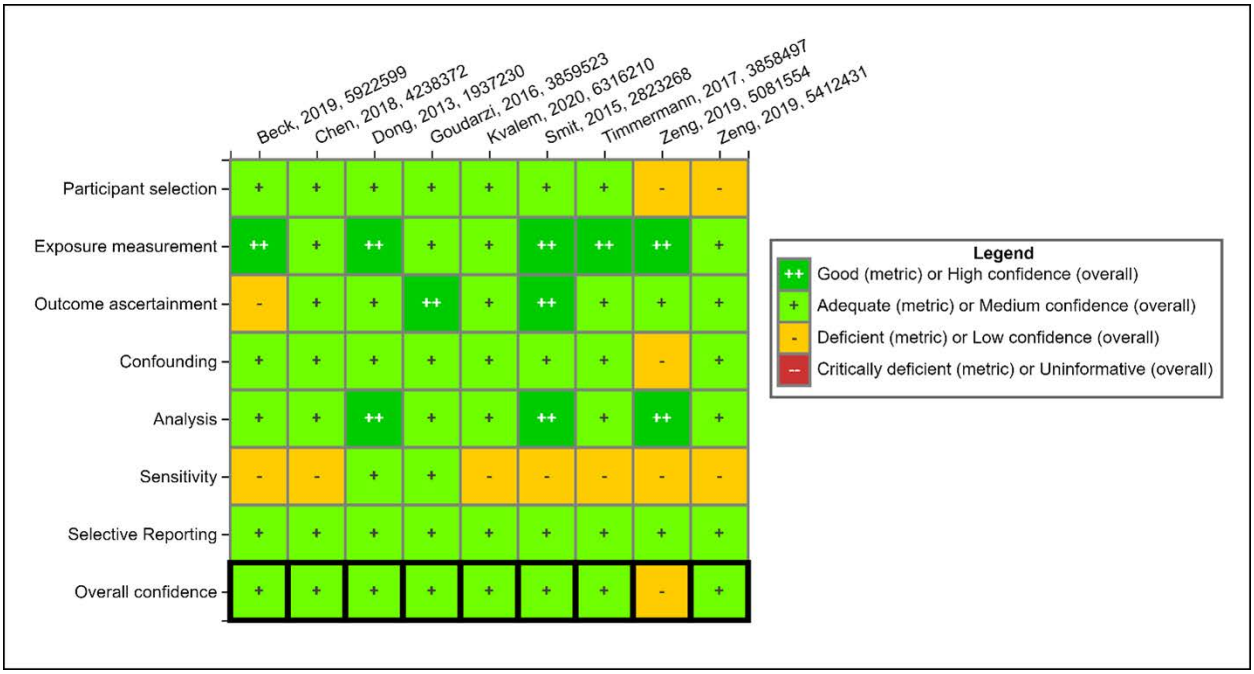


Figure 3-12. Summary of evaluation of epidemiology studies of PFDA and sensitization or allergic response. Refer to HAWC for details on the study evaluation review: HAWC Human Hypersensitivity Effects.

Functional immune measures of sensitization or allergic response

Asthma

Six studies (eight publications) evaluated any asthma-related outcome in relation to PFDA exposure. One case-control study in Taiwan examined asthma incidence (i.e., physician diagnosis within the past year, identified from two hospitals), which is the most specific measure but may result in under-ascertainment; this study was considered medium confidence (Zhou et al., 2017b; Zhu et al., 2016; Dong et al., 2013). Most available studies examined asthma prevalence (ever diagnosed asthma) and were also considered medium confidence including four birth cohorts with prenatal or cord PFDA blood measurements (Beck et al., 2019; Zeng et al., 2019a; Timmermann et al., 2017; Smit et al., 2015) and one study with PFDA exposure measured in childhood (Kvalem et al., 2020).

Positive associations with asthma were observed in Dong et al. (2013) and Timmermann et al. (2017) (see Table 3-15), including an exposure-response gradient observed in Dong et al. (2013). However, in Timmermann et al. (2017), the association was observed only in a small

number of subjects (4%, n = 22) that did not receive an MMR vaccine; the effects were statistically significant when both the outcome and PFDA exposure were evaluated when the children were 5 years of age. There remained an increased risk for asthma diagnosis when these same children were 13 years old. No association with childhood exposure was observed in the rest of the study population (that received MMR vaccine), but a positive association was suggested ($p > 0.05$) when using maternal PFDA concentrations as an indication of prenatal exposure (Timmermann et al., 2017). The Taiwan case-control study used the child's current PFDA concentrations and observed increased odds ratios in the highest quartile compared to the lowest quartile (concentrations not reported for the quartiles) and in boys and girls with low or high testosterone or high estradiol as well as in boys with low estradiol, indicating there was a modifying effect of sex hormones (Zhou et al., 2017b; Zhu et al., 2016; Dong et al., 2013). Associations were stronger in boys than girls. Dong et al. (2013) also observed a significant increase in asthma severity scores based on a 13-item questionnaire assessing frequency, use of medicine, and hospitalizations in the highest quartile with a significant increasing trend, but there was no difference in the asthma control test (five-item questionnaire assessing control of asthma symptoms). The other four studies study (Kvalem et al., 2020; Beck et al., 2019; Zeng et al., 2019a; Smit et al., 2015) reported no increase in asthma with PFDA exposure. The inconsistency may be accounted for at least in part by study sensitivity, as the Taiwan study with a clear association (Dong et al., 2013) had the highest PFDA exposure levels and was based on asthma incidence within the past year, a more specific definition, less likely to suffer from outcome misclassification, than whether the child ever had asthma ("ever asthma"). Still, overall, there is considerable uncertainty due to the lack of association with asthma in most studies.

Dermal allergic measures – eczema

Four *medium* confidence birth cohorts from different geographic locations in five publications (Chen et al., 2018a; Timmermann et al., 2017; Goudarzi et al., 2016; Smit et al., 2015; Okada et al., 2014) and one study with exposure measured in childhood (Kvalem et al., 2020) evaluated dermal allergic measures. While the studies used different terminology including eczema, atopic eczema, and atopic dermatitis, all assessed presence of an itchy rash that was coming and going for at least 6 months using the International Study of Asthma and Allergies in Childhood questionnaire with the exception of Kvalem et al. (2020) which used a different questionnaire. The dermal response conditions can represent hypersensitivity to antigen exposure by way of any exposure route. None of the studies found a significant association between PFDA exposure (either prior exposure, based on maternal or the child's earlier PFDA measurement, or current exposure) and dermal allergic effects (see Table 3-15). However, a non-statistically significant positive association for eczema was observed in Chen et al. (2018a) and for the children without MMR vaccine in Timmermann et al. (2017). An inverse association ($p > 0.05$) was observed in multiple studies (Kvalem et al., 2020; Timmermann et al., 2017; Smit et al., 2015; Okada et al.,

2014) (in children with MMR vaccine for Mamsen et al. (2017)). This inconsistency is not clearly explained by study confidence or other factors.

Allergic sensitization/ Skin prick test

Two *medium* confidence studies conducted skin prick tests. In Timmermann et al. (2017), they examined five common allergens (birch/grass pollen, dog/cat dander, and house dust mites) in 13-year old children from the Faroe Islands. A positive result was noted if the subjects developed a wheal ≥3 mm in diameter. In Kvalem et al. (2020), a positive result was noted if there was at least one positive test ≥3 mm at 10 and 16 years but the allergens tested were not described. The relative risk of a positive test was slightly higher ($p > 0.05$) with PFDA exposure in Kvalem et al. (2020) but there was no increase in the odds of having a positive test related to PFDA exposure regardless of when the PFDA was evaluated (i.e., maternal, child at 5 years of age, or current measurement at 13 years of age) in In Timmermann et al. (2017). Both studies had similar exposure contrast.

Observational immune measures of sensitization or allergic response

Two studies also analyzed observational measures including total IgE, eosinophil counts, or eosinophil cationic protein (Timmermann et al., 2017; Zhu et al., 2016; Dong et al., 2013); of these, IgE measures are considered the most informative. Dong et al. (2013) observed a statistically significant increase in total IgE, eosinophilic cationic protein concentration, and absolute eosinophilic count with increasing current child PFDA concentrations in asthmatics, as well as increased eosinophilic cationic protein concentrations in non-asthmatics in a population in Taiwan. In the same medium confidence study, Zhu et al. (2016) evaluated this further and found that the positive association with IgE was observed in boys and girls with asthma, but only statistically significant in boys. Zhu et al. (2016) expanded the evaluation to additional cytokines (IFN-γ, IL-2, IL-4, and IL-5) in subjects with and without asthma. While there were occasional statistically significant decreases in the lower quartiles compared to quartile 1 (i.e., IL-2 in males and IFN-γ in females) there was no consistency or trend. In the second medium confidence study, Timmermann et al. (2017) did not find any significant association between IgE levels in cord blood or blood samples from children at age 7 and PFDA concentrations (either maternal concentrations or child's concentration at age 5) in children from the Faroe Islands.

Table 3-14. Studies on PFDA and hypersensitivity-related outcomes in humans

Reference	Study design (location/study)	n	Exposure measure timing	Disease assessment timing	Hypersensitivity outcomes assessed	Study confidence
Maternal exposure						
Beck et al. (2019)	Prospective (Denmark birth cohort)	981	Maternal	Age 5	Asthma (ever)	*Medium*
Chen et al. (2018a) Zeng et al. (2019a)	Prospective (Shanghai Birth Cohort)	687	Cord blood (log transformed)	Age 2	Eczema	*Medium*
		358		Age 5	Asthma (ever)	
Goudarzi et al. (2016)	Prospective (Japan/Hokkaido Study of Environment and Children's Health cohort 2003–2013)	1,558	Maternal (quartiles)	Age 4	Total allergic disease, wheeze, eczema, rhinoconjunctivitis symptoms	*Medium*[a]
Okada et al. (2014)		2,062	Maternal (quartiles)	From birth to age 2	Wheeze, allergic rhinoconjunctivitis symptoms, eczema, total allergic diseases	
Smit et al. (2015)	Prospective (Greenland, Ukraine/ INUENDO birth cohort)	1,024	Maternal (log transformed)	Children age 5–9	Asthma (ever), eczema, wheeze	*Medium*
Timmermann et al. (2017)	Prospective (Faroe Island cohort; 1997–2000)	559	Maternal; child age 5, 13 (log transformed)	Age 5, 7, 13	Total IgE, asthma (ever), allergies, allergic rhinoconjunctivitis, eczema, skin prick test	*Medium* (*low* for asthma)
Child exposure						
Zhou et al. (2017b); Zhu et al. (2016); Dong et al. (2013)	Case-control (Taiwan/ Genetic and Biomarker study for Childhood Asthma)	asthma (231) non (225)	Child: current (quartiles)	Children age 10–15	Asthma incidence and control, total IgE, eosinophil count, eosinophil cationic protein	*Medium*
Kvalem et al. (2020)	Prospective (Norway Environment and Child Asthma)	378	Child: 10 years	Age 10 and 16	Asthma (ever/current), Eczema, skin prick test	*Medium*

[a]Medium vs. high confidence based primarily on sensitivity.

Table 3-15. Summary of PFDA and selected data on hypersensitivity in humans

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Asthma			
Smit et al. (2015)	Maternal, mean gest wk 24 or 25; geometric mean (5–95th percentile): Ukraine 0.16 (0.07–0.35) ng/mL, Greenland 0.42 (0.16–1.16) ng/mL	Child (age 5–9)	Ever asthma Ukraine: 0.80 (0.37, 1.75) per 1 SD change Greenland: 0.93 (0.73, 1.19) per 1 SD change Combined: 0.92 (0.73, 1.16) per 1 SD change
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10)	Ever asthma RR: 0.95 (0.78, 1.15)
		Child (age 10–16)	Asthma between 10 and 16 years RR: 0.89 (0.67–1.16)
		Child (age 16)	Current asthma (last 12 months) RR: 0.93 (0.71, 1.22)
Timmermann et al. (2017)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 5)	Ever asthma 1.09 (0.72, 1.65)
		Child (age 13)	1.26 (0.83, 1.92)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 5)	Ever asthma **No MMR: 4.04 (1.05, 15.50)**[c] Yes MMR: 0.71 (0.48, 1.06), **Interaction p = 0.02**
		Child (age 13)	No MMR: 2.87 (0.84, 9.79) Yes MMR: 0.71 (0.48, 1.06), **Interaction p = 0.03**
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	Ever asthma 0.84 (0.55, 1.29)
Beck et al. (2019)	Maternal, gest week 8–16; median (IQR): 0.3 (0.2–0.4) ng/mL	Child (age 5)	Ever doctor-diagnosed asthma 0.9 (0.60, 1.44) Ever self-reported asthma (≥episodes of wheezing lasting more than a day in past 12 months) 1.44 (0.87, 2.41)
Zeng et al. (2019a)	Cord blood median (IQR): 0.4 (0.2–0.5)	Child (age 5)	Ever asthma 0.63 (0.23, 1.72) Girls: 0.21 (0.03, 1.47) Boys: 1.09 (0.26, 4.50)
Dong et al. (2013)	Children, current; range: <0.1–5.0 ng/mL	Child (age 10–15)	Asthma incidence Q2: 1.02 (0.58, 1.80) Q3: 1.30 (0.72, 2.33) Q4: 3.22 (1.75, 5.94), *p*-trend < 0.001

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Zhou et al. (2017b)	Children, current; median (IQR): 1.1 (0.9–1.5) ng/mL with asthma, 1.0 (0.8–1.2) ng/nL without asthma	Child (age 10–15)	Asthma incidence Low Testosterone: M: 1.71 (0.75, 3.90); F: 1.24 (0.60, 2.56) High Testosterone: **M: 3.16 (1.21, 8.25)**; F: 1.37 (0.63, 3.02) Low Estradiol: M: 1.21 (0.60, 2.46); F: 0.76 (0.27, 2.20) High Estradiol: **M: 4.01 (1.46, 11.06); F:** 1.78 (0.94, 3.35) No significant interaction with sex hormone category
Zhu et al. (2016)	Children, current	Child (age 10–15)	Asthma incidence **Q4 vs, Q1** **M: 3.45 (1.51, 7.88); p-trend = 0.003** **F: 2.86 (1.16, 7.01); p-trend = 0.02**
Allergic sensitization (positive skin prick test)			
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10)	RR: 1.15 (0.99, 1.35)
		Child (age 16)	RR: 1.12 (0.87, 1.45)
Timmermann et al. (2017)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	1.02 (0.74, 1.41)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	0.79 (0.59, 1.05)
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	0.81 (0.59, 1.13)
Eczema			
Kvalem et al. (2020)	Child (age 10); median (IQR): 0.2 (0.1) ng/mL	Child (age 10)	Ever doctor diagnosed: RR: 0.93 (0.79, 1.09)
		Child (age 10–16)	Ever between 10 and 16 years RR: 0.86 (0.66, 1.12)
		Child (age 16)	Current (last 12 months) RR: 0.92 (0.68, 1.25)
Chen et al. (2018a)	Cord blood; median (range): 0.36 (<LOD–5.73) ng/mL	Child (age 2)	Ever: 1.22 (0.94, 1.58) per log unit increase Q2 0.94 (0.55, 1.60) Q3 1.15 (0.68, 1.95) Q4 1.58 (0.94, 2.65), p-trend = 0.06
Okada et al. (2014) Goudarzi et al. (2016)	Maternal, gest wk 28–32; median (range): 0.522 (<0.1–2.434) ng/mL	Child (age 1 or 2) Child (age 4)	Ever: Q2 0.80 (0.58, 1.10) Q3 0.78 (0.57, 1.08) Q4 0.85 (0.62, 1.17), p-trend = 0.3 Q2: 0.85 (0.59, 1.2) Q3: 0.82 (0.56, 1.18) Q4: 0.93 (0.64, 1.28), p-trend = 0.6

Reference	Exposure timing and concentration[a]	Hypersensitivity measurement timing	PFDA[b] OR (95% CI) or as specified
Smit et al. (2015)	Maternal, mean gest wk 24 or 25; geometric mean (5–95th percentile): Ukraine 0.16 (0.07–0.35) ng/mL, Greenland 0.42 (0.16–1.16) ng/mL	Child (age 5–9)	Current: 0.95 (0.75, 1.20) per 1 SD change Ever: 0.88 (0.73, 1.06) per 1 SD change
Timmermann et al. (2017)	Maternal, gest wk 34–36; median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	Ever: 0.92 (0.64, 1.32)
	Child (age 5); median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	Ever: 0.92 (0.64, 1.31)
	Child (age 13) median (IQR): 0.3 (0.2–0.4 ng/mL)	Child (age 13)	No MMR: 401.88 (0.09, 1.84 × 10^6) [c] Yes MMR: 0.88 (0.58, 1.34), p-interaction = 0.2

[a]Exposure timing is organized into groups based on maternal exposure (including cord blood), childhood exposure (including from birth through age 13), and adult exposure.
[b]All estimates are presented as OR (95% CI) for the odds of the outcome per 2-fold increase in PFDA concentration unless otherwise stated.
[c]Results provided broken down by MMR vaccination status; yes (n = 537) or no (n = 22) when provided; some results were not split by MMR vaccination status
Bold font indicates $p < 0.05$.

Animal studies

Animal toxicity studies examining effects on the immune system after PFDA exposure include two 28-day gavage studies using S-D rats and/or B6C3F1/N mice (Frawley et al., 2018; NTP, 2018) and a 14-day study in Balb/c mice (inferred as a gavage study based on information provided, although the method of chemical administration was not specified) (Lee and Kim, 2018). Immune effects reported in these studies are discussed according to the immunotoxicity categories and endpoint groupings outlined previously (see Section on Methodological considerations above for more details). Most of the available evidence, including host resistance, immune function, and observational assays, were conducted in female mice and rats, since female animals are preferred in immunotoxicity testing due to increased sensitivity (Kadel and Kovats, 2018; Klein and Flanagan, 2016). Further, no chemical-specific information on potential sex-specific differences was identified.

Immunosuppression

Host resistance

Host resistance assays measure the effects of toxicants on the overall immune function in response to a challenge, usually from an infectious agent, and these assays are considered highly relevant to the evaluation of immunotoxicity in the context of human health assessment (IPCS, 2012). Host resistance was evaluated in a 28-day gavage study in female B6C3F1/N mice

considered *medium* confidence primarily due to the lack of reporting on the blinding of investigators during assessment which raises some concerns for potential observational bias (Frawley et al., 2018) (see Figure 3-13). PFDA did not affect survival of mice challenged with three dilution levels of *Influenza* virus (groups A–C) during the observational period after exposures ended (Days 29–50 of the study); exposures ranged from 0.179–0.71 mg/kg-day (Refer to the interactive HAWC link for additional details). The only effect noted was a slight decrease (7.8%) in body weight at the highest exposure dose (0.71 mg/kg-day) on Day 29 in group C, the group challenged with the highest level of influenza. In summary, host resistance appeared to be unaffected by PFDA, although the evidence is limited to a single short-term study in mice.

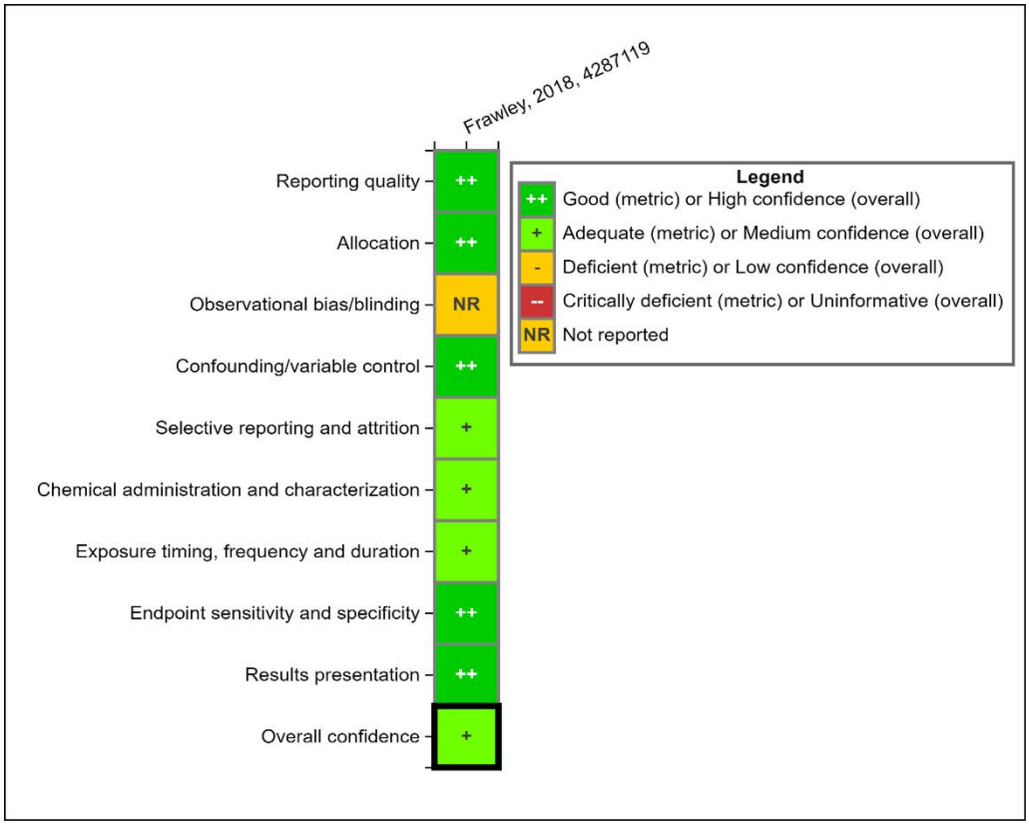


Figure 3-13. Evaluation results for animal study assessing effects of PFDA exposure on host resistance. Refer to HAWC for details on the study evaluation review.

Immune function assays

Markers of altered immune cell function or damage were evaluated in female B6C3F1/N mice and female S-D rats exposed to doses of 0.045–0.71 and 0.125–0.5 mg/kg-day, respectively, for 28 days via gavage (Frawley et al., 2018). Immune function assays included measures of: (1) innate immunity such as mononuclear phagocyte system (MPS) activity in rats and natural killer (NK) cell activity in rats and mice; (2) humoral-mediated immunity such as T-dependent antibody

responses in rats and mice; and (3) cell-mediated immunity such as mixed leukocyte response in mice and delayed-type hypersensitivity in rats and mice. These assays measure specific immune system responses to a stimulus both at the cellular and organism level and can provide clear and direct evidence of immunotoxicity (IPCS, 2012). Overall, study confidence in experiments conducted in both species was high for most endpoints, except delayed-type hypersensitivity (DTH). The absence of information on the blinding or any other strategy used to mitigate potential for observational bias resulted in a *medium* confidence rating for this endpoint (see Figure 3-14).

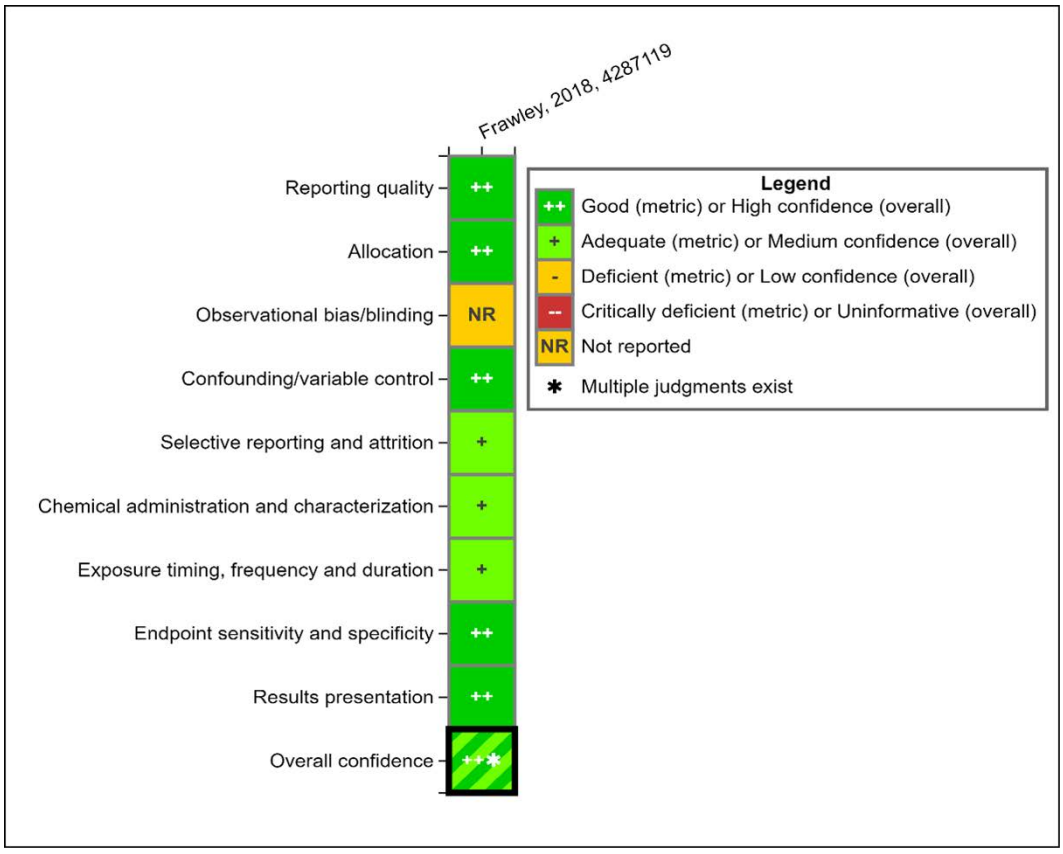


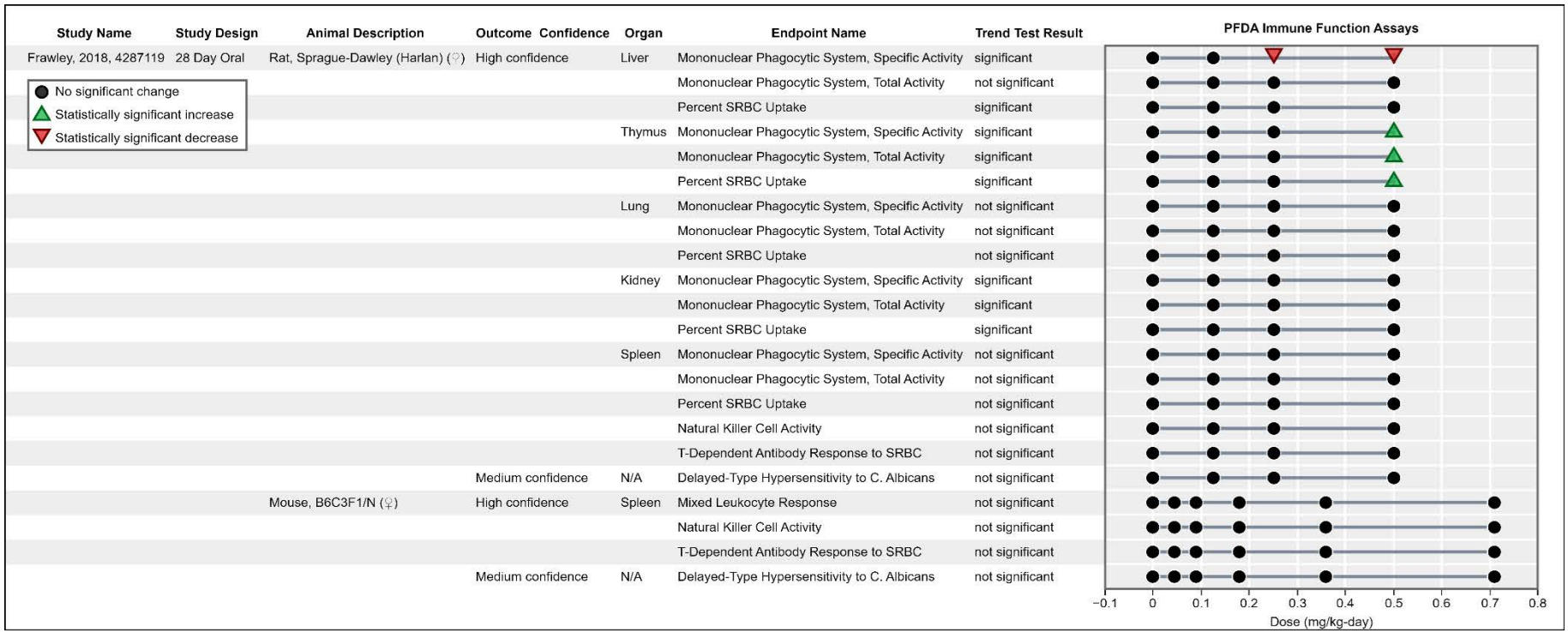
Figure 3-14. Evaluation results for animal studies assessing effects of PFDA exposure on immune function assays. Refer to HAWC for details on the study evaluation review.

Dose-related decreases in specific activity of the MPS (cpm/mg of tissue) were reported in rat liver (MPS was not examined in mice) at 0.125–0.5 mg/kg-day (15–45% compared to controls), reaching statistical significance at the two highest doses (see Figure 3-15). Alterations in phagocytic activity coincide with the liver histopathology (i.e., hepatocyte necrosis) and increased liver weight (see Section 3.2.1 on Liver effects for more details) observed in the exposed animals. Due to the increases in liver weight, it is possible that the effects on specific activity could represent changes in hepatocyte numbers/size rather than alterations in the functional activity of tissue macrophages (Frawley et al., 2018). However, a decreasing trend was also observed for total MPS

activity (p = 0.051) and percent (%) uptake of sheep red blood cell (SRBC) by macrophages in the liver (p = 0.029).

MPS activity was evaluated in other rat tissues such as the thymus, lung, kidney, and spleen. In the thymus, MPS activity (total, specific and % SRBC uptake) was significantly increased at the highest exposure dose (139–200% at 0.5 mg/kg-day) (Frawley et al., 2018) (see Figure 3-12). However, the values for total activity and % uptake were two orders of magnitude lower than the negative control tissue (kidney), which raises concerns about the biological significance of these results. No treatment-related effects were found in MPS activity in the lung and spleen of rats (see Figure 3-15).

Apart from the reduced MPS activity in rat liver after PFDA exposure, no treatment-related effects were observed in other immune function assays evaluated in rats and mice (i.e., NK cell activity and T-dependent antibody responses to SRBC in the spleen of rats and mice, mixed leukocyte response in mouse spleen and DTH response to C. albicans in rats and mice). Despite a general lack of findings from most immune function assays, the mild reductions in phagocytic activity in rat liver suggest potential suppression of innate immunity after short-term PFDA exposure, although uncertainties remain surrounding whether this finding might be attributable to the observed liver toxicity.



Study Name	Study Design	Animal Description	Outcome Confidence	Organ	Endpoint Name	Trend Test Result
Frawley, 2018, 4287119	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	High confidence	Liver	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	significant
				Thymus	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	significant
					Percent SRBC Uptake	significant
				Lung	Mononuclear Phagocytic System, Specific Activity	not significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	not significant
				Kidney	Mononuclear Phagocytic System, Specific Activity	significant
					Mononuclear Phagocytic System, Total Activity	significant
					Percent SRBC Uptake	significant
				Spleen	Mononuclear Phagocytic System, Specific Activity	not significant
					Mononuclear Phagocytic System, Total Activity	not significant
					Percent SRBC Uptake	not significant
					Natural Killer Cell Activity	not significant
					T-Dependent Antibody Response to SRBC	not significant
			Medium confidence	N/A	Delayed-Type Hypersensitivity to C. Albicans	not significant
		Mouse, B6C3F1/N (♀)	High confidence	Spleen	Mixed Leukocyte Response	not significant
					Natural Killer Cell Activity	not significant
					T-Dependent Antibody Response to SRBC	not significant
			Medium confidence	N/A	Delayed-Type Hypersensitivity to C. Albicans	not significant

Figure 3-15. Effects on immune function assays following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

General/observational immune assays

General or observational immune parameters were evaluated in two experiments (reported in one study) using female B6C3F1/N mice and female S-D rats after 28-day gavage exposure (Frawley et al., 2018). The 28-day experiments were *high* confidence for most endpoints, except bone marrow colony formation (see Figure 3-16). Key issues regarding observational bias/blinding and results presentation (i.e., ambiguity surrounding sample size) reduced confidence to *medium* for this endpoint. The assays included in the study are spleen cell immunophenotyping (rats and mice), anti-CD3+-mediated T-cell proliferation (rats and mice), bone marrow DNA synthesis (rats and mice) and bone marrow colony formation and differentials (rats only) (Frawley et al., 2018). These assays can indicate changes in immune cell populations and mediators and are often used in support of more predictive measures of immunotoxicity (i.e., host resistance and functional assays) (IPCS, 2012).

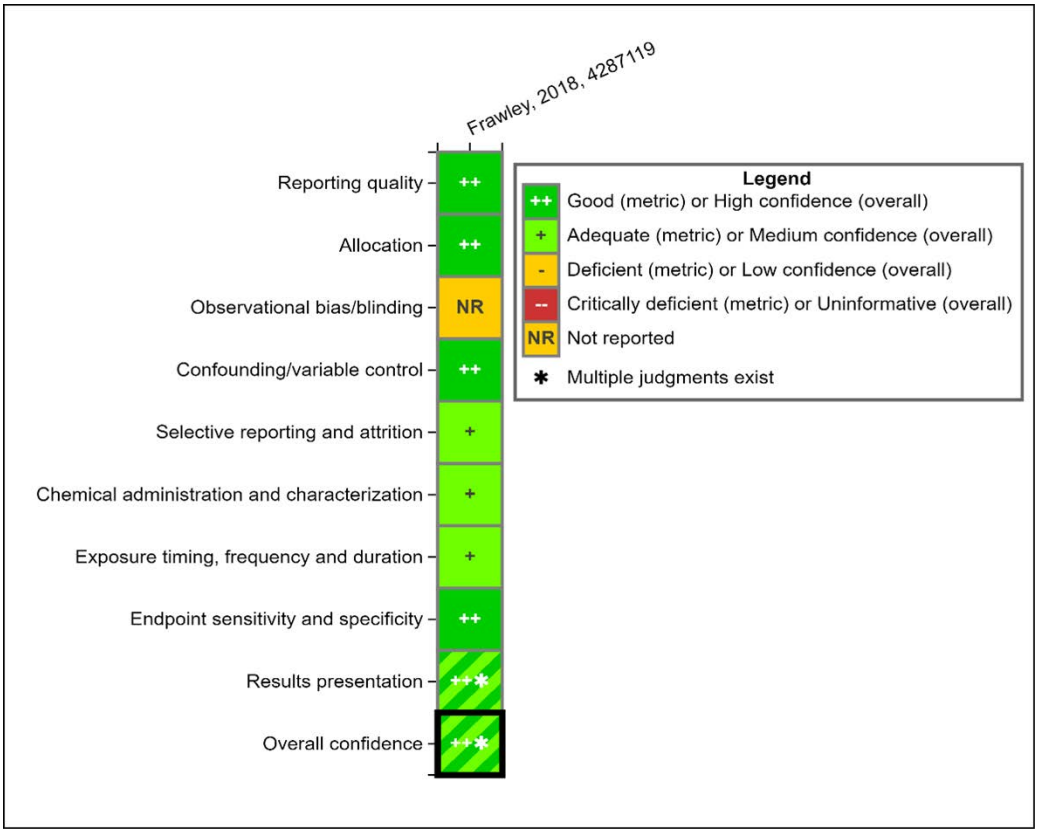


Figure 3-16. Evaluation results for animal studies assessing effects of PFDA exposure on general/observational immune assays. Refer to HAWC for details on the study evaluation review.

PFDA treatment caused dose-related reductions in absolute spleen cell numbers in mice reaching up to 24% decrease compared to controls at the highest dose (0.71 mg/kg-day) (see Table 3-16 and Figure 3-17). Likewise, absolute counts of splenic B-cells, T-cells, T-helper cells,

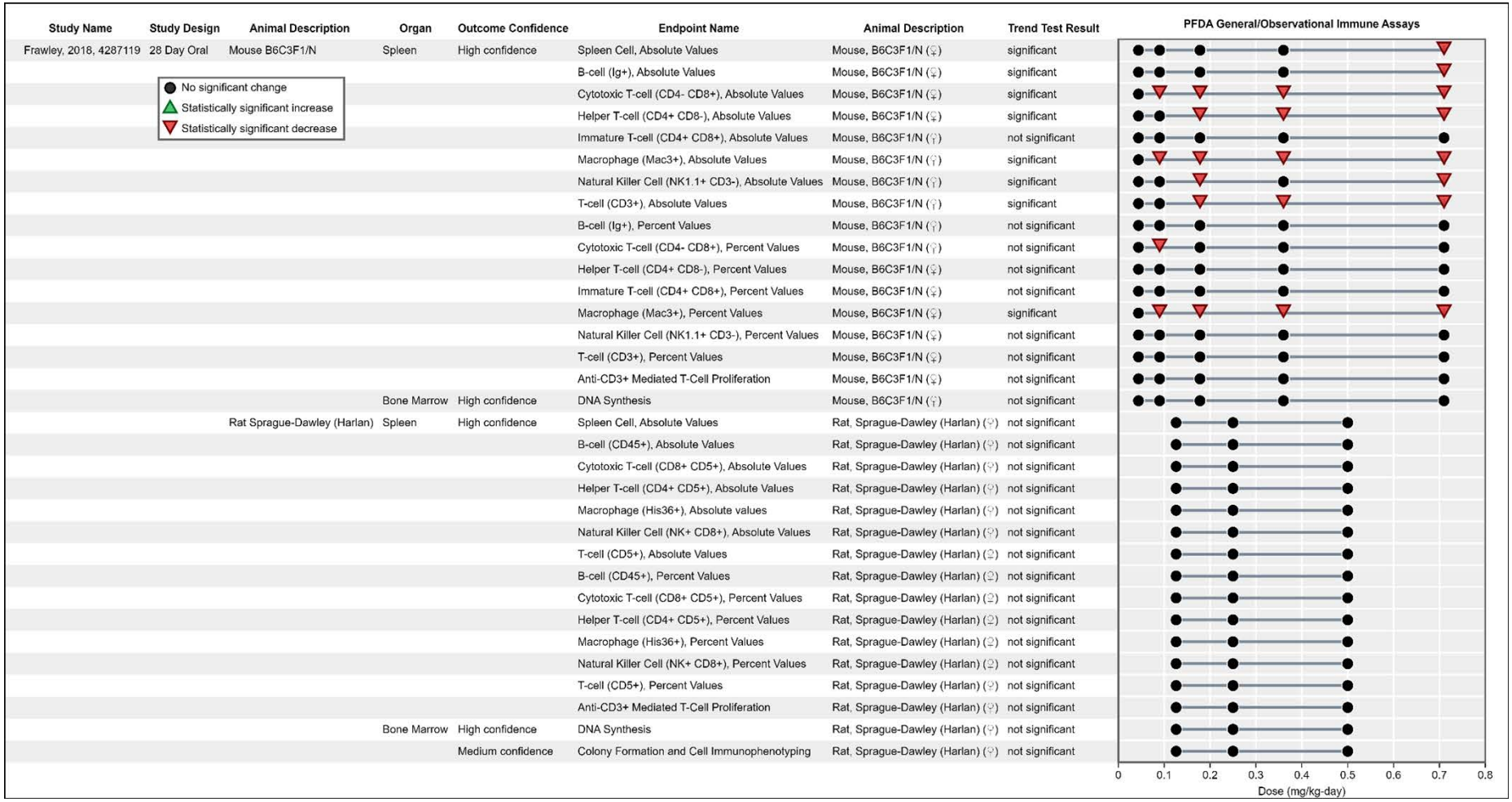
cytotoxic T-lymphocytes, NK cells, and macrophages displayed a decreasing trend and achieved statistical significance at doses ≥0.089 mg/kg-day; absolute counts of immature T-cells were not affected by PFDA exposure (percent changes from controls are summarized in Table 3-16). The relative percentages of spleen immune cell populations in mice were largely unchanged, except for macrophages, which showed, dose-related reductions at similar doses (13–19% relative to controls over 0.089–0.71 mg/kg-day). The mostly null findings in the relative percentage values of spleen cell immunophenotypes likely reflect the observed spleen atrophy in animals (i.e., decreases in spleen cell numbers and spleen weights [see synthesis on Histopathology and organ weights below for more details]) (Frawley et al., 2018). Furthermore, a lack of treatment-related effects was reported for other observational immune assays evaluated in mice (i.e., anti-CD3+-mediated T-cell proliferation and bone marrow DNA synthesis) (see Figure 3-17). In rats, results were null with PFDA exposure (0.125–0.5 mg/kg-day) in assays of spleen cell immunophenotyping (including spleen cell numbers and immune cell populations), anti-CD3+-mediated T-cell proliferation and bone marrow DNA synthesis, colony formation and progenitor cell populations (see Figure 3-17).

The reductions in absolute immune cell populations in mouse spleen provide evidence consistent with potential immunosuppression following short-term PFDA exposure, although uncertainties related to the overt organ toxicity (i.e., spleen atrophy) remain.

Table 3-16. Percent change relative to controls in absolute spleen cell population counts in female B6C3F1/N mice exposed to PFDA exposure for 28-days (Frawley et al., 2018)

Endpoint	Dose (mg/kg-d)				
	0.045	**0.089**	**0.179**	**0.36**	**0.71**
Spleen cell	−2	−8	−13	−8	**−24**
B-cell (Ig+)	3	0.3	−10	−4	**−27**
Cytotoxic T-cell ($CD4^-$ $CD8^+$)	−10	**−19**	**−22**	**−10**	**−28**
Helper T-cell ($CD4^+$ $CD8^-$)	−11	−13	**−19**	**−12**	**−29**
Immature T-cell ($CD4^+$ $CD8^+$)	−21	−53	−21	−16	−53
Macrophage ($Mac3^+$)	−13	**−21**	**−31**	**−25**	**−39**
Natural Killer Cell ($NK1.1^+$ $CD3^-$)	−15	−15	**−18**	−16	**−18**
T-cell ($CD3^+$)	−9	−15	**−22**	**−14**	**−28**

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



Study Name	Study Design	Animal Description	Organ	Outcome Confidence	Endpoint Name	Animal Description	Trend Test Result
Frawley, 2018, 4287119	28 Day Oral	Mouse B6C3F1/N	Spleen	High confidence	Spleen Cell, Absolute Values	Mouse, B6C3F1/N (♀)	significant
					B-cell (Ig+), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					Cytotoxic T-cell (CD4- CD8+), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					Helper T-cell (CD4+ CD8-), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					Immature T-cell (CD4+ CD8+), Absolute Values	Mouse, B6C3F1/N (♀)	not significant
					Macrophage (Mac3+), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					Natural Killer Cell (NK1.1+ CD3-), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					T-cell (CD3+), Absolute Values	Mouse, B6C3F1/N (♀)	significant
					B-cell (Ig+), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					Cytotoxic T-cell (CD4- CD8+), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					Helper T-cell (CD4+ CD8-), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					Immature T-cell (CD4+ CD8+), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					Macrophage (Mac3+), Percent Values	Mouse, B6C3F1/N (♀)	significant
					Natural Killer Cell (NK1.1+ CD3-), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					T-cell (CD3+), Percent Values	Mouse, B6C3F1/N (♀)	not significant
					Anti-CD3+ Mediated T-Cell Proliferation	Mouse, B6C3F1/N (♀)	not significant
			Bone Marrow	High confidence	DNA Synthesis	Mouse, B6C3F1/N (♀)	not significant
		Rat Sprague-Dawley (Harlan)	Spleen	High confidence	Spleen Cell, Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					B-cell (CD45+), Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Cytotoxic T-cell (CD8+ CD5+), Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Helper T-cell (CD4+ CD5+), Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Macrophage (His36+), Absolute values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Natural Killer Cell (NK+ CD8+), Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					T-cell (CD5+), Absolute Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					B-cell (CD45+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Cytotoxic T-cell (CD8+ CD5+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Helper T-cell (CD4+ CD5+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Macrophage (His36+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Natural Killer Cell (NK+ CD8+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					T-cell (CD5+), Percent Values	Rat, Sprague-Dawley (Harlan) (♀)	not significant
					Anti-CD3+ Mediated T-Cell Proliferation	Rat, Sprague-Dawley (Harlan) (♀)	not significant
			Bone Marrow	High confidence	DNA Synthesis	Rat, Sprague-Dawley (Harlan) (♀)	not significant
				Medium confidence	Colony Formation and Cell Immunophenotyping	Rat, Sprague-Dawley (Harlan) (♀)	not significant

Figure 3-17. Effects on general/observational immune assays following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Blood leukocyte counts

Hematological evaluations of potential alterations in blood leukocyte (white blood cell) counts with PFDA treatment comes from three *high* confidence experiments (reported in two studies) with gavage exposure for 28 days: one in female B6C3F1/N mice (Frawley et al., 2018) and two in male and female S-D rats (Frawley et al., 2018; NTP, 2018) (see Figure 3-18). The parameters evaluated included leukocyte counts and differentials (basophils, eosinophils, lymphocytes, monocytes, and neutrophils). For lymphocytes, both absolute counts and total counts (absolute plus large lymphocytes such as lymphoblasts or reactive lymphocytes) were provided.

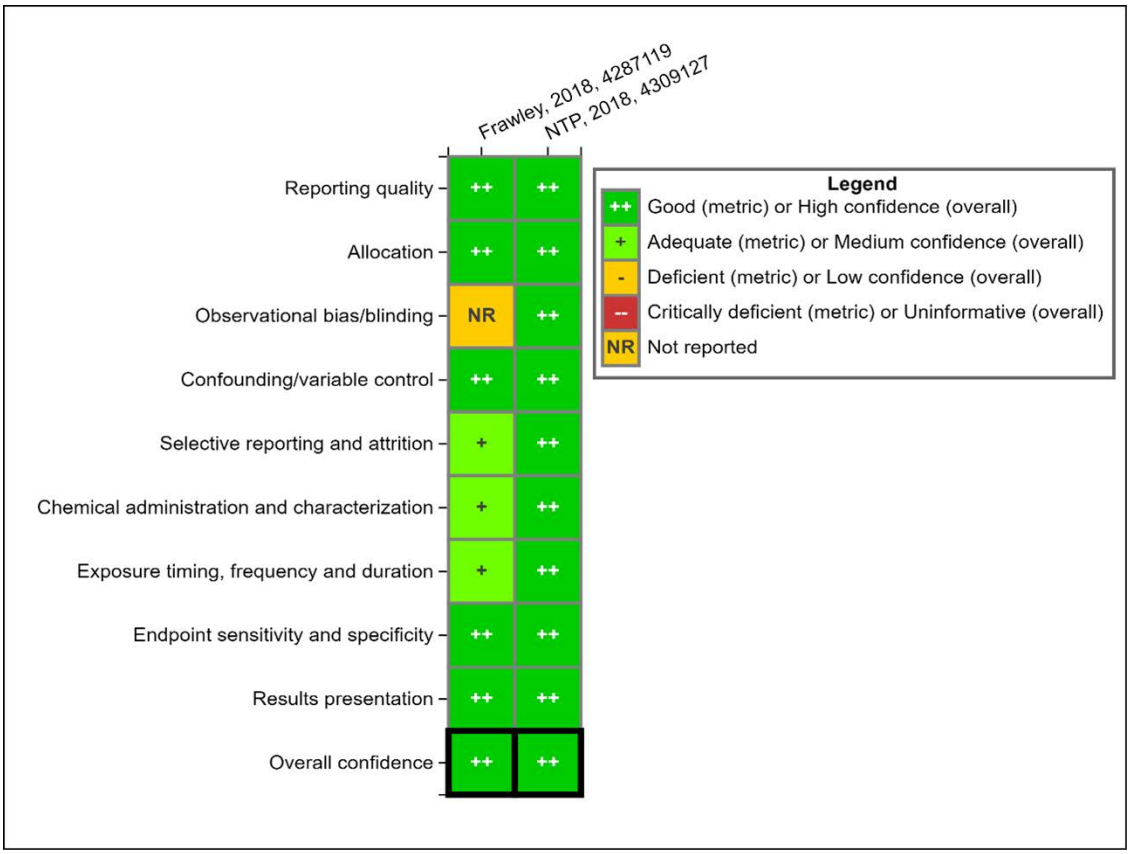


Figure 3-18. Evaluation results for animal studies assessing effects of PFDA exposure on blood leukocyte counts. Refer to HAWC for details on the study evaluation review.

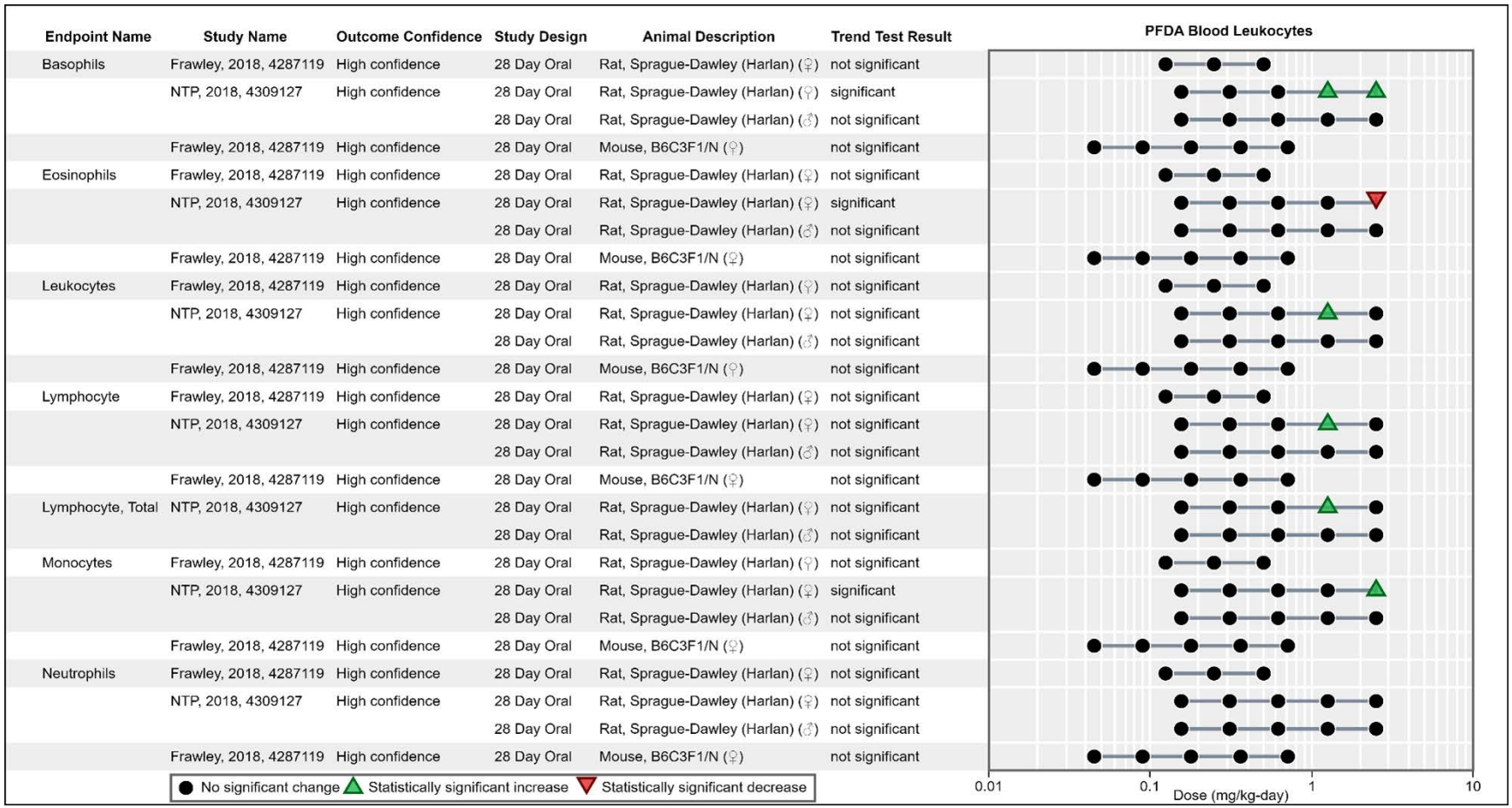
The effects of PFDA exposure on blood leukocyte counts in animals are unclear (see Table 3-17 and Figure 3-19). Frawley et al. (2018) found no treatment-related effects on blood leukocyte numbers and differentials in female mice and female rats with exposures up to 0.71 and 0.5 mg/kg-day, respectively (males were not examined). In a separate study by NTP (2018), statistically significant changes were noted in circulating leukocytes in female rats (but not males) at higher doses (≥1.25 mg/kg-day). Specifically, the number of basophils increased by 157% and

71% compared to controls at doses of 1.25 and 2.5 mg/kg-day, respectively, while the number of monocytes increased by 41% at the high-dose group (2.5 mg/kg-day). Leukocyte and lymphocyte (total and absolute) numbers were elevated at 1.25 mg/kg-day (37–41% compared to controls) but not at 2.5 mg/kg-day (0% compared to controls). Conversely, eosinophil counts decreased up to 64% at a dose of 2.5 mg/kg-day. In general, the hematological data suggests increases in blood leukocyte counts and populations in female rats. The biological significance of these findings is uncertain given the inconsistencies in the directionality of changes across dose groups in the (Frawley et al., 2018) study and, more importantly, the lack of coherent evidence in other endpoints supportive of a potential immunostimulatory response following PFDA exposure. Additionally, the observed hematological changes occurred mostly at high PFDA doses (≥1.25 mg/kg-day) associated with adverse systemic effects (see Section 3.2.9 on General toxicity effects for more details).

Table 3-17. Percent change relative to controls in blood leukocyte counts in female S-D rats exposed to PFDA exposure for 28-days (NTP, 2018)

Endpoint	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Basophils	61	14	43	**157**	**71**
Eosinophils	−27	−27	−18	−9	**−64**
Leukocytes	15	11	2	**37**	0
Lymphocyte (absolute)	18	14	3	**41**	0
Lymphocyte (total)	19	15	3	**41**	0
Monocytes	0	6	−12	24	**41**
Neutrophils	−9	−17	−6	9	0

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



Endpoint Name	Study Name	Outcome Confidence	Study Design	Animal Description	Trend Test Result
Basophils	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
Eosinophils	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
Leukocytes	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
Lymphocyte	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
Lymphocyte, Total	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
Monocytes	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
Neutrophils	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
			28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant

Figure 3-19. Effects on blood leukocyte counts following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Histopathology and organ weights

The data on immune histopathology and organ weights is described in one study using female B6C3F1/N mice (Frawley et al., 2018) and two studies in male and female rats (Frawley et al., 2018; NTP, 2018) exposed to PFDA for 28 days via gavage. The NTP (2018) study was *high* confidence for histopathology and organ weight measures. Frawley et al. (2018) was considered *high* confidence for the evaluation of organ weight but exhibited deficiencies in the presentation and discussion of histopathological findings (lack of quantitative data), which resulted in a *medium* confidence rating for this endpoint (see Figure 3-20).

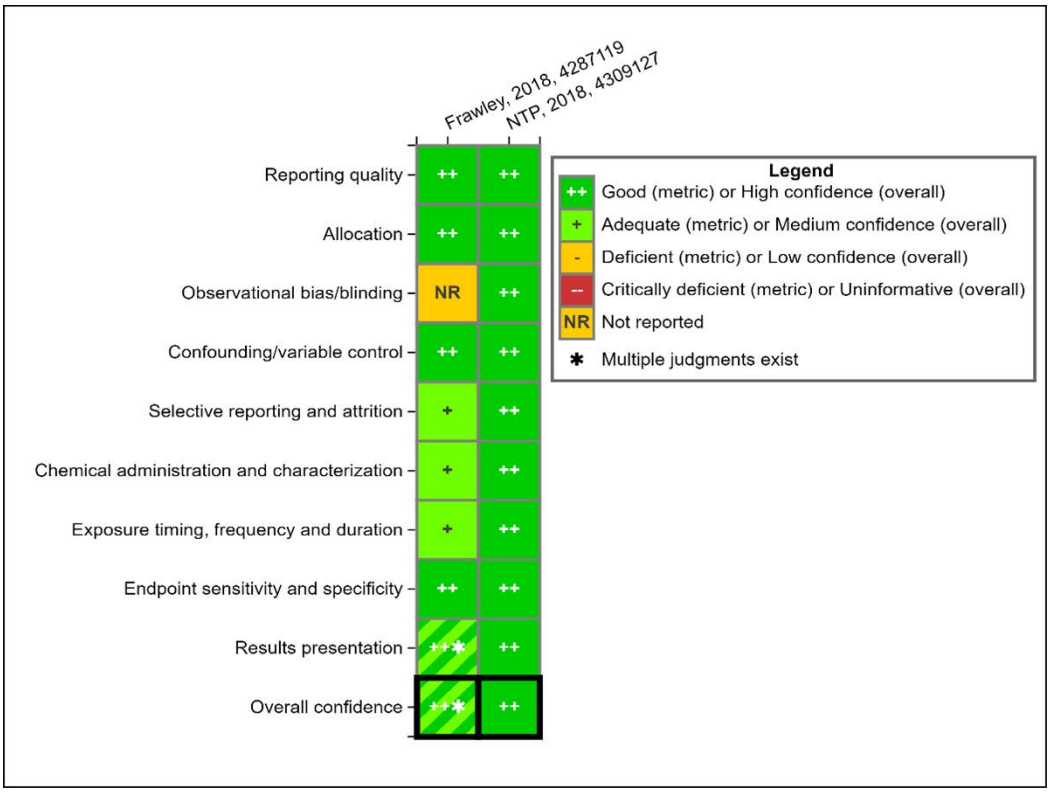


Figure 3-20. Evaluation results for animal studies assessing effects of PFDA exposure on immune histopathology and organ weights. Refer to HAWC for details on the study evaluation review.

Animal toxicity studies provide some evidence of immune organ histopathology (see Figure 3-21). The bone marrow, lymph nodes, spleen and thymus were examined histologically in male and female rats exposed to doses ranging from 0.125–2.5 mg/kg-day (Frawley et al., 2018; NTP, 2018). No treatment-related effects were found in any of these organs at doses ≤0.625 mg/kg-day across the two rat studies, but morphological changes were observed in bone marrow and thymus in the study that tested higher doses (≥1.25 mg/kg-day) (NTP, 2018). Increased incidences of bone marrow hypocellularity (10/10 in males and females) and thymic atrophy (9/10 in males and 8/10 in females) were observed in rats at the highest dose

(2.5 mg/kg-day), while incidence of lymphocyte apoptosis in the thymus was increased in males only at a dose of 1.25 mg/kg-day (8/10 rats). The aforementioned lesions ranged from mild to moderate in severity and did not occur in the controls or in other exposure groups.

Changes in immune organ weights were reported in female mice and male/female rats across three, 28-day gavage studies (Frawley et al., 2018; NTP, 2018) (see Table 3-18 and Figure 3-17). The rat study by Frawley et al. (2018) included three cohorts exposed to similar experimental conditions. Statistically significant decreases in spleen weights (absolute and relative) were observed across species and sexes at ≥0.179 mg/kg-day, reaching 55% in rats and 22% in mice relative to controls at the highest doses tested (2.5 and 0.71 mg/kg-day, respectively) (Frawley et al., 2018; NTP, 2018). Although there were no notable histopathological findings in the spleen, the organ weight reductions in mice are concordant with alterations in spleen cell numbers and populations previously described (see synthesis on General/observational immune assays above for additional details). Absolute and relative thymus weights decreased in a dose-dependent manner (29–75% compared to controls) at ≥1.25 mg/kg-day in rats that exhibited thymic lesions (atrophy and apoptosis) and marked body weight reductions in one study (NTP, 2018). In contrast, another study reported increases in absolute and relative thymus weights in rats at lower PFDA doses (0.125–0.5 mg/kg-day) but the results were not consistent across study cohorts and in most cases did not show a dose-response dependency (Frawley et al., 2018) (see Table 3-21). As such, the significance of the increases in thymus weights in rats is uncertain. Thymus weights in mice were not impacted by PFDA treatment (up to 0.71 mg/kg-day) in one study (Frawley et al., 2018).

In summary, histopathological lesions were found in the bone marrow and thymus of rats and decreased spleen and thymus weights were reported in mice and/or rats after short-term PFDA exposure. The effects on spleen weights are coherent with reductions in spleen cell counts and populations in mice at ≥0.089 mg/kg-day. The bone marrow and thymus lesions in rats were only observed in the presence of marked reductions in body weight (12–38% relative to controls) at PFDA doses ≥1.225 mg/kg-day, which provides a significant source of uncertainty. Indeed, bone marrow hypocellularity and thymic atrophy have been linked to diet restriction in short-term rat studies (Levin et al., 1993) and PFDA-induced wasting syndrome characterized by decreased food consumption and rapid weight loss has been well documented in animals (see Section 3.2.10 on General toxicity for more details). As such, the toxicological significance of the histopathological findings is uncertain.

Organ	Endpoint Name	Study Name	Outcome Confidence	Study Type	Animal Description	Trend Test Result
Bone Marrow	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Hypocellularity	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
					Rat, Sprague-Dawley (Harlan) (♂)	significant
Lymph Node	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
		NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
					Rat, Sprague-Dawley (Harlan) (♂)	not applicable
Spleen	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
		NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
					Rat, Sprague-Dawley (Harlan) (♂)	not applicable
Thymus	Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Atrophy	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
					Rat, Sprague-Dawley (Harlan) (♂)	significant
	Lymphocyte Apoptosis	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant

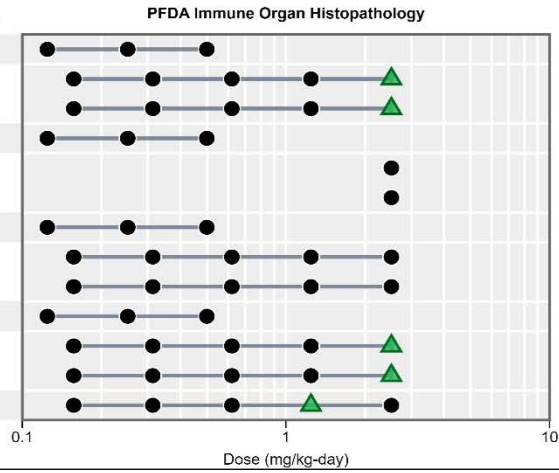


Figure 3-21. Effects on immune organ histopathology following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Table 3-18. Percent change relative to controls in immune organ weights in short-term animal studies after exposure to PFDA

Animal group	Dose (mg/kg-d)						
	0.045	0.089	0.125–0.179	0.25–0.36	0.5–0.71	1.25	2.5
Spleen weight (absolute) Male S-D rats NTP (2018)			11	0	−4	**−26**	**−49**
Spleen weight (absolute) Female S-D rats NTP (2018)			1	−2	−9	**−36**	**−55**
Spleen weight (absolute) Female C57BL/6N mice Frawley et al. (2018)	−3	2.8	−18	−6	**−20**		
Spleen weight (relative) Male S-D rats NTP (2018)			7	1	−1	**−6**	**−19**
Spleen weight (relative) Female S-D rats NTP (2018)			−3	−5	**−9**	**−27**	**−30**
Spleen weight (relative) Female C57BL/6N mice Frawley et al. (2018)	−3	−6	**−16**	**−9**	**−22**		
Thymus weight (absolute) Male S-D rats NTP (2018)			1	0	−1	**−44**	**−75**
Thymus weight (absolute) Female S-D rats NTP (2018)			5	18	9	−20	**−65**
Thymus weight (absolute) Female S-D rats; MPS cohort Frawley et al. (2018)			13	23	13		
Thymus weight (absolute) Female S-D rats; Histopathology cohort Frawley et al. (2018)			−5	3	−1		
Thymus weight (absolute) Female S-D rats; TDAR to SRBC cohort Frawley et al. (2018)			**34**	**30**	21		
Thymus weight (relative) Male S-D rats NTP (2018)			−2	0	3	**−29**	**−61**
Thymus weight (relative) Female S-D rats NTP (2018)			1	12	9	−8	**−46**
Thymus weight (relative) Female S-D rats; MPS cohort Frawley et al. (2018)			18	27	18		
Thymus weight (relative) Female S-D rats; Histopathology cohort Frawley et al. (2018)			−7	0	0		
Thymus weight (relative) Female S-D rats; TDAR to SRBC cohort Frawley et al. (2018)			**36**	**21**	14		

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

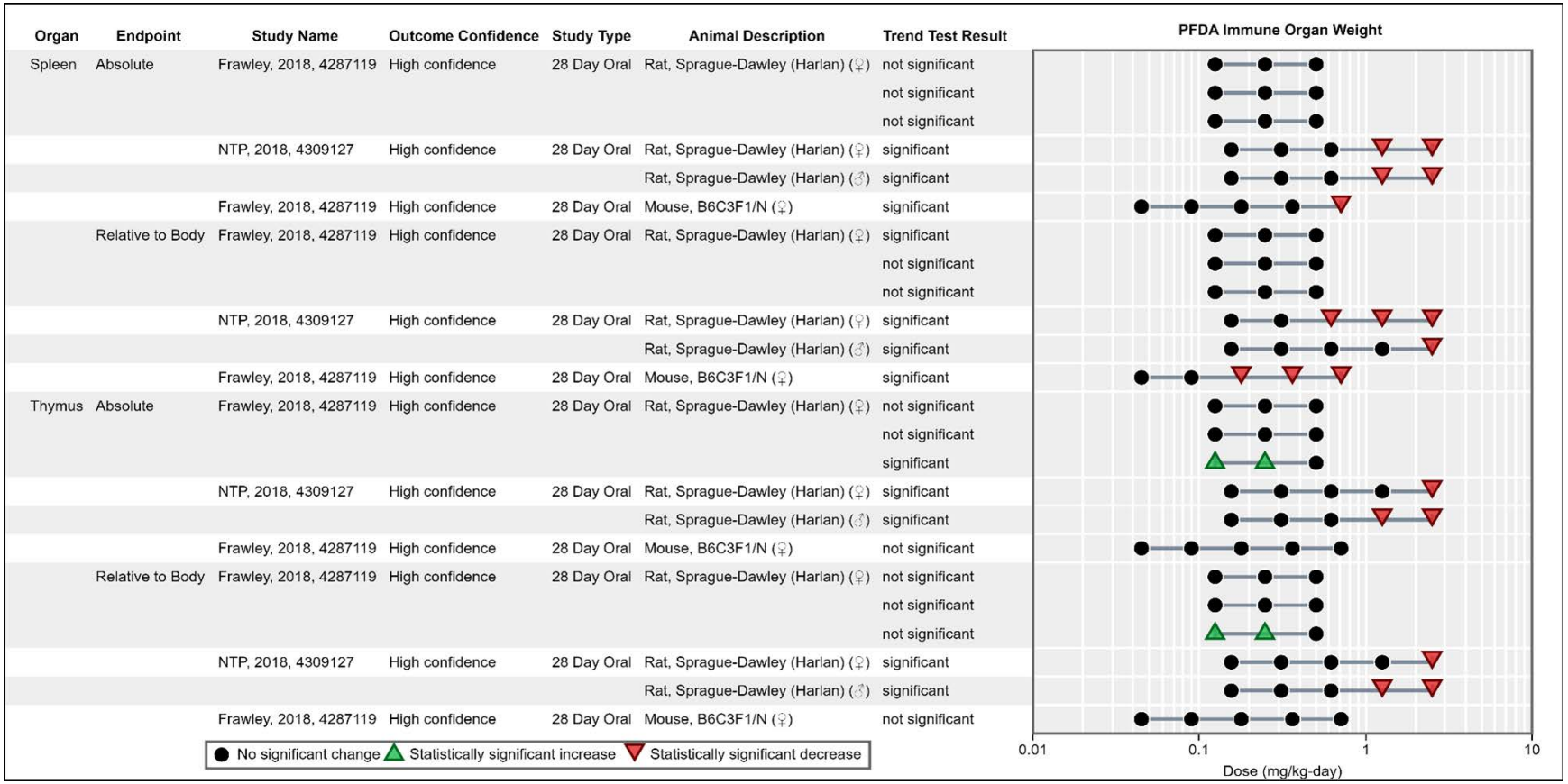


Figure 3-22. Effects on immune organ weights following exposure to PFDA in short-term oral studies in animals. The rat study by **Frawley et al. (2018)** included three cohorts exposed to similar experimental conditions. Results can be viewed by clicking the HAWC link).

Sensitization and allergic response

Immune function assays

A 14-day study in male ICR mice exposed to a dose of 21.4 mg/kg-day examined the effect of PFDA treatment on OVA-induced active systemic anaphylaxis (Lee and Kim, 2018), a well-accepted model for evaluating mast cell function and allergic reactions (Je et al., 2015; Evans et al., 2014; Ribeiro-Filho et al., 2014). The study was rated as low confidence due to issues with reporting on potential confounding effects (no information on general systemic toxicity measures; this excessive dose would be expected to cause significant, overt toxicity given observations, including "wasting syndrome", from other short-term studies with similar dosing paradigms; see Section 3.2.10 on GENERAL TOXICITY EFFECTS for more details), experimental groups (no indication of randomization) and the characterization of the test compound (no information on analytical verification or specific method of administration) (see Figure 3-23).

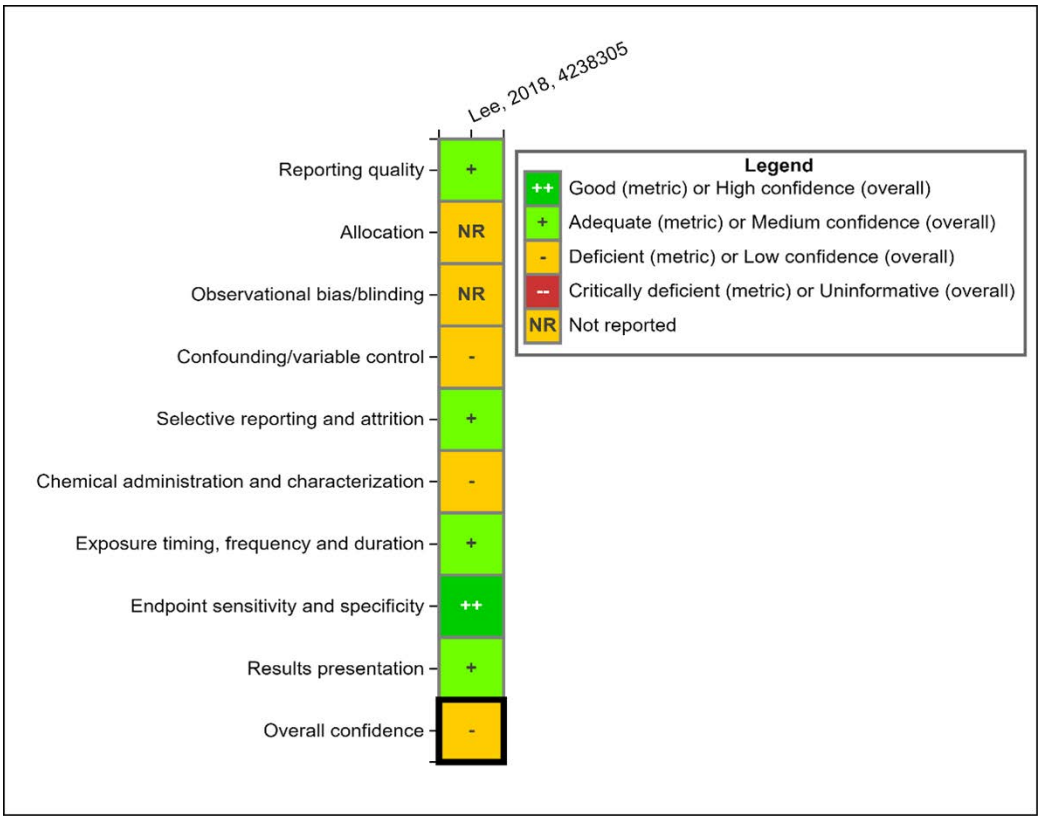


Figure 3-23. Evaluation results for animal study assessing effects of PFDA exposure on immune function assays for sensitization and allergic response. Refer to HAWC for details on the individual study evaluation review.

PFDA (21.4 mg/kg-day) exacerbated the response to OVA-induced active systemic anaphylaxis in mice as indicated by a significant decrease in rectal temperature (i.e., hypothermia) and significant elevation in serum levels of inflammatory mediators such histamine, TNFα and

immunoglobulins (IgE and IgG1) compared to OVA treatment alone (Lee and Kim, 2018). Histamine is released in response to mast cell degranulation and plays a key role in immediate-type hypersensitivity (Amin, 2012). The findings from the Lee and Kim (2018) study suggest possible induction of immediate-type hypersensitivity, although the exposure dose was high compared to doses associated with immunosuppressive responses in animals (0.089–2.5 mg/kg-day) and raises concerns over potential confounding with general toxicity effects. Although the study provided no information on general toxicity measures, PFDA exposure was associated with significant body weight reductions at doses ≥1.25 mg/kg-day in oral exposure studies and the induction of wasting syndrome in acute, i.p. injection studies at doses ≥20 mg/kg (see Section 3.2.10 on General toxicity for more details).

Mechanistic studies and supplemental evidence

The available supplemental evidence most relevant to interpretation consists of an acute i.p. injection study evaluating immunotoxicity endpoints in exposed rats and a few in vitro studies in human and animal models examining possible mechanisms of immunotoxicity following PFDA exposure.

An acute i.p. injection study investigating potential immune effects of PFDA exposure (20 and 50 mg/kg) in Fischer 344 rats showed reductions in the antibody (i.e., serum KLH-specific IgG2a levels) and DTH responses to Keyhole limpet hemocyanin (KLH) in exposed animals (Nelson et al., 1992); the effects on the DTH response were not statistically significant but showed a decreasing trend with increase in dose at each timepoint (40–46% and 38–47% compared to ad libitum-fed controls after 8 and 30 days respectively). In addition, NK cell activity was increased in rats after PFDA treatment (Nelson et al., 1992). Exposure to PFDA altered immune responses in comparison to both ad libitum- and pair- fed controls with the exception of NK activity, which was similarly elevated in PFDA-exposed rats and pair-fed (but not ad libitum) controls (Nelson et al., 1992). The acute toxicity of PFDA is characterized by a wasting syndrome, which induces rapid and severe reductions in food consumption and body weight in rats at doses similar to those associated with the immunomodulatory effects described above (20–100 mg/kg) (see Section 3.2.9 on General toxicity effects for more details). The findings suggest that the antibody and DTH responses are directly related to PFDA exposure, while the NK activity is likely a secondary effect of chemical-induced wasting syndrome. Functional alterations in antibody and DTH responses after acute i.p. exposure is supportive of the immunomodulatory effects observed after short-term PFDA administration (see synthesis of Animal studies in this Section for more details).

Using an in vitro model to study mast cell functions and allergic inflammation, Lee and Kim (2018) showed that PFDA exposure can elevate markers of mast cell degranulation (histamine, β-hexosaminidase and intracellular calcium levels), increase gene expression and secretion of pro-inflammatory cytokines involved in immune cell recruitment and activation (TNF-α, IL-1β, IL-6, and IL-8) and induce NF-kB transactivation in IgE-stimulated rat basophilic leukemia (RBL-2H3) cells

(Lee and Kim, 2018). The data are consistent with the exacerbation of hypothermia and allergic inflammatory mediators (histamine, TNFα, IgE and IgG1 levels) in OVA-stimulated mice following continuous high-dose oral exposure to PFDA (see synthesis of Animal studies in this Section for more details) and suggest a plausible mechanism for PFDA-induced immediate-type hypersensitivity.

Other potential mechanisms of PFDA-induced immune effects were evaluated in two studies conducted in human and animal in vitro cell models. No effects on IgM secretion and surface membrane expression were observed in human (F4 and Hurtwitz) or murine (HPCM2) B cell lines at non-cytotoxic PFDA concentrations, but detergent-like activity (i.e., solubilization of cell membranes) was reported in these lymphoblastoid cell lines at doses that caused significant cytotoxicity (Levitt and Liss, 1986). Another study evaluated the effects of PFDA on cytokine release in human primary and cultured leukocytes (Corsini et al., 2012). Decreases in pro-inflammatory (TNF-α and interleukin [IL]-6) and anti-inflammatory (IL-10 and interferon gamma [IFN-γ]) cytokine levels were reported in human peripheral blood leukocytes stimulated with lipolysaccharide (LPS) and phytohemagglutinin, respectively, following PFDA exposure (Corsini et al., 2012). Leukocytes from female donors were generally more susceptible to alterations in cytokine production (primarily TNFα) compared to male counterparts, although differential responses across cytokine measures were apparent and may be explained in part by variability in cell donors (Corsini et al., 2012). Similarly, PFDA decreased TNFα levels and NF-kB activation (measured as I-kB degradation, p65 phosphorylation and NF-kB gene reporter activity) in human promyelocytic THP-1 cells stimulated with LPS but had no effects on PPARα-mediated transactivation (Corsini et al., 2012). Cell viability measured via the lactase dehydrogenase assay was unaffected in this cell line by PFDA treatment (Corsini et al., 2012). The data suggest that PFDA suppresses cytokine release (i.e., TNFα) by interfering with the NF-kB pathway in stimulated immune cells and that such effects may occur independently of PPARα activation.

Collectively, the mechanistic data indicate that PFDA can modulate NF-kB activation to induce both pro- and anti- inflammatory responses in cultured immune cells, which may have implications for the mechanisms of immunotoxicity of this compound.

Evidence Integration

Studies in humans and animals exposed to PFDA are available for the evaluation of potential immunosuppression and sensitization or allergic responses.

The evidence of an association between PFDA exposure and immunosuppressive effects in human studies is *moderate*. This is based on largely consistent decreases in antibody response following vaccination (against two different infectious agents) in two *medium* confidence studies describing results from two independent birth cohorts in the Faroe Islands with outcome measurement in childhood. Reduced antibody response is an indication of immunosuppression and may result in increased susceptibility to infectious disease (IPCS, 2012). The antibody results

present a consistent pattern of findings that higher prenatal, childhood, and adult serum concentrations of PFDA were associated with suppression of at least one measure of the anti-vaccine antibody response to common vaccines in two well-conducted birth cohorts in the Faroe Islands and supported by a *low* confidence study in adults. An inverse association was observed in 21 of 26 evaluations, with a minimum of a 2% decrease in antibody concentration per doubling of PFDA concentration at levels consistent with the general population in NHANES; six of these evaluations were statistically significant and exhibited a large magnitude of effect (i.e., >18% decrease in response). These associations were observed despite poor study sensitivity, which increases confidence in the findings. There is some remaining uncertainty resulting from variability in the response, including positive associations in a few exposure-outcome combinations, differences in the responses by age of exposure and outcome measures as well as timing of vaccination (initial and boosters), from potential confounding across PFAS, and from inconsistency in two other *medium* confidence studies with outcome measurement in adults and cross-sectional exposure measurement in children. Overall, the evidence supports an association with immunosuppressive-type effects. These results are consistent with hazard identification conclusions from the NTP (2016) monograph on immunotoxicity associated with exposure to PFOS and PFOA, which concluded that PFOA and PFOS are *presumed to be an immune hazard to humans* based largely on evidence of suppression of antibody responses in both human and animal studies (NTP, 2016). Although no effects were observed in T-dependent antibody responses with PFDA in one rat and one mouse study (both *high* confidence), other immunomodulatory responses were observed in animals that indicate potential for immunosuppression (see summary of animal evidence below for more details).

The database of animal studies examining PFDA-induced immunosuppressive responses consists of two *high* or *medium* confidence studies in B6C3F1/N mice (Frawley et al., 2018) and/or S-D rats (Frawley et al., 2018; NTP, 2018) exposed via gavage for 28 days. PFDA did not elicit a strong immunotoxic response in animals as evidenced by the absence of treatment-related effects in a host resistance assay and most immune function assays (NK cell activity and T-dependent antibody responses to SRBC, mixed leukocyte response and DTH response to C. albicans). Nevertheless, coherent responses that suggest potential immunosuppression by PFDA exposure were observed, which is consistent with the human evidence. The immunomodulatory responses included dose-related decreases in phagocytic activity of rat liver macrophages (MPS activity) at ≥ 0.25 mg/kg-day and in immune cell population counts in mouse spleen at ≥0.089 mg/kg-day (Frawley et al., 2018), but issues regarding overt organ toxicity (increased liver weight and hepatocyte necrosis and spleen atrophy, respectively) introduce significant uncertainty (Frawley et al., 2018). Additionally, morphological changes occurred in the bone marrow (hypocellularity) and thymus (atrophy and lymphocyte apoptosis) of rats at PFDA doses associated with systemic toxicity (i.e., decreased body weights at ≥1.25 mg/kg-day) (NTP, 2018); the changes are consistent with the wasting syndrome that PFDA elicits and could represent secondary effects of the accompanying

systemic toxicity. In light of the uncertainties in the available database, the evidence for potential immunosuppression from short-term animal studies is considered *slight*.

Mechanistic evidence from a high-dose, i.p. injection study is supportive of potential PFDA-induced immunosuppression (i.e., decreased antibody and DTH responses) in rats at ≥20 mg/kg (Nelson et al., 1992). Furthermore, an in vitro study using stimulated human primary and cultured leukocytes suggests that PFDA is capable of inhibiting NF-κB transcription and suppressing cytokine production (Corsini et al., 2012), which may be relevant to its mechanisms of immunotoxicity. Limitations in the mechanistic information include issues interpreting the exposure context (i.e., acute, high-dose exposure) of the i.p. injection study and general lack of studies in animal and human models that can provide support for the biological plausibility of putative immunosuppression observed in human and animal studies.

There is *slight* evidence for sensitization and allergic responses from studies in humans, but notable limitations and uncertainties in the evidence base remain. In human studies, the available evidence for infectious disease and hypersensitivity was less consistent than the evidence on immunosuppression and had more uncertainties resulting from a limited number of studies, unexplained heterogeneity in outcome or results, variable exposure assessment approaches that considered exposure at different times in relation to outcomes, and in some cases self-reported outcomes. For asthma, two of the three available studies reported no association with PFDA exposure. However, significant associations with asthma diagnosis and asthma-related outcomes, including an exposure response gradient, were observed in one well-conducted (*medium* confidence) study with adequate sensitivity (Dong et al., 2013). This study also had the most specific outcome definition, based on asthma incidence in the past year. These differences could account for the inconsistency with other asthma studies, including the other *medium* confidence study which examined "ever asthma". In addition, increases in biomarkers related to asthma were reported in this study, providing biological plausibility to the apical association. Still, the number of available studies is small, and poor sensitivity makes the null results difficult to interpret.

In animals, the single, short-term, *low* confidence study that examined endpoints relevant to sensitization and allergic responses reporting findings coherent with immediate-type hypersensitivity (i.e., exacerbation of hypothermia and markers of mast cell-mediated allergic inflammation in OVA-induced mice) (Lee and Kim, 2018); however, the high exposure dose used (21.4 mg/kg-day) raises significant concerns about potential confounding effects by indirect systemic toxicity and thus these coherent results were not interpreted to provide biological plausibility for the findings in humans and the animal evidence was considered *indeterminate* (Lee and Kim, 2018).

Altogether, considering the available evidence from human, animal and mechanistic studies, the ***evidence indicates*** that PFDA exposure is likely to cause adverse immune effects, specifically immunosuppression, in humans, given sufficient exposure conditions[3] (see Table 3-19). The hazard judgment is driven primarily by consistent evidence of reduced antibody response from human

epidemiological studies (mostly from two birth cohort studies) at levels of 0.3 ng/mL (median exposure in studies observing an adverse effect). The evidence in animals showed coherent immunomodulatory responses at ≥0.089 mg/kg-day that are consistent with potential immunosuppression and supportive of the human studies, although issues with overt organ/systemic toxicity raise concerns about the biological significance of some of these effects. A small number of studies conducted via i.p. injection and in vitro exposure in human and rodent cell culture models add some support for the biological plausibility of the observed phenotypic effects. While there is some evidence that PFDA exposure might also have the potential to affect sensitization and allergic responses, the human evidence underlying this possibility is uncertain and without support from animal or mechanistic studies. Based on the antibody response data in humans, children and young individuals exposed during critical developmental windows may represent a potential susceptible population for the immunosuppressive effects of PFDA. The absence of additional epidemiological studies or any long-term/chronic exposure studies in animals examining alterations in immune function or immune-related disease outcomes during different developmental lifestages represents a major source of uncertainty in the immunotoxicity database of PFDA.

Table 3-19. Evidence profile table for PFDA exposure and immune effects

Evidence stream summary and interpretation					Inferences and summary judgment
Evidence from studies of exposed humans (see Section 3.2.2: Human Studies)					⊕⊕⊙ ***Evidence indicates* (likely)** *Primary basis*: Evidence of immunosuppression from human studies indicating reduced antibody response in children at levels of approximately 0.3 ng/mL (moderate evidence) and some coherent findings in animals (*slight* evidence) at ≥0.089 mg/kg-d. Overall, other forms of potential PFDA-induced immunotoxicity, including *slight* human evidence for hypersensitivity-related outcomes, were interpreted with less certainty. *Human relevance*: Coherent effects in human and animal studies *Cross-stream coherence*: Evidence of immunosuppression in both animals and humans.
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Immunosuppression (Antibody response) 4 medium confidence studies (3 in children) and 1 *low confidence* study	• Three studies in children and one in adults reported *decreased* antibody response following vaccination with higher PFDA exposure	• *Consistency* overall across vaccine type, timing of vaccination, and age at antibody response measurement including in two *medium confidence* studies with prospective exposure measurement and outcomes in children • Associations observed despite limited sensitivity	• Potential for confounding across PFAS	⊕⊕⊙ *Moderate* Generally consistent evidence for decreased antibody responses. The inconsistent and *low confidence* evidence on infectious disease did not influence this judgment.	
Immunosuppression (Infectious diseases) 3 *medium* and 2 *low confidence* cohort studies	• Positive association with infectious diseases in one *medium* and two *low confidence* studies, but inconsistency across studies of the same infections/symptoms.	• No factors noted	• *Unexplained inconsistency*, though limited sensitivity may contribute • Imprecision		

Evidence stream summary and interpretation					Inferences and summary judgment
<u>Sensitization and allergic response</u> 7 *medium* confidence studies in children	• Significantly higher odds of asthma (OR = 3.2) in one medium confidence study. One additional study reported *increased* odds of asthma with higher PFDA exposure, but only in a small sub-group that did not receive MMR vaccine before age 5 • Other studies reported no association with hypersensitivity outcomes	• Large effect size for asthma incidence in the only study with adequate sensitivity (based on exposure contrast and outcome definition) • Exposure-response gradient across quartiles in same study	• Potential for confounding across PFAS • Unexplained inconsistency across studies	⊕⊙⊙ *Slight* Sparse evidence for hypersensitivity with some concerns for unexplained inconsistency and potential confounding	*Susceptible populations and lifestages*: Based on the antibody response data in humans, children and fetuses may be at higher risk of adverse effects. *Other inferences*: MOA is unknown, but some uncertain evidence from human and animal in vitro studies suggests a possible role for NFκB in both pro- and anti-inflammatory responses that may be relevant to the mechanism(s) of immunotoxicity of PFDA.
Evidence from in vivo animal studies (see Section 3.2.2: Animal Studies)					
Studies and confidence	**Summary and Key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
<u>Immunosuppression</u> 2 *high/medium* confidence studies in mice and/or rats • 28-day gavage (2x)	• Decreased hepatic MPS activity in rats at ≥0.5 mg/kg-d in the presence of liver toxicity (increased liver weight and hepatocyte necrosis) • Decreased absolute spleen cell population counts in mice at ≥0.89 mg/kg-d in the presence of	• Coherence across immune responses (i.e., MPS activity in rats and spleen cell population counts and spleen weights in mice) • Dose- response gradient for MPS activity, absolute spleen cell counts and spleen weights	• Lack of effects on host resistance and most immune function assays • Potential confounding with overt organ or systemic toxicity.	⊕⊙⊙ *Slight* Coherent evidence of potential immunosuppression in rats and mice at doses ≥0.089 mg/kg-d across two *high/medium* confidence studies; however, there is uncertainty due to potential confounding effects with overt organ/systemic toxicity.	

Evidence stream summary and interpretation					Inferences and summary judgment
	spleen atrophy (decreased spleen weights and total cell counts) • Bone marrow and thymic lesions and decreased thymus weights in rats at ≥1.25 mg/kg-d in the presence of marked body weight reductions • No effects in a host resistance assay in mice or other immune function assays conducted in rats and mice at doses up 0.71 mg/kg-d	• *High/medium* confidence studies			
<u>Sensitization and allergic response</u> 1 *low* confidence study in mice • 14-day	• Exacerbation of hypothermia and release of serum inflammatory markers (i.e., histamine, TNFα, IgE and IgG1) in OVA-stimulated mice at 21.4 mg/kg-d	• *Coherence* across markers of allergic inflammation and hypersensitivity	• Potential for confounding by systemic toxicity	⊙⊙⊙ *Indeterminate* *Low confidence* evidence with considerable uncertainty due to potential confounding effects due to high dose systemic toxicity.	
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**			**Evidence stream judgment**	
<u>Mast cell function and allergic response</u>	*Interpretation*: PFDA may induce mast cell-mediated allergic inflammation via NFκB activation.			A small number of mechanistic studies in human	

Evidence stream summary and interpretation			Inferences and summary judgment
	Key findings: • Increases in markers of mast cell degranulation (histamine, β-hexosaminidase and intracellular calcium levels), immune cell recruitment and activation (TNF-α, IL-1β, IL-6, and IL-8 levels) and NF□B transactivation in IgE-stimulated rat RBL-2H3 cells. *Limitations*: Single study available.	and rodent in vitro models suggest a possible involvement of NF□B in pro- and anti-inflammatory responses that may be relevant to the mechanisms of immunotoxicity of PFDA. Supportive evidence of immunosuppression in rats was reported in an acute, i.p. injection study. Although the available evidence is limited introducing significant uncertainty, the findings provide some support for the biological plausibility of the immune-related responses in humans and animals.	
Other mechanisms	*Interpretation*: PFDA may suppress cytokine production by inhibiting NFκB activation. *Key findings*: • Attenuation of cytokine release (including TNFα) in stimulated human peripheral blood leukocytes (leukocytes from female donors appeared to be more susceptible to these effects); decreases in TNFα release and NF□B activation but no effects on PPARα transactivation in stimulated human promyelocytic THP-1 cells (Corsini et al., 2012). • No effects on IgM secretion and surface expression in human and murine B cell lines exposed to noncytotoxic PFDA concentrations (Levitt and Liss, 1986). *Limitations*: Few studies available; cell donor variability introduces some uncertainty in interpreting sex-specific differences in cytokine release from exposed human primary leukocytes.		
Other evidence	*Interpretation*: Results are consistent with immunosuppressive responses observed in oral exposure studies. *Key findings*: • Decreases in antibody response and DTH in KLH-stimulated rats compared to libitum and pair-fed controls; Increase in NK cell activity may be attributable to PFDA-induced anorexia. *Limitations*: Single study with high-dose, one-time i.p. exposure.		

3.2.3. DEVELOPMENTAL EFFECTS

Human studies

Studies of developmental endpoints related to PFDA are available for fetal and post-natal growth restriction, spontaneous abortion, anogenital distance, birth defects, and gestational duration outcomes (i.e., preterm birth and gestational age). Given that spontaneous abortion and preterm birth could be driven by either female reproductive or developmental toxicity, these endpoints are also discussed in the context of coherence in Section 3.2.5 on Female reproductive effects.

Forty-eight epidemiological publications (across 46 different studies) examining examined PFDA exposures in relation to developmental endpoints were identified in the literature search. This included the following: eight studies on postnatal growth, 12 studies on gestational duration, six on fetal loss, three on anogenital distance, two studies on birth defects, and 31 publications (across 29 different studies) examined fetal growth restriction. Publications based on overlapping populations in the same cohort were included in the synthesis only if they provided some unique data for different endpoints. For example, the Bjerregaard-Olesen et al. (2019) study from the Aarhus birth cohort also provided birth length and head circumference measures in the overall population and across sex that were not included in the main study by Bach et al. (2016). Therefore, it is included in the fetal growth restriction count above and considered one study (population) from two publications with separate analyses. This synthesis, and especially the evaluation of consistency across studies, focuses on a primary study to avoid duplicative analyses or overweighting of one study population. Although the results for the smaller sample size in this study are not plotted, in this instance divergent primary birthweight (BWT) results are presented for comparison in the text. Another study by Gyllenhammar et al. (2018) was supplemented by a second publication (Swedish Environmental Protection Agency, 2017) that provided mean BWT data on a larger population from the same cohort. Supplemental data and communication from study authors were used if they provided additional data or information, and US EPA calculated confidence intervals and rescaled study results to provide comparisons based on a ln-unit change to increase comparability.

Additional Methodological Considerations

As detailed in the PFAS Systematic Review Protocol (Appendix A) and Section 1.2.2, there were multiple outcome-specific considerations for study evaluation that influenced the domain ratings and the overall study confidence. For the confounding domain, downgrading of studies occurred when key confounders of the fetal growth and PFAS relationship, such as parity, were not considered. Pregnancy hemodynamics represent a source of uncertainty as PFAS biomarkers sampled late in pregnancy may be prone to bias potentially from either confounding or reverse causality (see Appendix F for detailed discussion). Among the few fetal growth studies [e.g.,

([Gyllenhammar et al., 2018](#)) for PFDA] examining the potential for confounding by measures of pregnancy hemodynamics (e.g., plasma albumin or GFR measures), there is very little direct evidence that these measures were important confounders ([Gyllenhammar et al., 2018](#); [Meng et al., 2018](#); [Sagiv et al., 2018](#); [Whitworth et al., 2012](#)) across different PFAS. Sample timing patterns across studies were considered here to see if results among studies with early sampling (i.e., studies with any trimester 1 sampling) differed from those with later sampling (i.e., maternal samples exclusively from trimester 2 through trimester 3, umbilical cord, placental or post-partum maternal samples). More research is needed especially amongst studies with early samples and/or with repeated measures during different stages of pregnancy to further clarify any potential impact of this source of uncertainty in epidemiological studies using biomarkers. There is additional uncertainty across all health endpoints due to potential confounding by co-occurring PFAS (see Appendix A and F for methods and analyses, respectively). For fetal growth restriction and other developmental endpoints, there may be more concern over potential PFAS co-exposure confounding due to PFNA given higher correlations with PFDA and associations that are fairly comparable in consistency and magnitude, as detailed in Appendix F. Although there is some uncertainty as to whether other PFAS are plausible confounders here, studies were downgraded if the authors did not rule out or account for these or other covariates that may be confounders.

For the exposure domain, all the available studies analyzed PFAS in serum or plasma using standard methods. Given the long half-life of PFDA, samples collected during all three trimesters (and shortly after birth) were considered representative of the most critical in utero exposure windows for fetal growth and gestational duration measures. Various measures of postnatal growth were included based on an assumed fetal programming mechanism (i.e., Barker hypothesis) where in utero perturbations or exposures, such as poor nutrition, can lead to developmental effects such as fetal growth restriction and ultimately adult-onset metabolic-related disorders (see more on this topic in [De Boo and Harding (2006)](#) and [Perng et al. (2016)](#) syntheses for metabolic disorders for other PFAS). There is some evidence that birth weight deficits from in utero exposures can be followed by increased weight gain during rapid growth catch-up periods in early childhood ([Perng et al., 2016](#)). Therefore, the most critical exposure window for measures of postnatal (and early childhood) weight and height change is assumed to be in utero. Thus, studies were downgraded if exposure data were collected later during childhood concurrent with outcome assessment (i.e., cross-sectional analyses).

Studies were also downgraded for study sensitivity, for example, if they had limited exposure contrasts (i.e., limited exposure ranges or distributions) or small sample sizes, since this can impact the ability of studies to detect statistically significant associations that may be present (especially when sample size is reduced by estimating stratum-specific results such as by sex). In the outcome domain, specific considerations included accuracy of fetal and early childhood anthropometric measures and adequacy of case ascertainment for dichotomized (i.e., binary) outcomes. Mismeasurement and incomplete case ascertainment can affect the accuracy of effect

estimates by impacting both precision and validity. For example, the spontaneous abortion studies were downgraded for incomplete case ascertainment in the outcome domain given that some pregnancy losses go unrecognized early in pregnancy (e.g., before implantation). This incomplete ascertainment, referred to as left truncation, can result in decreased study sensitivity and loss of precision. Often, this type of error can result in bias towards the null if ascertainment of fetal loss is not associated with PFDA exposures (i.e., non-differential). In some situations, differential loss is possible and bias away from the null and can manifest as an apparent protective effect. Anogenital distance (AGD) is an externally visible marker that has been shown in animal studies to be a sensitive indicator of prenatal androgen exposure (lower androgen levels associated with decreased AGD). It is associated with other reproductive tract abnormalities, including hypospadias and cryptorchidism in human and animal males (Liu et al., 2014; Sathyanarayana et al., 2010; Salazar-Martinez et al., 2004). The primary outcome-specific criteria for this outcome are the use of clearly defined protocols for measurement, ideally multiple measures of each distance (averaged), and minimal variability in the age of participants at measurement. In boys, measures can be taken from the center of the anus to the posterior base of the scrotum (ASD) or from the center of the anus to the cephalad insertion of the penile (APD).

Fetal and childhood growth restriction was examined through several endpoints including low birth weight (LBW), small for gestational age (SGA), abdominal and head circumference, as well as upper arm/thigh length, mean height/length, and mean weight either at birth or later during childhood. The developmental effects synthesis is largely focused on the higher-quality endpoints (i.e., considered good in the outcome domain) that were measured in multiple studies to allow for an evaluation of consistency and any heterogeneity across studies that may be present). Some of the adverse endpoints of interest examined here included fetal growth restriction endpoints based on birth weight such as mean birth weight reductions (or variations of this endpoint such as standardized birthweight z-scores), as well as categorical measures such as SGA births (e.g., lowest decile of birthweight stratified by gestational age and other covariates) and LBW (i.e., typically defined as <2,500 grams). Overall, birthweight measures are considered very accurate and, in these studies, were derived predominately from medical records; therefore, the outcome domain judgments reflect the high reliability of these endpoints. Sufficient details on the SGA percentile definitions and stratification factors as well as sources of standardization for z-scores were necessary for these endpoints to be considered good. LBW is a less preferred measure of fetal growth restriction than SGA, especially if analyses include both term and preterm neonates. This is because birth weight is dependent on both the rate of fetal growth and gestational duration, and perturbation in each may arise from different etiologies.

Gestational duration measures were examined in epidemiological studies as either continuous (i.e., per each gestational week) or dichotomized categorical endpoints such as preterm birth (typically defined as gestational age <37 weeks). Although gestational age dating methods, such as ultrasounds early in pregnancy are preferred, this and other approaches (e.g., last

menstrual period recall), are expected to result in some decreased sensitivity as measurement error could impact classification of SGA as well as PTB. Gestational duration measures were, therefore, downgraded if based solely on last menstrual period estimates or if the method(s) were not reported, and less uncertainty is anticipated in studies that compare and adjust for differences between last menstrual period and ultrasound measurements. Any sources of error noted in the classification of these endpoints are anticipated to be non-differential with respect to PFNA exposure and, therefore, would not be considered a major concern for risk of bias, but could impact precision and study sensitivity. Other measures of fetal growth may be subject to measurement error (e.g., head circumference and body length measures) if the measures are less reproducible (i.e., are subject to more interobserver differences). Thus, unless multiple measurements were taken, these endpoints were given a rating of adequate (Shinwell and Shlomo, 2003). Additional details for domain-specific evaluation of epidemiological studies can be found in the PFAS Systematic Review Protocol, Appendix A.

Growth Restriction – Neonatal Anthropometric Measures

Birth Weight

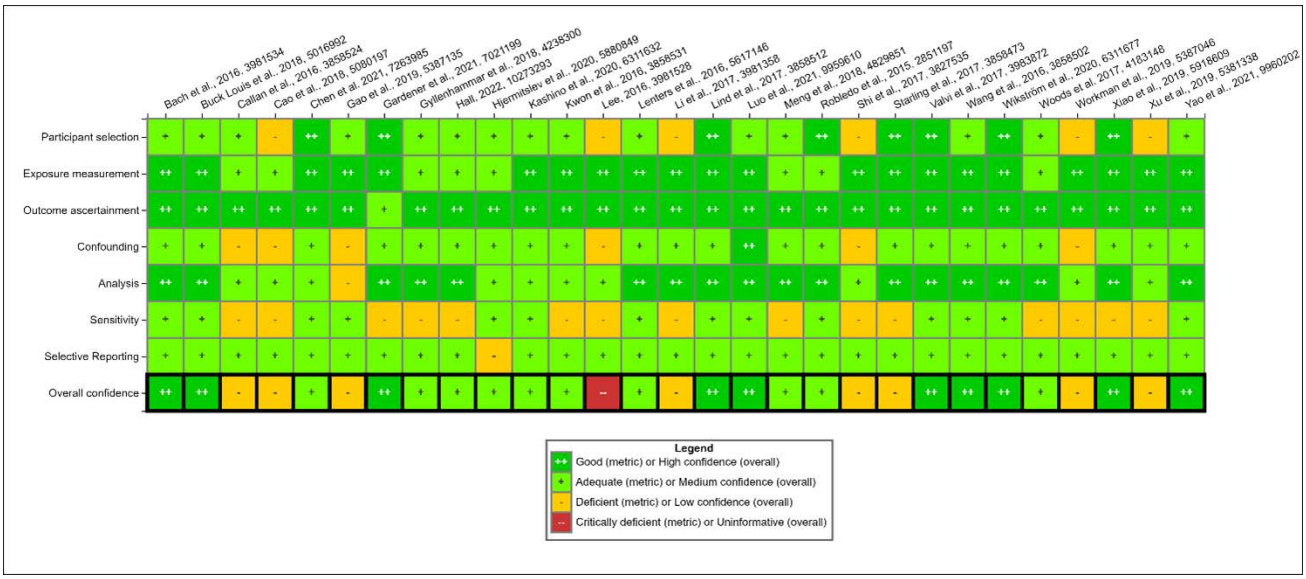


Figure 3-24. Study evaluation results for twenty-nine epidemiological studies of birth weight and PFDA. Refer to HAWC for details on the study evaluation review: HAWC Human Birth Weight.

*Confidence descriptors based on the mean birth weight or birth weight z-score endpoints.

As shown in Figure 3-24, 29 different studies examined birth weight measures (either mean BWT differences or standardized BWT scores) in relation to PFDA exposures. One uninformative study (Lee et al., 2016) due to several critical study deficiencies in confounding, selection participation and study sensitivity is not considered further below. Among the 28 included studies based on maternal, umbilical cord or placental measures, eight reported standardized BWT measures such as BWT z-scores (Gardener et al., 2021; Wikström et al., 2020; Workman et al., 2019; Xiao et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Bach et al., 2016; Wang et al., 2016) with all but two (Gardener et al., 2021; Xiao et al., 2019) of these reporting both standardized and mean BWT measures (see Figure 3-25). Twenty-six studies examined mean BWT either in the overall population (i.e., both girls and boys) or both sexes including four (Hall et al., 2022; Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015) that reported sex-specific analyses only. Fourteen studies in total reported sex-specific results in both sexes.

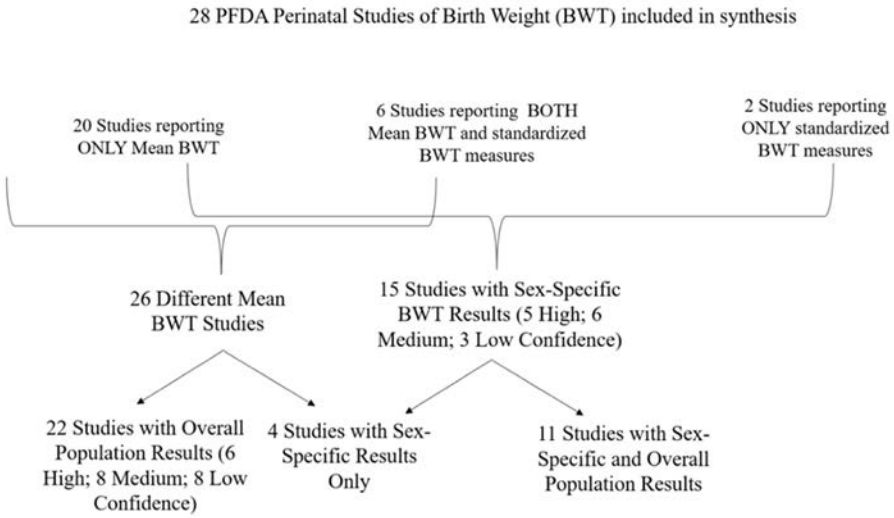


Figure 3-25. Twenty-eight perinatal studies of birth weight measures and subsets considered for different analyses.

Twenty-two of the 28 studies examining either standardized or mean BWT were prospective birth cohort studies, while the remaining six (Xu et al., 2019b; Gyllenhammar et al., 2018; Li et al., 2017; Shi et al., 2017; Callan et al., 2016; Kwon et al., 2016) were cross-sectional studies (see Figure 3-25). For evaluation of patterns, studies that collected biomarker samples concurrently or after birth were considered to be cross-sectional analyses [e.g., (Hall et al., 2022)]. Five of the 28 PFDA studies relied on umbilical cord samples (Xu et al., 2019b; Cao et al., 2018; Li et al., 2017; Shi et al., 2017; Kwon et al., 2016), and the recent medium confidence study by (Hall et al., 2022) based their exposure characterization on PFDA placental measures sampled at birth. Twenty studies had maternal blood measures that were sampled preconception (Robledo et al., 2015) or during trimester one (Buck Louis et al., 2018; Lind et al., 2017a; Bach et al., 2016), trimester three (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Kashino et al., 2020; Gao et al., 2019; Xiao et

al., 2019; Valvi et al., 2017; Callan et al., 2016; Wang et al., 2016) across multiple trimesters (Chen et al., 2021; Hjermitslev et al., 2020; Wikström et al., 2020; Workman et al., 2019; Meng et al., 2018; Starling et al., 2017; Woods et al., 2017; Lenters et al., 2016), after delivery (Gyllenhammar et al., 2018). The study by Meng et al. (2018) pooled samples from umbilical cord and multiple maternal samples during trimesters 1 and 2.

Ten of the 28 included studies examining different BWT indices were rated *high* confidence (Gardener et al., 2021; Luo et al., 2021; Yao et al., 2021; Wikström et al., 2020; Xiao et al., 2019; Buck Louis et al., 2018; Lind et al., 2017a; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016), while 10 were *medium* confidence (Hall et al., 2022; Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018; Woods et al., 2017; Kwon et al., 2016; Lenters et al., 2016; Robledo et al., 2015) and eight were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019b; Cao et al., 2018; Li et al., 2017; Shi et al., 2017; Starling et al., 2017; Callan et al., 2016). Among the 28 studies with mean BWT measures, 14 each had adequate and deficient study sensitivity (see Figure 3-24). The evidence syntheses for mean BWT differences detailed below primarily emphasizes the results from the twenty *high* or *medium* confidence studies.

Standardized BWT Measures

Three of the eight studies reported smaller standardized BWT scores in relation to PFDA exposures including one *medium* (Gyllenhammar et al., 2018) and two *high* (Wikström et al., 2020; Xiao et al., 2019) confidence studies (see Figure 3-26). One study not plotted by Gardener et al. (2021) reported positive associations with increasing PFDA exposures, while four studies reported null associations (Workman et al., 2019; Meng et al., 2018; Bach et al., 2016; Wang et al., 2016). One of the studies showing a null association in the quartile 4 (relative to quartile 1) and per each ln-unit increase did show elevated but non-significant BWT scores of -0.10 and -0.13 for quartiles 2 and 3 (Bach et al., 2016). Two of the studies (Wikström et al., 2020; Gyllenhammar et al., 2018) with inverse associations in the overall population reported statistically significant BWT z-scores similar in magnitude (β range: -0.14 to -0.15 per each ln-unit increase). The *high* confidence (Xiao et al., 2019) study reported associations about twice as large as these other studies (β = -0.39; 95%CI: -0.94, 0.16) and were largely driven by associations in girls (β = -0.62; 95%CI: -1.28, 0.03) (see Figure 3-27). One (Wikström et al., 2020) of two studies with categorical data showed evidence of an inverse exposure-response relationship.

Study sensitivity did not seem to explain the four null studies as two were adequate (Bach et al., 2016; Wang et al., 2016) and two were deficient (Workman et al., 2019; Meng et al., 2018). No pattern in study results by exposure contrasts was evident either. There may be some evidence of potential impact of pregnancy hemodynamics, as two of these three studies were based on later biomarker samples.

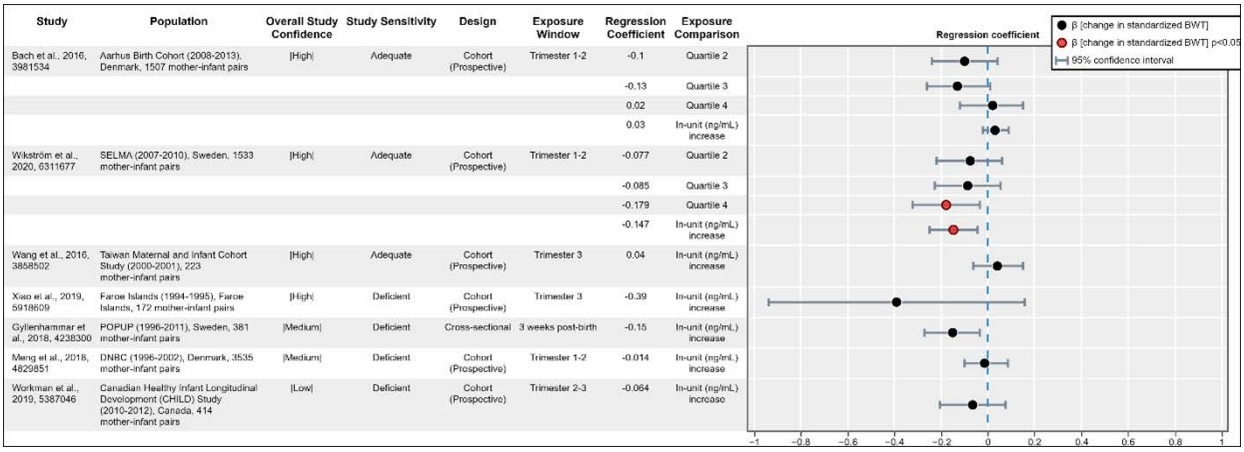


Figure 3-26.PFDA and birth weight z-scores (overall population)[a]. Refer to Birth Weight-Z for details on the individual study evaluation review.

Abbreviations: BWT = Birth Weight
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

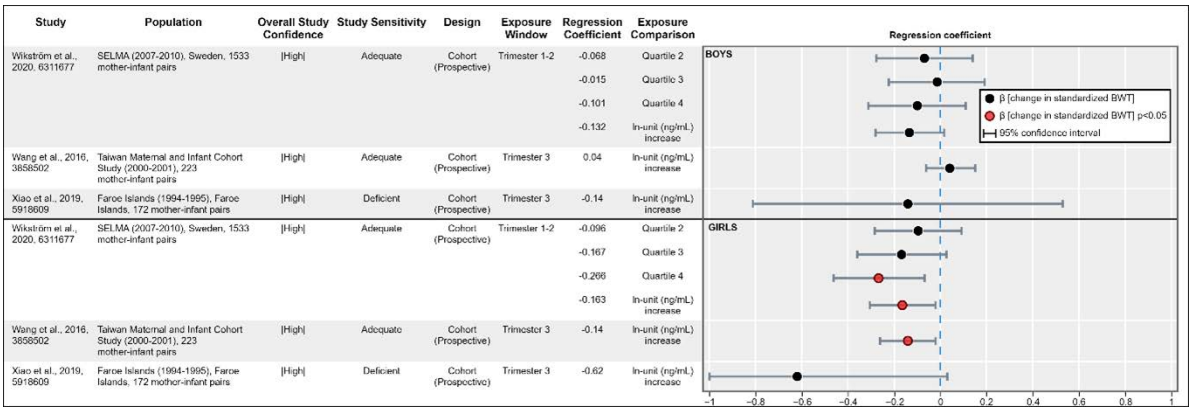


Figure 3-27.PFDA and birth weight z-score (sex-stratified)[a]. Refer to Birth Weight-Z Score Sex-Stratified for details on the individual study evaluation review

Abbreviations: BWT = Birth Weight
[a]Studies are sorted first by overall study confidence level then by Exposure Window examined.

Overall Population Results

Twenty-two studies (6 *high* and 8 each *medium* and *low* confidence) examined mean BWT differences in the overall population (see Figure 3-28). Although some of these were not statistically significant, 11 of the 22 studies reported some deficits including 4 *high*, 5 *medium*, and 2 *low* confidence studies. Eight studies in the overall population were null (Chen et al., 2021; Buck Louis et al., 2018; Meng et al., 2018; Shi et al., 2017; Starling et al., 2017; Woods et al., 2017; Bach et al., 2016; Callan et al., 2016) and three others reported increased mean BWT with increasing PFDA exposures (Gao et al., 2019; Xu et al., 2019b; Cao et al., 2018). Five of the six studies with categorical

data did not show definitive BWT deficits; however, the one study that reported deficits did demonstrate an exposure-response relationship in the overall population (Wikström et al., 2020).

There was considerable variability in BWT deficits (β range: -29 to -101 g) per ln-unit increases, with eight studies ranging from 29 to 72 grams. The *high* confidence study by Luo et al. (2021) showed a statistically significant larger BWT deficit (β = -96.8 g; 95%CI: −178.0, −15.5 per each ln-unit PFDA increase). For each ln-unit PFDA increase, statistically significant reductions similar in magnitude were reported by the *medium* confidence studies by Swedish Environmental Protection Agency (2017) (β = −94 g; 95%CI: −163, −25) and Kwon et al. (2016) (β = −101 g; 95%CI: −184.8, −17.7). The *medium* confidence study by Kashino et al. (2020) reported a large deficit between PFDA exposure in the overall population (β = −31.4 g; 95%CI: −60.0, −2.7 per each ln-unit increase). For each ln-unit PFDA increase, smaller non-statistically significant BWT deficits in two *high* confidence studies by Yao et al. (2021) (β = −46.3 g; 95%CI: −131.1, 38.5) and Valvi et al. (2017) (β = −59 g; 95%CI: −147, 26). The medium confidence study by Lenters et al. (2016) detected a BWT deficit (β = −31 g; 95%: −75, 12 for each ln-unit PFDA increase) in single pollutant multivariate models, although PFDA was not selected as an important independent predictor in their multi-pollutant elastic net model adjusting for other PFAS exposures and phthalate metabolites (see more details in Appendix F). The associations noted in many studies were evident despite some limitations, such as low exposure levels and/or narrow contrasts which can decrease study sensitivity and statistical power. In contrast to the medium and high confidence studies which exhibited associations in the overall population, there was more heterogeneity in the *low* confidence studies often noted by imprecision. Overall, 10 of the 22 studies of the overall population with mean BWT data were deficient in study sensitivity given very low PFDA ranges and median values (from 0.08 to 0.24 ng/mL) (see Table 3-20); this included 5 of the 8 null studies (Meng et al., 2018; Shi et al., 2017; Starling et al., 2017; Woods et al., 2017; Callan et al., 2016). Two (Buck Louis et al., 2018; Bach et al., 2016) of the remaining three null studies also reported low median and IQR values (0.20–0.30); thus, study sensitivity may partially explain some of these null associations given the limited exposure contrasts.

Sex-Specific Results

Although they were not always consistent across sexes within each study, most studies showed some mean BWT deficits in either or both sexes (see Figure 3-29). For example, nine studies each in girls and boys showed some BWT reductions in relation to PFDA, including six out of eleven *medium* and *high* confidence studies in boys and seven out of eleven *medium* and *high* confidence studies in girls. Null associations were reported in two studies each for boys (Meng et al., 2018; Robledo et al., 2015) and girls (Hjermitslev et al., 2020; Swedish Environmental Protection Agency, 2017), while increased BWT were reported in three studies in girls (Cao et al., 2018; Lind et al., 2017a; Shi et al., 2017) and boys (Cao et al., 2018; Bach et al., 2016; Wang et al., 2016).

Males

Among the five (2 *high*, 2 *medium*, and 1 *low* confidence) studies showing BWT deficits in both sexes, three studies reported larger mean BWT deficits in boys (Hall et al., 2022; Kashino et al., 2020; Valvi et al., 2017) while two did in girls (Wikström et al., 2020; Li et al., 2017). The deficits across sexes were quite variable per each unit change in PFDA exposures; with mean BWT deficits ranging from -20 g (Hjermitslev et al., 2020) to -156 g (Swedish Environmental Protection Agency, 2017) in boys. Smaller per ln-unit PFDA changes of -24 g were noted in two studies (Kashino et al., 2020; Meng et al., 2018) for girls compared to very large changes of -140 g (Wang et al., 2016) and -254 g observed in Robledo et al. (2015). The medium confidence study by Hall et al. (2022) reported non-significant deficits only in tertile 3 for boys (β = −73.2 g; 95% CI: −307.2, 160.8) and girls (β = −50.3 g; 95%CI: -185.3, 84.7) relative to tertile 1.

Females

The *high* confidence study by Wang et al. (2016) reported a mean birth weight decrease among girls only (β = −140 g; 95% CI: −260, −20) per each ln increase. Among these girls, they also reported large mean BWT deficits in PFDA quartiles 3 (β = −120 g; 95%CI: -330, 100) and 4 (β = −230 g; 95% CI: −440, −10) compared to the Q1 referent. The *high* confidence study by (Wikström et al., 2020) reported an exposure-response relationship among girls with BWT deficits ranging from -42 to -116 g but only in quartile 4 (β = -27 g; 95%CI: −118, 64) for boys. Although deficits were not seen in the *high* confidence (Bach et al., 2016) study among 743 girls based on continuous exposure expressions, large non-monotonic deficits were noted across all three upper PFDA quartiles. In contrast, their sister publication (not shown on Figure 3-29) by Bjerregaard-Olesen et al. (2019) did report BWT deficits of 43 g (95%CI: −102, 16) per each ln-unit increase in a subset of 334 girls

Overall, there was limited patterns in results across sexes or across study characteristics. Among the studies showing mean BWT associations, six of nine studies in girls and five of nine studies in boys were based on biomarker samples later in pregnancy or post-partum. This might be indicative of potential bias related to pregnancy hemodynamics. Study sensitivity was limited in half the studies but did not appear to explain the four null studies (two each were adequate and deficient).

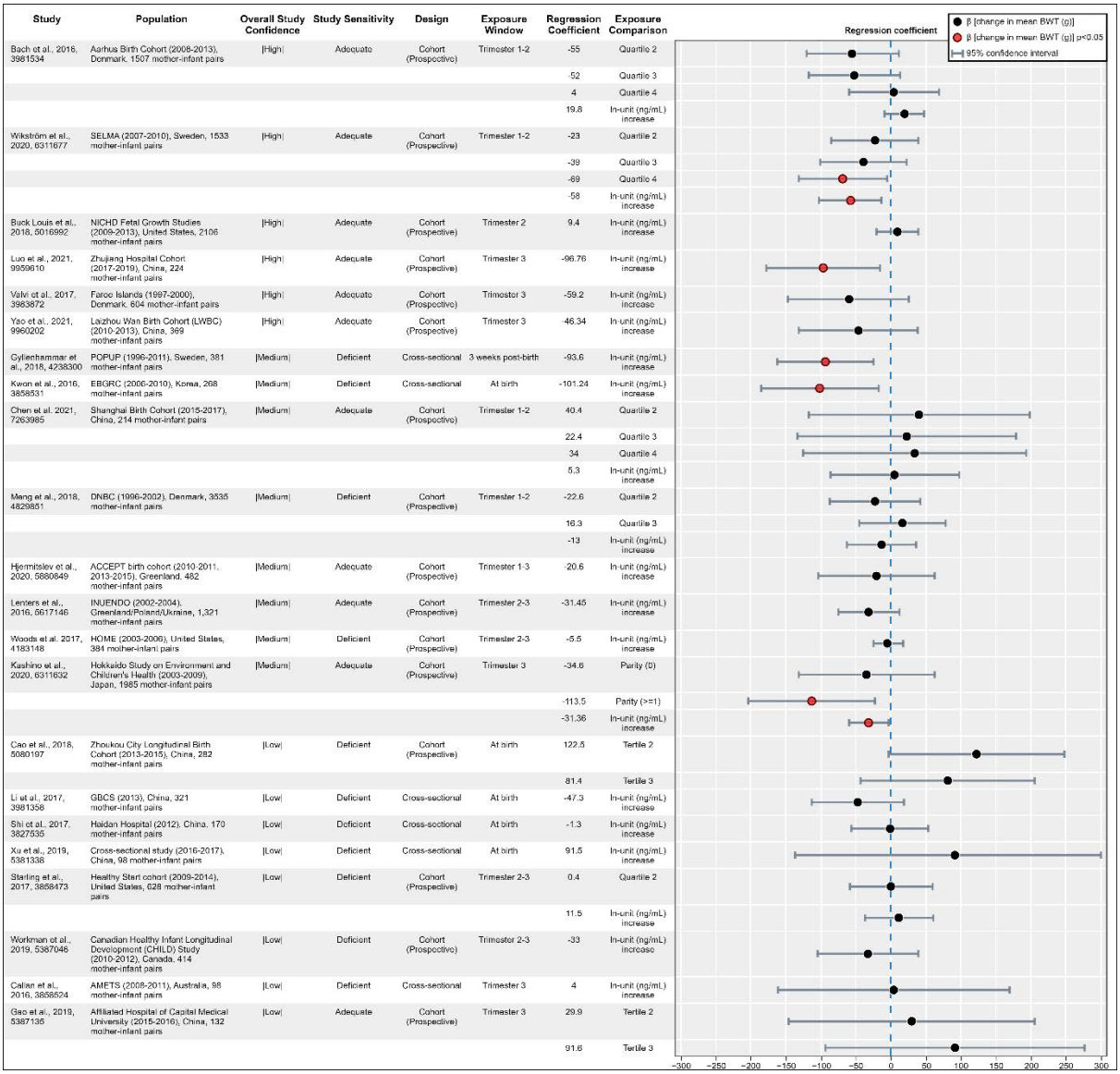
BWT Summary

Eighteen of 28 studies examining mean or standardized BWT measures in the overall population or each sex including 17 of the 26 studies examining mean BWT measures. Eleven of 22 studies (and 9 of 14 *medium* and *high* confidence) examining mean BWT in the overall population. Although there was not a clear sex-specific effect of PFDA, eight studies each in girls and boys showed some mean BWT reductions; four studies showed deficits in both sexes. Few studies examined non-linear relationships between PFDA and mean BWT. The lone study that reported

deficits across categories, demonstrated an exposure-response relationship for mean BWT, while one of two studies showed this for standardized BWT measures.

Eleven of the 22 studies of the overall population were deficient in study sensitivity with very low PFDA contrasts which may partially explain some of these null associations. For example, among the eight null studies examining mean BWT measures in the overall population, there was a slight preponderance of deficient study sensitivity (five compared to three with adequate study sensitivity). There was a definitive pattern by sampling timing as only two of the eleven studies (including two of nine medium/high studies) reporting BWT deficits in the overall population had early sampling biomarkers measures during pregnancy. The majority of sex-specific studies reporting BWT deficits were also based on later biomarker sampling (defined here as trimester 2 exclusive onward).

Although the collective evidence is fairly consistent of an association between BWT and PFDA there is considerable uncertainty given that pregnancy hemodynamic factors related to sample timing may explain some of the reported BWT deficits.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Bach et al., 2016, 3981534	Aarhus Birth Cohort (2008-2013), Denmark, 1507 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	-55	Quartile 2
						-52	Quartile 3
						4	Quartile 4
						19.8	ln-unit (ng/mL) increase
Wikström et al., 2020, 6311677	SELMA (2007-2010), Sweden, 1533 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	-23	Quartile 2
						-39	Quartile 3
						-69	Quartile 4
						-58	ln-unit (ng/mL) increase
Buck Louis et al., 2018, 5016992	NICHD Fetal Growth Studies (2009-2013), United States, 2106 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 2	9.4	ln-unit (ng/mL) increase
Luo et al., 2021, 9959610	Zhujiang Hospital Cohort (2017-2019), China, 224 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-96.76	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-59.2	ln-unit (ng/mL) increase
Yao et al., 2021, 9960202	Laizhou Wan Birth Cohort (LWBC) (2010-2013), China, 369 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	46.34	ln-unit (ng/mL) increase
Gyllenhammar et al., 2018, 4238300	POPUP (1996-2011), Sweden, 381 mother-infant pairs	Medium	Deficient	Cross-sectional	3 weeks post-birth	-93.6	ln-unit (ng/mL) increase
Kwon et al., 2016, 3858531	EBGRC (2006-2010), Korea, 268 mother-infant pairs	Medium	Deficient	Cross-sectional	At birth	-101.24	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-2	40.4	Quartile 2
						22.4	Quartile 3
						34	Quartile 4
						5.3	ln-unit (ng/mL) increase
Meng et al., 2018, 4829851	DNBC (1996-2002), Denmark, 3535 mother-infant pairs	Medium	Deficient	Cohort (Prospective)	Trimester 1-2	-22.6	Quartile 2
						16.3	Quartile 3
						-13	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-3	-20.6	ln-unit (ng/mL) increase
Lenters et al., 2016, 5617146	INUENDO (2002-2004), Greenland/Poland/Ukraine, 1,321 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 2-3	-31.45	ln-unit (ng/mL) increase
Woods et al. 2017, 4183148	HOME (2003-2006), United States, 384 mother-infant pairs	Medium	Deficient	Cohort (Prospective)	Trimester 2-3	-5.5	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	-34.6	Parity (0)
						-113.5	Parity (>=1)
						-31.36	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Low	Deficient	Cohort (Prospective)	At birth	122.5	Tertile 2
						81.4	Tertile 3
Li et al., 2017, 3981358	GBCS (2013), China, 321 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	-47.3	ln-unit (ng/mL) increase
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	-1.3	ln-unit (ng/mL) increase
Xu et al., 2019, 5381338	Cross-sectional study (2016-2017), China, 98 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	91.5	ln-unit (ng/mL) increase
Starling et al., 2017, 3858473	Healthy Start cohort (2009-2014), United States, 628 mother-infant pairs	Low	Deficient	Cohort (Prospective)	Trimester 2-3	0.4	Quartile 2
						11.5	ln-unit (ng/mL) increase
Workman et al., 2019, 5387046	Canadian Healthy Infant Longitudinal Development (CHILD) Study (2010-2012), Canada, 414 mother-infant pairs	Low	Deficient	Cohort (Prospective)	Trimester 2-3	-33	ln-unit (ng/mL) increase
Callan et al., 2016, 3858524	AMETS (2008-2011), Australia, 98 mother-infant pairs	Low	Deficient	Cross-sectional	Trimester 3	4	ln-unit (ng/mL) increase
Gao et al., 2019, 5387135	Affiliated Hospital of Capital Medical University (2015-2016), China, 132 mother-infant pairs	Low	Adequate	Cohort (Prospective)	Trimester 3	29.9	Tertile 2
						91.6	Tertile 3

Figure 3-28. Overall study population mean birth weight results for 22 PFDA epidemiological studies[a-e]. (results can be viewed by clicking the HAWC link).

Abbreviation: BWT = Birth Weight

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during the first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c.] If a study presented regression coefficients for continuous exposure with multiple exposure units, only one unit change is shown (e.g., (Bach et al., 2016), with the exception of (Li et al., 2017), which displays both IQR and ln-unit (ng/mL) values.

[d.] The results displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared to a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[e.] Xu et al. (2019a) results are truncated for the 210.7 gram increase; the complete 95% CI ranges from -314.3 to 735.8 grams.

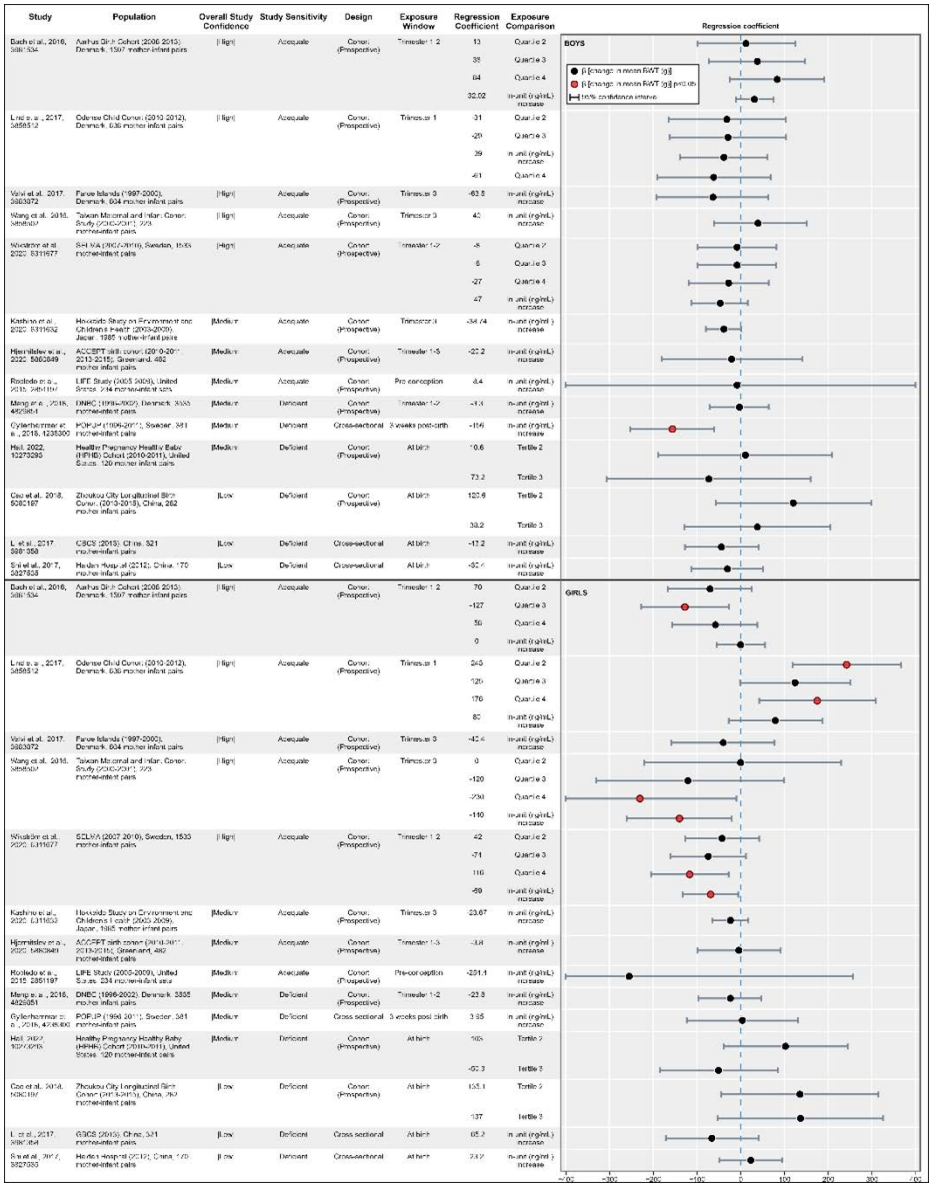


Figure 3-29. Sex-specific mean birth weight results for 14 PFDA epidemiological studies: boys are above reference line, girls are below [a-g]. (results can be viewed by clicking the HAWC link).

Abbreviation: BWT = Birth Weight

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Meng et al. (2018) pooled samples from umbilical cord blood and maternal plasma during first and second trimesters. The remaining studies were all based on either one umbilical or maternal sample.

[c.] If a study presented regression coefficients for continuous exposure with multiple exposure units, only one unit change is shown.

[d.] The results displayed here for mean birth weight in the POPUP Cohort are from a larger population of participants (Swedish Environmental Protection Agency, 2017) compared to a sample size of 381 in their 2018 publication Gyllenhammar et al. (2018).

[e.] (Robledo et al., 2015) regression coefficients for maternal serum PFDA are displayed. The complete 95% CI for the male-8.4 gram difference ranges from -434.3 to 417.6 grams; the complete 95% CI for the female -254.4 gram difference ranges from -766.7 to 258.1 grams.

[f.] (Wang et al., 2016) quartile results are truncated; the complete 95% CI for the -230 gram difference (Quartile 4) ranges from -440 to -10 grams. Quartile results reported for females only.

[g.] For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses (e.g., Hall et al. (2022)).

Small for Gestational Age and Low Birth Weight

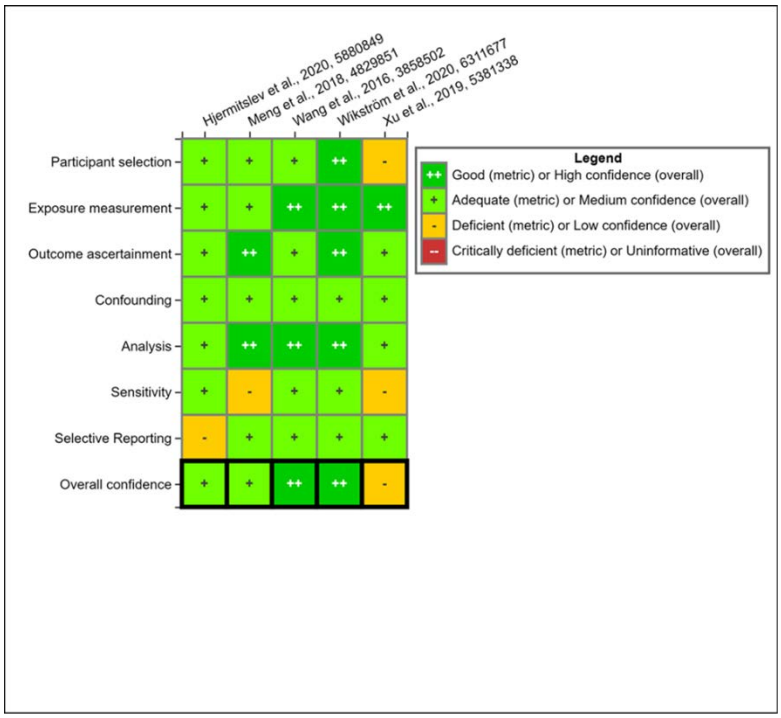


Figure 3-30. Low birth weight/small for gestational age heatmap. Results can be viewed by clicking the HAWC link.

Five epidemiological studies included here examined associations between PFDA exposure and different dichotomous fetal growth restriction endpoints, such as SGA (or related intrauterine growth retardation endpoints) (Wikström et al., 2020; Xu et al., 2019a; Wang et al., 2016) or low birth weight (LBW) (Hjermitslev et al., 2020; Meng et al., 2018). Two studies were *high* confidence (Wikström et al., 2020; Wang et al., 2016), two were *medium* confidence (Hjermitslev et al., 2020; Meng et al., 2018) and one study was *low* confidence (Xu et al., 2019a). Three of these studies had *adequate* study sensitivity (Hjermitslev et al., 2020; Wikström et al., 2020; Wang et al., 2016) while two were deficient (Xu et al., 2019a; Meng et al., 2018) (Figure 3-30).

Two (Wikström et al., 2020; Wang et al., 2016) of three SGA studies showed some adverse associations, while one study was null (Xu et al., 2019a) (Figure 3-31). The *high* confidence study by Wang et al. (2016) reported a statistically significant increased odds ratio (OR) (3.14; 95%CI: 1.07, 9.19) for SGA per each ln-unit PFDA increase among females. Increased risks were not

detected among males (OR = 0.71; 95%CI: 0.33, 1.52). The *medium* confidence study by (Wikström et al., 2020) showed that PFDA was associated with SGA based on a continuous measure (OR = 1.46; 95%CI: 1.06, 2.01 per each ln-unit increase), as well as categorical exposures (Q4: OR = 1.50; 95%CI: 0.94, 2.38 compared to Q1 referent). Results were stronger among females (OR = 1.62; 95%CI: 0.98, 2.67) than males (OR = 1.36; 95%CI: 0.90, 2.07) per each ln-unit increase.

Two studies reported relatively small ORs that were not statistically significant between PFDA and risk of LBW, while another study showed an 80% increased risk of very LBW per each ln-unit increase. The *medium* confidence study by (Meng et al., 2018) reported a larger risk (OR = 1.8; 95%CI: 0.9, 4.0 per each ln-unit increase) for a very LBW (i.e., <2,260 grams) measure compared to the typical LBW definition of <2,500 grams (OR = 1.3; 95%CI: 0.7, 2.15). There was also no evidence of increased risk across PFDA quartiles or an exposure-response relationship, but the study may have been impacted by sparse cell bias. A nonsignificant increased odds (OR = 1.15; 95%CI: 0.57, 2.33) was reported in the *medium* confidence study by Hjermitslev et al. (2020) per each PFDA ln-unit increase.

SGA/LBW Summary

Although they were not always statistically significant, three (Wikström et al., 2020; Meng et al., 2018; Wang et al., 2016) of the five studies examining either SGA, LBW, or very LBW showed some increased risks with increasing PFDA exposures. There was no evidence of an exposure-response relationships based on categorical data in one SGA and one LBW study. The relative risks reported in the two LBW studies based on either categorical or continuous exposures (per each unit increase) were consistent in magnitude (OR range: 1.2-1.3), while a larger risk was found (1.8) for the very LBW endpoint. SGA results were more variable based on sex-specific findings but both studies showed larger risks among females. Two of the three studies with stronger results were based on early biomarker sampling.

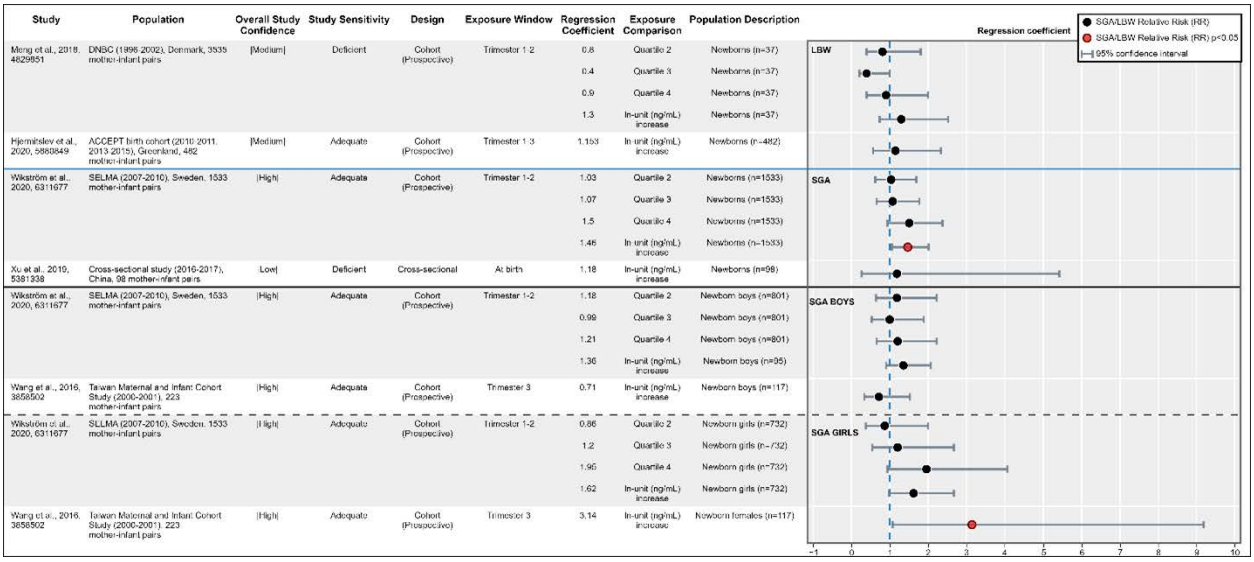


Figure 3-31. Dichotomous fetal growth restriction (small for gestational age and low birth weight) forest plot[a-d]. Results can be viewed at the HAWC link.

Abbreviations: SGA = Small for Gestational Age; LBW = Low Birth Weight

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Low birth weight overall population data above blue reference line.

[c.] Overall population SGA data above Black reference line; sex-stratified SGA data below reference line.

[d.] Sex-Stratified SGA; Boys Above Dotted Line, Girls Below.

Birth Length Measures

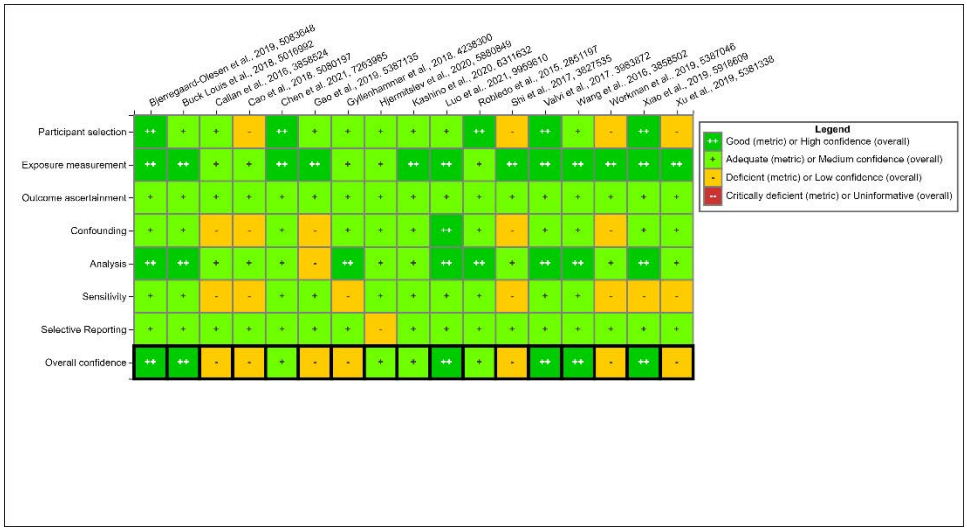


Figure 3-32. Study evaluation results for 17 epidemiological studies of birth length and PFDA. Refer to HAWC for details on the study evaluation review: HAWC Human Birth Length.

Seventeen studies examined the relationship between PFDA exposures and mean or standardized birth length measures including 15 studies that examined changes in the overall

population and ten that examined sex-specific changes (see Figure 3-32). Two of these 10 reported sex-specific analyses only (Wang et al., 2016; Robledo et al., 2015). Most of the studies reported mean birth length differences in relation to PFDA exposures, but two reported standardized birth length measures across the sexes only (Gyllenhammar et al., 2018) or in both sexes as well as the overall population (Xiao et al., 2019).

Six of the 17 studies examining birth length measures in relation to PFDA were classified as *high* confidence (Luo et al., 2021; Xiao et al., 2019; Buck Louis et al., 2018; Valvi et al., 2017; Bach et al., 2016; Wang et al., 2016), four were *medium* confidence (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Robledo et al., 2015), and seven were *low* confidence (Gao et al., 2019; Workman et al., 2019; Xu et al., 2019b; Cao et al., 2018; Gyllenhammar et al., 2018; Shi et al., 2017; Callan et al., 2016) (see Figure 3-32). All but one of the ten *medium* and *high* confidence studies were considered to have adequate study sensitivity, whereas the remaining six *low* confidence studies were classified as deficient.

Birth Length: Overall Population

Four (1 *high* and 3 *low* confidence studies (Bjerregaard-Olesen et al., 2019; Xu et al., 2019b; Cao et al., 2018; Callan et al., 2016) of the 15 studies examining the overall population reported increased birth length in relation to PFDA, while six studies were null (Hjermitslev et al., 2020; Kashino et al., 2020; Gao et al., 2019; Buck Louis et al., 2018; Shi et al., 2017; Valvi et al., 2017) (see Figure 3-33). Five (2 *high*, 1 *medium* and 2 *low* confidence) of the 15 studies reported reduced birth length in the overall population. The *high* confidence study by Buck Louis et al. (2018) also did not show an association for birth length and PFDA in the overall population. They did report that each standard deviation increase in PFDA was associated with reductions in upper arm length (β = −0.09 cm; 95% CI: −0.14, −0.04); these were largely due to associations detected among White (β = −0.21 cm; 95% CI: −0.31, −0.11) and Asian neonates (β = −0.15 cm; 95% CI: −0.25, −0.05). They also reported reductions in upper thigh length (β = −0.14 cm; −0.21, −0.07) in the NICHD cohort with the largest associations detected among White (β = −0.32 cm; 95% CI: −0.45, −0.19) and Asian neonates (β = −0.18 cm; 95% CI: −0.30, −0.06).

The *high* confidence study by Xiao et al. (2019) reported similar birth length z-scores in the overall population (β = -0.49; 95%CI: -1.00, 0.01), girls (β = -0.46; 95%CI: -1.07, 0.14), and boys (β = -0.53; 95%CI: -1.17, 0.10). The *high* confidence study by Luo et al. (2021) showed a non-significant birth length deficit (β = -0.23 cm; 95%CI: −0.64, 0.19) per each ln-unit PFDA increase. The *medium* confidence study by Chen et al. (2021) detected a statistically significant birth length deficit (-0.27 cm; 95%CI: -0.53, -0.01 per each ln-unit increase), while the *low* confidence study by Workman et al. (2019) reported a nonsignificant birth length deficit (β = -0.3 cm; 95%CI: -0.8, 0.2 per each ln-unit increase). A small but precise deficit of 0.19 cm (95%CI: -0.36, 0.02 per each ln-unit) was reported in the *low* confidence study by Gyllenhammar et al. (2018) for their standardized birth length measures.

Among these five studies (two *high*, one *medium* and two *low* confidence) showing some evidence of birth length deficits, there was limited evidence of exposure-response relationships with only study (Chen et al., 2021) examining categorical data showing deficits in quartile 4 only (-0.46 cm; 95%CI: -0.91, -0.01). There was a preponderance (four of five studies) of birth length reductions in the overall population from studies based on later sampled biomarkers which may be indicative of an impact of pregnancy hemodynamics. Study sensitivity did not seem to explain null results, as five of these six studies had adequate ratings.

Birth Length: Sex-specific Results

Among the 10 studies with sex-specific results, seven different ones (4 *high*, 3 *medium* confidence) showed some evidence of birth length deficits in relation to PFDA. This included four studies each in girls and in boys (see Figure 3-34). Only the *high* confidence study by Xiao et al. (2019) noted above found reduced standardized birth length measures in both girls and boys. Three studies in girls were null (Hjermitslev et al., 2020; Kashino et al., 2020; Robledo et al., 2015) and two (Cao et al., 2018; Valvi et al., 2017) showed increases in birth length with increasing PFDA exposures. Four studies (Chen et al., 2021; Cao et al., 2018; Shi et al., 2017; Wang et al., 2016) in boys were null and two (Hjermitslev et al., 2020; Bjerregaard-Olesen et al., 2019) showed slight non-significant increases in birth length.

In addition to the *high* confidence study by Xiao et al. (2019) noted above, three other studies reported any suggestion of smaller birth length among boys. The *medium* confidence study by Robledo et al. (2015) reported a non-statistically significant reduction among boys (β = −1.15 cm; 95% CI: −3.65, 0.96 per each ln-unit based on maternal serum measures). Smaller birth length deficits per each PFDA ln-unit increase were detected in the *high* confidence study by Valvi et al. (2017) (β = −0.23 cm; 95%CI: −0.68, 0.22) and the *medium* confidence study by Kashino et al. (2020) (β = −0.16 cm; 95%CI: −0.38, 0.07).

Including the Xiao et al. (2019) data above, four of the 10 studies in females reported some birth length reductions. The *high* confidence study by Wang et al. (2016) reported non-statistically significant deficits were detected among girls in quartile 4 (β = −0.75 cm; 95% CI: −2.09, 0.59) and for each PFDA ln-unit increase (β = −0.47 cm; 95% CI: −1.23, 0.30). Birth length deficits similar in magnitude (β = −0.44 cm; 95%CI: −0.79, −0.09 per each ln-unit PFDA increase) were detected among girls in the *medium* confidence study by Chen et al. (2021). Smaller birth length changes were detected among girls (β = −0.22 cm; 95%CI: −0.86, 0.43) in the Aarhus Birth Cohort Bjerregaard-Olesen et al. (2019) study.

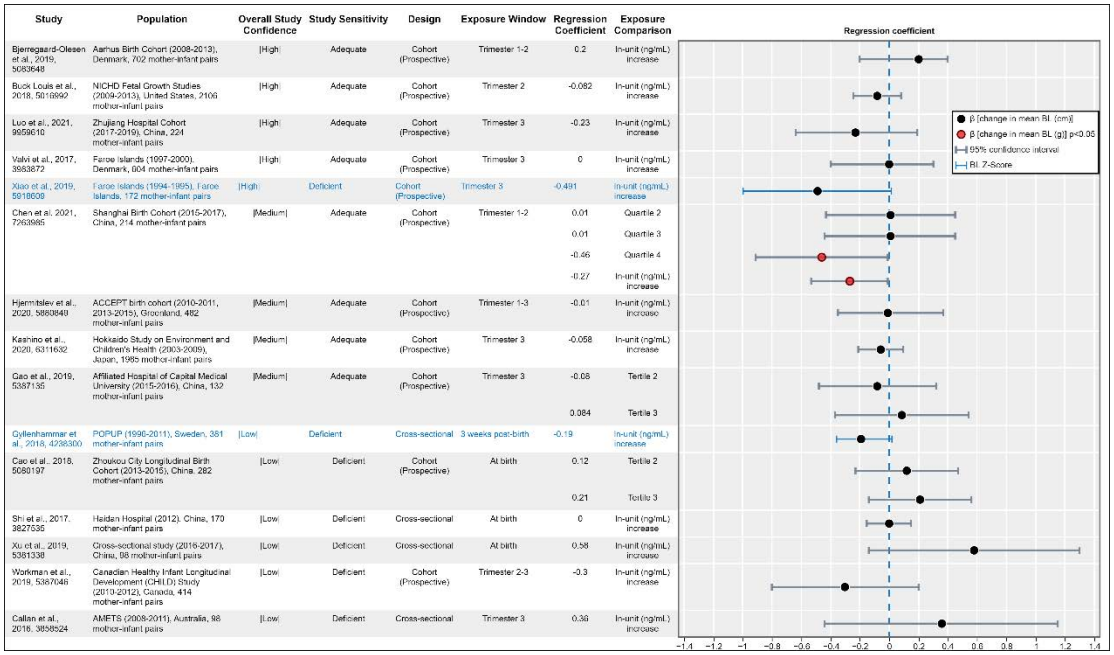
Among the 10 studies in total, 7 different ones reported some evidence of sex-specific associations between PFDA and reduced birth length, including 4 studies each in girls and in boys. Few patterns were evident across study characteristics and study sensitivity did not appear to be an explanatory factor for null studies. Sample timing also did not appear to be a strong determinant

of the sex-specific study results as four of the seven different studies reporting reductions were based on later biomarker sampling.

Birth Length Summary

Overall, ten different studies among the 16 in total showed some evidence of birth length deficits in relation to PFDA exposures in either the overall population or in one/both sexes. Five (2 *high*, 1 *medium,* and 2 *low* confidence) of the 14 studies examining the overall population reported birth length deficits that were consistent in magnitude (mean birth length deficit range: 0.19 to 0.30 per each unit increase). Birth length changes were a bit more variable in the seven studies (four *high*, three *medium* confidence) that reported sex-specific deficits. Although three studies reported mean birth length reductions around 0.20 cm, the remaining sex-specific studies ranged from -0.44 to -1.15 cm per each ln-unit PFDA increase. Four (2 *high* and 2 *medium* confidence) of the 10 studies each in boys and girls (3 *high* and 1 *medium* confidence) reported birth length deficits in relation to PFDA.

Although some of these studies reported large differences in birth length, there was no direct evidence exposure-response relationships in the few studies with categorical data. However, the Wang et al. (2016) analysis in girls did show some large gradients in birth length among the upper two quartiles. The (Chen et al., 2021) also showed larger deficits in quartile 4 relative to quartile 1. Few patterns were evident across study characteristics, and study sensitivity did not appear to be an explanatory factor for null studies. Seven of ten different studies reporting some birth length reductions in the overall population (four of five) and across sexes (four of seven) were based on later biomarker samples. This may be indicative of potential bias due to the impact of pregnancy hemodynamics and adds some uncertainty.



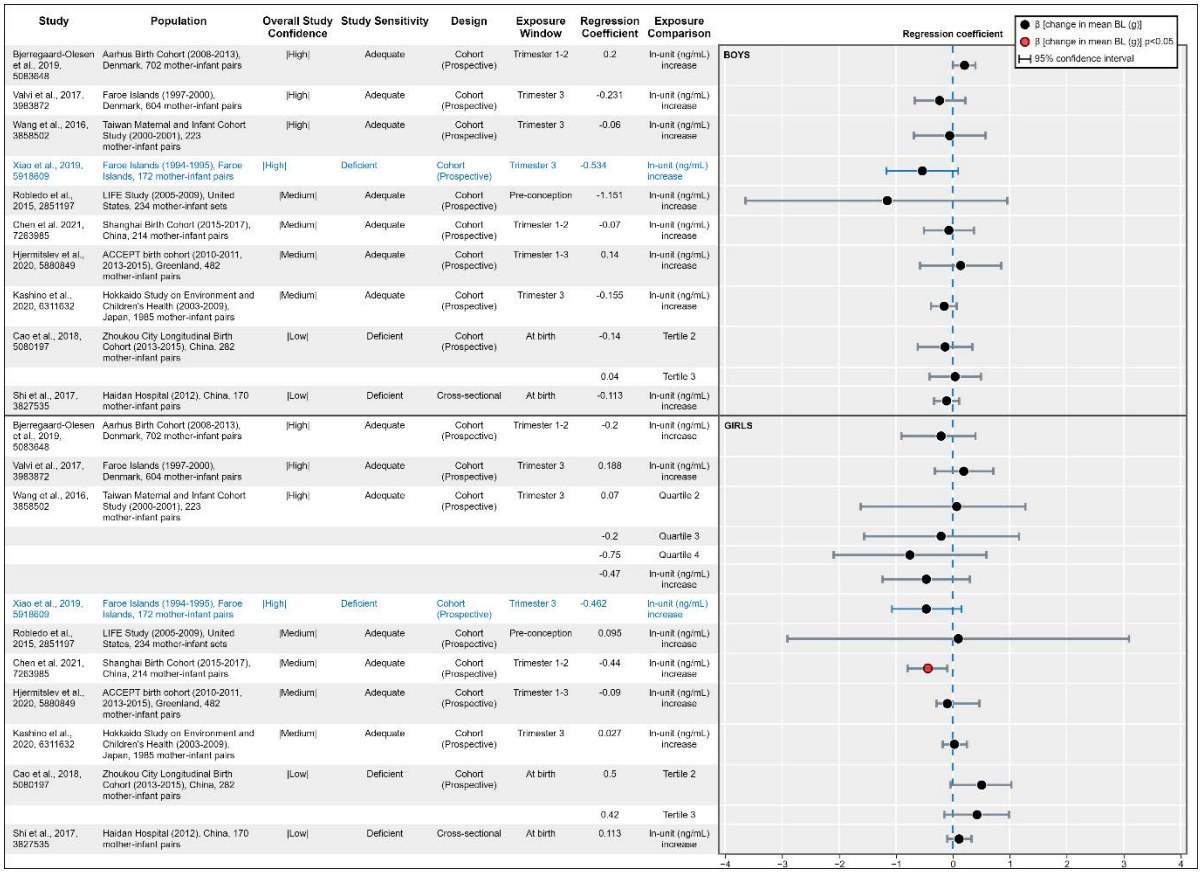
Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
Bjerregaard-Olesen et al., 2019, 5083648	Aarhus Birth Cohort (2008-2013), Denmark, 702 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	0.2	ln-unit (ng/mL) increase
Buck Louis et al., 2018, 5010892	NICHD Fetal Growth Studies (2009-2013), United States, 2106 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 2	-0.082	ln-unit (ng/mL) increase
Luo et al., 2021, 9959610	Zhujiang Hospital Cohort (2017-2019), China, 224 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-0.23	ln-unit (ng/mL) increase
Volvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	0	ln-unit (ng/mL) increase
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), Faroe Islands, 172 mother-infant pairs	High	Deficient	Cohort (Prospective)	Trimester 3	-0.491	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-2	0.01	Quartile 2
						0.01	Quartile 3
						-0.46	Quartile 4
						-0.27	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5860649	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 462 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-3	-0.01	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	-0.058	ln-unit (ng/mL) increase
Gao et al., 2019, 5387135	Affiliated Hospital of Capital Medical University (2015-2016), China, 132 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	-0.08	Tertile 2
						0.084	Tertile 3
Gyllenhammar et al., 2018, 4238300	POPUP (1996-2011), Sweden, 381 mother-infant pairs	Low	Deficient	Cross-sectional	3 weeks post-birth	-0.19	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Low	Deficient	Cohort (Prospective)	At birth	0.12	Tertile 2
						0.21	Tertile 3
Shi et al., 2017, 3827536	Haidan Hospital (2012), China, 170 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	0	ln-unit (ng/mL) increase
Xu et al., 2019, 5381338	Cross-sectional study (2016-2017), China, 98 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	0.58	ln-unit (ng/mL) increase
Workman et al., 2019, 5367046	Canadian Healthy Infant Longitudinal Development (CHILD) Study (2010-2012), Canada, 414 mother-infant pairs	Low	Deficient	Cohort (Prospective)	Trimester 2-3	-0.3	ln-unit (ng/mL) increase
Callan et al., 2016, 3858524	AMETS (2008-2011), Australia, 98 mother-infant pairs	Low	Deficient	Cross-sectional	Trimester 3	0.39	ln-unit (ng/mL) increase

Figure 3-33. Overall study population mean birth length results for 15 PFDA epidemiological studies[a,b]. (Results can be viewed by clicking the HAWC link)

Abbreviation: BL = Birth Length

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Xiao et al. (2019) and Gyllenhammar et al. (2018) report birth length z-score data.



Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison
BOYS							
Bjerregaard-Olesen et al., 2019, 5083648	Aarhus Birth Cohort (2008-2013), Denmark, 702 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	0.2	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-0.231	ln-unit (ng/mL) increase
Wang et al., 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001), 223 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	-0.06	ln-unit (ng/mL) increase
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), Faroe Islands, 172 mother-infant pairs	High	Deficient	Cohort (Prospective)	Trimester 3	-0.534	ln-unit (ng/mL) increase
Robledo et al., 2015, 2851197	LIFE Study (2005-2009), United States, 234 mother-infant sets	Medium	Adequate	Cohort (Prospective)	Pre-conception	-1.151	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-2	-0.07	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-3	0.14	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	-0.155	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Low	Deficient	Cohort (Prospective)	At birth	-0.14	Tertile 2
						0.04	Tertile 3
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	-0.113	ln-unit (ng/mL) increase
GIRLS							
Bjerregaard-Olesen et al., 2019, 5083648	Aarhus Birth Cohort (2008-2013), Denmark, 702 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 1-2	-0.2	ln-unit (ng/mL) increase
Valvi et al., 2017, 3983872	Faroe Islands (1997-2000), Denmark, 604 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	0.188	ln-unit (ng/mL) increase
Wang et al., 2016, 3858502	Taiwan Maternal and Infant Cohort Study (2000-2001), 223 mother-infant pairs	High	Adequate	Cohort (Prospective)	Trimester 3	0.07	Quartile 2
						-0.2	Quartile 3
						-0.75	Quartile 4
						-0.47	ln-unit (ng/mL) increase
Xiao et al., 2019, 5918609	Faroe Islands (1994-1995), Faroe Islands, 172 mother-infant pairs	High	Deficient	Cohort (Prospective)	Trimester 3	-0.462	ln-unit (ng/mL) increase
Robledo et al., 2015, 2851197	LIFE Study (2005-2009), United States, 234 mother-infant sets	Medium	Adequate	Cohort (Prospective)	Pre-conception	0.095	ln-unit (ng/mL) increase
Chen et al. 2021, 7263985	Shanghai Birth Cohort (2015-2017), China, 214 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-2	-0.44	ln-unit (ng/mL) increase
Hjermitslev et al., 2020, 5880849	ACCEPT birth cohort (2010-2011, 2013-2015), Greenland, 482 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 1-3	-0.09	ln-unit (ng/mL) increase
Kashino et al., 2020, 6311632	Hokkaido Study on Environment and Children's Health (2003-2009), Japan, 1985 mother-infant pairs	Medium	Adequate	Cohort (Prospective)	Trimester 3	0.027	ln-unit (ng/mL) increase
Cao et al., 2018, 5080197	Zhoukou City Longitudinal Birth Cohort (2013-2015), China, 282 mother-infant pairs	Low	Deficient	Cohort (Prospective)	At birth	0.5	Tertile 2
						0.42	Tertile 3
Shi et al., 2017, 3827535	Haidan Hospital (2012), China, 170 mother-infant pairs	Low	Deficient	Cross-sectional	At birth	0.113	ln-unit (ng/mL) increase

Figure 3-34. Sex-stratified birth length results for 10 PFDA epidemiological studies[a,b]. Results can be viewed by clicking the HAWC link.

Abbreviation: BL= Birth Length

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b] Xiao et al. (2019) reports birth length z-score data.

Head Circumference

Fourteen studies examined PFDA levels in relation to head circumference including five *high* confidence studies (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018; Valvi et al., 2017; Wang et al., 2016) and five *medium* confidence studies (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Lind et al., 2017a; Robledo et al., 2015) (see Figure 3-35). The four *low confidence* studies (Workman et al., 2019; Xu et al., 2019b; Gyllenhammar et al., 2018; Callan et al., 2016) as well as (Xiao et al., 2019) were considered deficient in the study sensitivity domain largely due to low exposure levels and/or narrow contrasts. The remaining nine *medium* and *high* confidence studies had adequate ratings in the sensitivity domain.

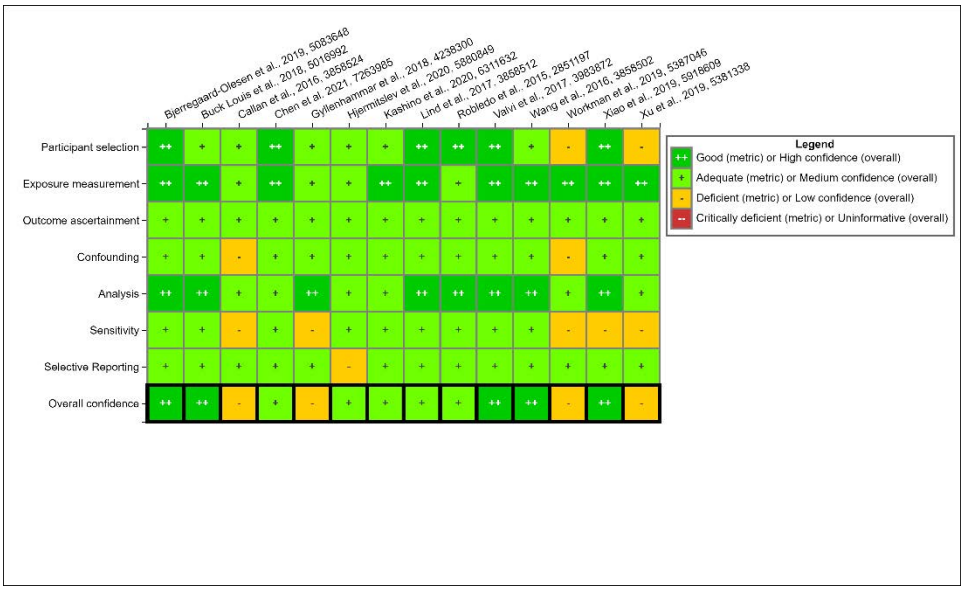


Figure 3-35.Study evaluation results for 14 epidemiological studies of head circumference and PFDA. Refer to HAWC for details on the study evaluation review**:** HAWC Human Head Circumference**.**

One study provided standardized head circumference data (Xiao et al., 2019), while the other 13 included in Figures 3-36 and 3-37 are based on mean head circumference differences. Eight studies examined sex-specific results in both boys and girls (Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Lind et al., 2017a; Valvi et al., 2017; Wang et al., 2016; Robledo et al., 2015) including three with sex-specific data only (Lind et al., 2017a; Wang et al., 2016; Robledo et al., 2015). Eleven studies reported head circumference results in the overall population (Chen et al., 2021; Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Workman et al., 2019; Xiao et al., 2019; Xu et al., 2019b; Buck Louis et al., 2018; Gyllenhammar et al., 2018; Valvi et al., 2017; Callan et al., 2016).

Head Circumference-Overall Population Results

Only 2 of 11 studies in the overall population showed head circumference associations with PFDA exposures. The *medium* confidence study by Hjermitslev et al. (2020) reported a nonsignificant decreased head circumference in the overall population (β = -0.15 cm; 95%CI: -0.37, 0.07 per each ln-unit increase). Slightly smaller but precise head circumference deficits were reported in the *medium* confidence study by Kashino et al. (2020) (β = -0.10 cm; 95%CI: -0.24, 0.003 per each ln-unit PFDA increase). In contrast, nonsignificant increased head circumference in the overall population was reported in relation to PFDA in three studies (Chen et al., 2021; Workman et al., 2019; Valvi et al., 2017). No associations were reported between PFDA exposures and mean or standardized head circumference measures in 6 of the 11 studies based on the overall population, including three studies each with *high* (Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Buck Louis et al., 2018) and *low* confidence (Xu et al., 2019b; Gyllenhammar et al., 2018; Callan et al., 2016) (see Figure 3-36).

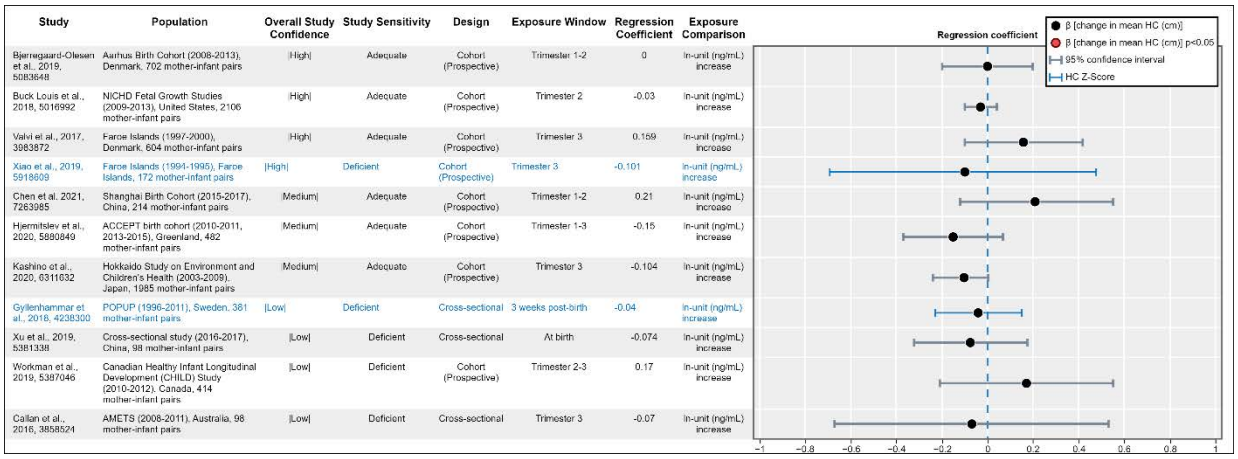


Figure 3-36. Overall population head circumference results in 11 epidemiological studies[a,b]. Refer to the HAWC link.

Abbreviation: HC = Head Circumference

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Xiao et al. (2019) reports head circumference z-score data.

Head Circumference-Sex-specific Results

Among the eight studies (Hjermitslev et al., 2020; Kashino et al., 2020; Bjerregaard-Olesen et al., 2019; Xiao et al., 2019; Lind et al., 2017a; Valvi et al., 2017; Wang et al., 2016; Robledo et al., 2015) reporting sex-specific head circumference results in both male and female neonates, three studies in girls and one in boys reported reductions with increasing PFDA exposures (see Figure 3-37). The Lind et al. (2017a) study reported an exposure-response relationship based on PFDA quartiles (range: −0.1. to −0.3 cm) in boys, but there was not much evidence of associations when scaled to each ln-unit increase (β = −0.10 cm; 95%CI: −0.5, 0.3). The *high* confidence study by Wang

et al. (2016) detected a non-significant decrease (β = −0.37 cm; 95% CI: −0.85, 0.10 per each ln-unit increase) in mean head circumference only among girls. The *medium* confidence study by Robledo et al. (2015) reported larger but very imprecise head circumference reductions for girls (β = −0.62 cm; 95% CI: −2.4, 1.2 per each ln-unit PFDA increase). The *high* confidence study by Bjerregaard-Olesen et al. (2019) showed a smaller non-significant result (β = −0.22 cm; 95%CI: -0.65, 0.22 per each ln-unit increase). In contrast, one *high* (Valvi et al., 2017); β = 0.51 cm; 95% CI: 0.13, 0.90) and one *medium* (Lind et al., 2017a); β = 0.3 cm; 95% CI: -0.1, 0.7) confidence study each reported increased birth length for female neonates for each ln-unit PFDA increase, as did the Bjerregaard-Olesen et al. (2019) study (β = 0.19 cm; 95%CI: −0.19, 0.38 each ln-unit increase) in males. Null associations were reported per each ln-unit increase in five studies in boys (Hjermitslev et al., 2020; Kashino et al., 2020; Xiao et al., 2019; Valvi et al., 2017; Wang et al., 2016) and three in girls (Hjermitslev et al., 2020; Kashino et al., 2020; Xiao et al., 2019).

Four of eight available studies reported some head circumference reductions among boys or girls including three that were based on early biomarker samples. In addition to the Lind et al. (2017a) study noted in boys above, four null studies examining different head circumference measures in relation to continuous exposures reported non-significant and imprecise deficits around -0.1 cm per each unit increase for either or both sexes (Hjermitslev et al., 2020; Kashino et al., 2020; Valvi et al., 2017; Wang et al., 2016).

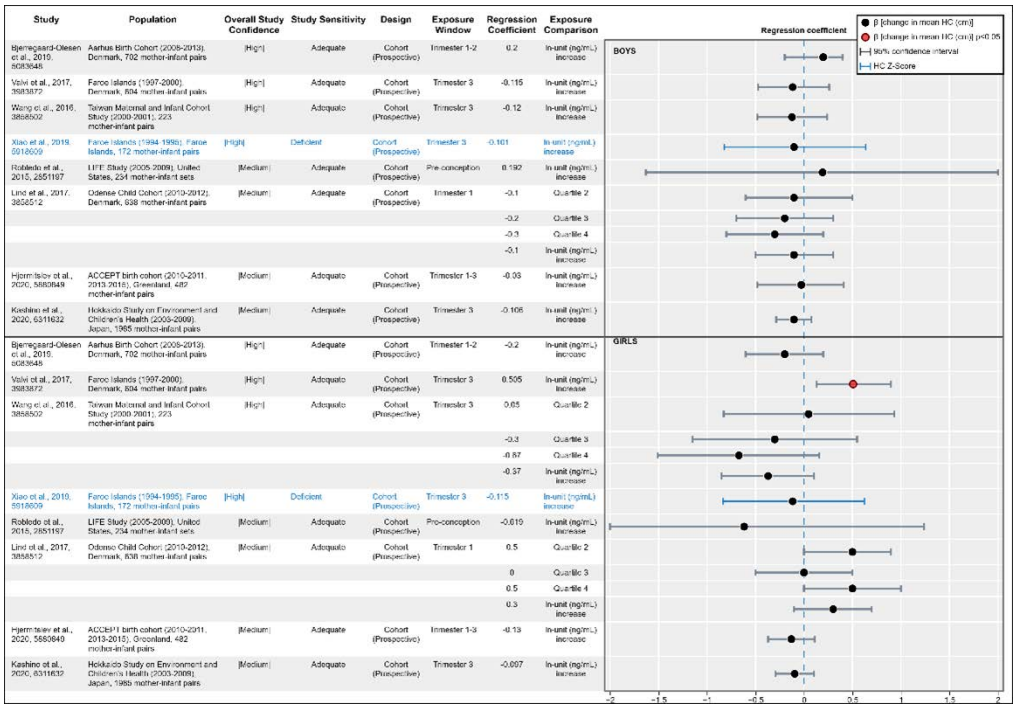


Figure 3-37. Sex-stratified head circumference results in 8 epidemiological studies [a,b]. Refer to the HAWC link.

Abbreviation: HC = Head Circumference

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b.] Xiao et al. (2019) reports head circumference z-score data.

Head Circumference-Summary

There was limited evidence of associations between PFDA and head circumference with 6 (2 *high* and 4 *medium* confidence) out of 14 studies reporting reductions in head circumference in the overall population or either or both sexes. These reductions were reported in the over one-half (6 of 10) of the *high* and *medium* confidence studies. Very limited evidence was found in the overall population with only 2 of 11 studies. Four of eight sex-specific studies reported some head circumference reductions with three of these occurring among female neonates. Four of these six studies that reported some head circumference reductions in the overall population or either sex were based on early biomarker sampling during or prior to pregnancy. In contrast to the null sex-specific studies where only one of five studies had deficient study sensitivity, nearly all (five of six) null studies in the overall population were rated as deficient. Narrow exposure contrasts in many studies of PFDA, likely limited statistical power and may have precluded the ability to detect statistically significant associations that are small in magnitude.

Fetal Growth Restriction Summary

Eighteen of the 28 studies examining different BWT measures in relation to PFDA measures in the overall population or either/both sexes, reported some evidence of associations. This included 11 different studies (and nine of fourteen *medium* and *high* confidence) out of 22 examining mean BWT in the overall population. There was considerable variability in BWT deficits (β range: -29 to -101 g per ln-unit increases) in the overall population, with seven studies ranging from 31 to 59 g deficits per each ln-unit increase. These deficits were seen despite low exposure levels and contrasts in many studies (Table 3-20). For example, among the nine *medium* and *high* studies reporting it, the PFDA IQR in the overall study populations ranged from 0.07 to 0.37 ng/mL and the median levels ranged from 0.11 to 0.55 ng/mL. Few studies examined nonlinear relationships between PFDA and mean BWT. The lone study that reported deficits across categories, demonstrated an exposure-response relationship for mean BWT, while one of two studies showed this for standardized BWT measures. Twelve of the 13 studies reporting sex-specific results showed some evidence of BWT deficits in either or both sexes. However, there was not a clear sex-specific effect of PFDA. Eight studies each in girls and boys showed some reductions and only four studies showed deficits in both sexes.

Although there was no evidence on an exposure-response relationships in the few studies with categorical data, the majority of studies reporting results for either SGA, LBW, or very LBW showed some increased risks with increasing PFDA exposures. Relative risks generally were fairly modest in magnitude ranging from 1.2 to 1.8, with more variable and larger risks for SGA results denoted among females.

Results were more mixed for birth length and head circumference. Ten of 16 studies in total including reported birth length in relation to increasing PFDA exposures. This included 5 (2 *high*, 1 *medium* and 2 *low* confidence) of the 14 studies that reported birth length deficits fairly consistent in magnitude in the overall population (range: 0.13 to 0.30 per each unit increase). In comparison to the overall population results, birth length changes were more variable in the 10 studies that examined stratified results by sex. Four out 10 studies each in boys (2 *high* and 2 *medium* confidence) and girls (3 *high* and 1 *medium* confidence) birth length deficits in relation to PFDA. The four studies that showed birth length deficits in girls were generally more consistent in magnitude; one study reported mean birth length reductions of 0.20 cm and, the three others ranged from -0.44 to -0.75 cm per each ln-unit increase. There was no direct evidence exposure-response relationships in the few birth length studies with categorical data; but one analysis in girls did show some large gradients in birth length among the upper two quartiles.

Five (2 *high*; 3 *medium*) out of 14 overall PFDA studies reported reductions in head circumference in the overall population (2 of 11 studies) or either sex (3 of 7 studies); the 5 studies showing some reductions accounted for one-half of the 10 *high* and *medium* confidence studies. Head circumference results were less consistent but were comparable in magnitude to those seen for birth length. The one analysis in boys that reported head circumference reductions did show an exposure-response relationship. Although not monotonic across all quartiles, another study in girls did show some large gradients in head circumference among the upper two quartiles.

Few explanatory factors were consistently identified by general study characteristics across the FGR endpoints including exposure levels, study sensitivity, and sex differences. Given limited exposure contrasts in many of the studies, this likely precluded sufficient statistical power to detect associations small in magnitude and, especially when stratified by sex. There was a definitive pattern by sampling timing as only two of the eleven studies (including two of nine *medium/high* studies) reporting BWT deficits in the overall population had early sampling biomarkers measures during pregnancy. Although there was no pattern in the sex-specific studies of birth length, most (four of five) of the studies reporting some birth length reductions in the overall population were based on later biomarker samples. The opposite was seen for studies of head circumference with three of four studies in the overall population or either sex based on early samples. Although these patterns were not consistent across endpoints, the dearth of birth weight and length results in the overall study populations based on early or prepregnancy measures might be indicative of potential bias due to the impact due to pregnancy hemodynamics on PFDA levels. Despite fairly consistent evidence of an association between PFDA and different BWT-related measures, and more mixed for other endpoints, there is considerable uncertainty given that some sample timing differences may explain some of the reported fetal growth restriction deficits examined here.

Table 3-20. Summary of 33 studies (from 35 publications) of PFDA exposure in relation to fetal and postnatal growth restriction measures sorted by overall confidence[a]

Author (Year)	Study location/ Years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
High Confidence Studies								
Wang et al. (2016)	Taiwan, 2000–2001	223	0.46/0.56–Boys 0.43/0.48–Girls (0.16–1.57)	↑ **SGA (Girls)*** Ø (Boys)	**– Girls*[b]** Ø Boys	– Girls Ø Boys	– Girls Ø Boys	**– Wt Girls*** + Wt Boys **– Ht Girls*** – Ht Boys
Bjerregaard-Olesen et al. (2019); Bach et al. (2016)	Denmark, 2008–2013	1533	0.30/0.20 (<LOD–2.87)		Ø All – Girls + Boys	+ All – Girls + Boys	Ø All – Girls + Boys	
Lind et al. (2017a)	Denmark, 2010–2012	636	0.30/0.10 (0.1–1.8)		**+ Girls*** – Boys		+ Girls Ø Boys [b]	
Valvi et al. (2017)	Faroe Islands, 1997–2000	604	0.28/0.16 (0.22–0.38) [c]		– All – Girls – Boys	Ø All	+ All	
Buck Louis et al. (2018)	USA, 2009–2013	2106	0.25/0.26 (0.16–0.42) [c]		Ø All	– All		
Gardener et al. (2021)	USA, 2009-2013	354	0.2/0.2 (LOD-2.6)		+ All			
Luo et al. (2021)	China, 2017-2019	224	0.50/0.28 (N/A)		– All	– All		
Wikström et al. (2020)	Sweden, 2007-2010	1533	0.26/0.15 (N/A)	↑ SGA (All)* ↑ SGA (Girls) ↑ SGA (Boys)	**– All*[b]** – Girls – Boys			
Xiao et al. (2019)	Faroe Islands, 1994-1995	140	N/A/N/A (0.1, 0.9)		– All – Girls – Boys		Ø All Ø Girls Ø Boys	

Author (Year)	Study location/ Years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Yao et al. (2021)	China, 2010-2013	369	0.55/0.37 (0.09-3.77)		− All			
Gao et al. (2022)	China, 2013-2016	1350	1.82/1.44 (0.21, 26.6)					Ø RWG-Wt (All)* ↓RWG-Wt (Girls) ↑ RWG-Wt (Boys) ↑ RWG-Ht (All)* ↑ RWG-Ht (Girls) ↑ RWG-Ht (Boys)
Starling et al. (2019)	USA, 2009-2014	1410	0.1/0.1 (N/A)					Ø -Wt ↑RWG-Wt + Adiposity (All/Boys/Girls)
Zhang et al. (2022)	China, 2013-2016	2395	1.72/1.38 (0.21, 27.8)					Ø All Ø Girls Ø Boys
Medium Confidence Studies								
Robledo et al. (2015)	MI/TX, USA, 2005–2009	234	0.45–Boys [d] 0.40–Girls [d] (N/A)		− Girls Ø Boys	Ø Girls - Boys	− Girls Ø Boys	
Lenters et al. (2016)	Ukraine/ Poland/ Greenland, 2002–2004	1,321	0.16–0.40 (0.07–1.18) range across 3 countries		− All			

Author (Year)	Study location/ Years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Gyllenhammar et al. (2018); Swedish Environmental Protection Agency (2017)[e]	Sweden, 1996–2001	381/587	0.24/0.14 (LOD–1.1)		**– All*** Ø Girls **– Boys***	– All	Ø All	Ø Wt, Ht
Woods et al. (2017)	OH, USA, 2003–2006	272	0.20/0.10 (0.2–0.3)[f]		Ø All			
Meng et al. (2018)	Denmark, 1996–2002	2,120	0.20/0.10 (N/A)	↑ LBW (All) ↑ VLBW (All)	– All – Girls Ø Boys			
Kwon et al. (2016)	S. Korea, 2006–2010	268	0.11/0.07 (0.04–0.41)		**– All***			
Chen et al. (2021)	China, 2013-2015	214	1.73/1.47 (N/A)		Ø All	– All* **– Girls*** Ø Boys		
Gao et al. (2019)	China, 2015-2016	132	0.47 (LOD-3.15)		+ All	Ø All		
Hall et al. (2022)					Ø Girls – Boys			
Hjermitslev et al. (2020)	Greenland, 2010-2011;2013-2015	266	0.71/N/A (0.12–7.84)	↑ LBW (All)	– All Ø Girls – Boys	Ø All	– All Ø Girls Ø Boys	
Kashino et al. (2020)	Japan, 2003-2009	1591	0.6/0.5 (LOD-2.4)		– All – Girls – Boys	Ø All	– All Ø Girls Ø Boys	
Low Confidence Studies								
Xu et al. (2019b)	China, 2016–2017	98	0.21/0.15 (0.1–0.58)[f]	Ø SGA	+ All	+ All		

Author (Year)	Study location/ Years	n	Exposure median/IQR (range) in ng/mL	SGA/LBW	Birth weight	Birth length	HC	Postnatal measures (Wt, Ht)
Li et al. (2017)	China, 2013	321	0.15/0.16 (LOD–2.12)		− All − Girls − Boys			
Lee et al. (2018)	South Korea, 2012–2013	361	0.37/0.36 (0.04–1.25)					**− Wt***, − Ht [b]
Callan et al. (2016)	W. Australia 2003–2004	98	0.12/N/A (0.03–0.39)		Ø All	Ø All	Ø All	
Cao et al. (2018)	China, 2013–2015	337	0.10/0.09 (0.04–0.22) [g]		+ All + Girls Ø Boys	Ø All + Girls Ø Boys	Ø All − Girls Ø Boys	+ Wt Girls − Wt Boys Ø Ht Girls Ø Ht Boys
Starling et al. (2017)	CO, USA, 2009–2014	598	0.10/0.10 [c] (LOD–3.5)		Ø All			
Shi et al. (2017)	China, 2012	170	0.08/0.10 (LOD–0.60)		Ø All + Girls − Boys	Ø All Ø Girls − Boys		Ø All Ø Girls Ø Boys
Jensen et al. (2020a)	Denmark, 2010-2012	589	0.26/N/A (N/A)					+ Adiposity (All)
Workman et al. (2019)	Canada, 2010-2011	414	0.13/N/A (LOD-1.4)		− All	− All	+ All	

Abbreviations: LOD = limit of detection; N/A: not available; All = Overall population of boys and girls; IQR = interquartile range; HC = head circumference; SGA = small for gestational age; LBW = low birth weight; VLBW = very low birth weight; Ht = height; Wt = weight; RWG = rapid weight gain.

Symbols: Ø: null association; + : positive association; − : negative association; ↑: increased odds ratio; ↓: decreased odds ratio.

*Statistically significant findings based on $p < 0.05$.

[a]Overall confidence descriptor is for the birth weight endpoints when studies included prenatal and postnatal growth measures; four other studies had only postnatal data Gao et al. (2022); Zhang et al. (2022); Starling et al. (2019); Lee et al. (2018).

[b]Exposure-response relationships detected for categorical data.

[c]IQR calculated by subtracting the 25th percentile from the 75%; the 25th percentile estimated here as 0 given it was below the detection limit.

[d]Robledo et al. (2015) regression coefficients for maternal serum PFDA are displayed.

[e]Swedish Environmental Protection Agency (2017) results are displayed here for mean birth weight among 587 overall population participants in the POPUP Cohort compared to a smaller sample size of 381 in the 2018 publication by Gyllenhammar et al. (2018).

[f]5th–95th percentiles.

[g]10th–90th percentiles.

Note: "Developmental effects" indicated by increased odds ratio (↑) for dichotomous outcomes, (+) for adiposity/body mass index and waist circumference, and negative associations (−) for the other outcomes.

Growth Restriction – Postnatal Growth (Infancy and early childhood up to 2 years of age)

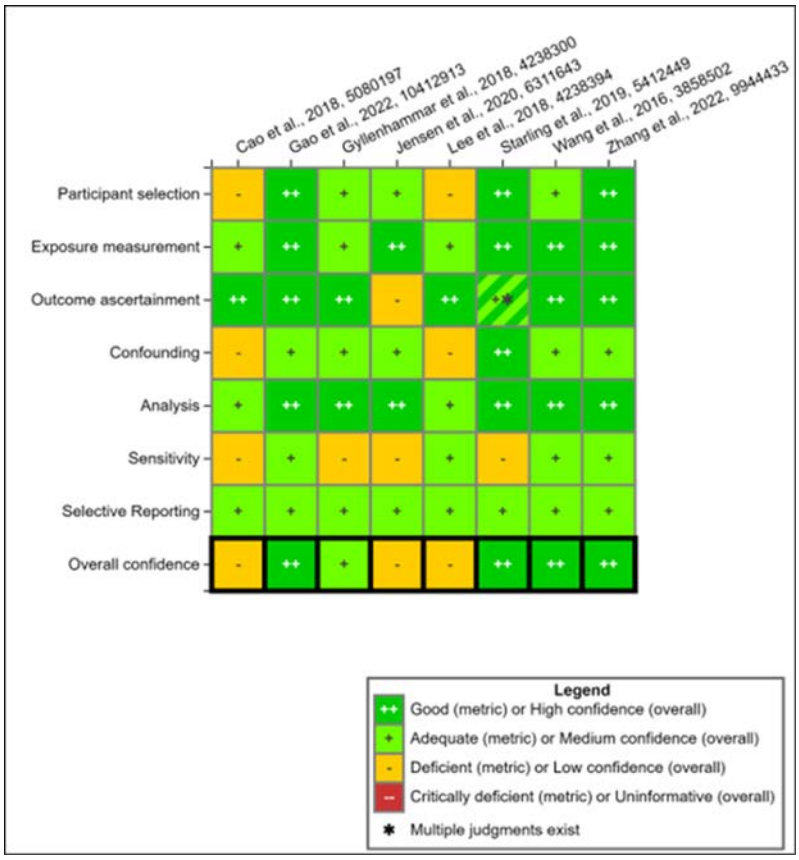


Figure 3-38. Study evaluation results for four epidemiological studies of postnatal growth and PFDA[a,b]. Refer to HAWC for details on the study evaluation review: HAWC Human Postnatal Growth

[a.] In Gyllenhammar et al. (2018), the outcomes height, weight, and body mass index are rated as Good, while the outcome head circumference is rated as Adequate.

[b.] In Starling et al. (2019), the outcome weight-for-age z-score (at 5 months) rated as Good, while the outcomes length-for-age z-score and adiposity/fat mass at 5 months were rated as Adequate.

Eight studies were identified that assessed postnatal growth in relation to PFDA (see Figure 3-38) with each of these examining some measures of childhood weight and/or height in relation to PFDA. Four studies were considered *high* (Gao et al., 2022; Zhang et al., 2022; Starling et al., 2019; Wang et al., 2016), one was *medium* (Gyllenhammar et al., 2018) and three were *low* confidence (Jensen et al., 2020a; Cao et al., 2018; Lee et al., 2018). Of the eight postnatal growth studies, four each had adequate (Gao et al., 2022; Zhang et al., 2022; Lee et al., 2018; Wang et al., 2016) and deficient (Jensen et al., 2020a; Starling et al., 2019; Cao et al., 2018; Gyllenhammar et al., 2018) study sensitivity ratings largely owing to small exposure contrasts. Although there was some

overlap across studies, limited serial measures during infancy as well as inconsistent age at examinations and analyses may limit some comparisons here. For example, (Zhang et al., 2022) examined growth up to 12 months and (Starling et al., 2019) took measurements at 5 months only. Both (Wang et al., 2016) and (Lee et al., 2018) examined postnatal growth at 2 years, while (Cao et al., 2018) analyses were based on a mean of 19 months in participants. (Gyllenhammar et al., 2018) had serial measures of postnatal growth at 3, 6, 12 and 18 months. (Jensen et al., 2020a) examined different adiposity measures at 3 and 18 months, while (Gao et al., 2022) examined growth trajectory based on serial measurements at five time periods within the first 2 years (at birth, 42 days, 6 months, 12 months, and 24 months).

Postnatal Weight

Postnatal Weight: Overall Population

In the overall population, five postnatal studies (two *high*, one *medium*, and two *low* confidence) examined PFDA in relation to either standardized (Zhang et al., 2022; Starling et al., 2019; Gyllenhammar et al., 2018) or mean weight measures in two low confidence studies (Cao et al., 2018; Lee et al., 2018). All three standardized weight studies reported null associations including (Gyllenhammar et al., 2018) for PFDA exposures and standard deviation scores (SDS) for weight measured at 3 to 18 months (Figure 3-39). Similar to findings from (Zhang et al., 2022) examining growth up to 12 months, (Starling et al., 2019) also detected no difference in the overall population at 5 months for either weight-for-age and weight-for-length z-scores across PFDA tertiles and for each ln-unit increase.

Two *low* confidence studies examining mean weight differences in the overall population during early childhood showed some deficits related to upper PFDA exposure (Figure 3-40) around 2 years of age. (Cao et al., 2018) reported a non-significant and imprecise postnatal weight change (β= −130 g; 95%CI: −579, 319) in the overall population (mean age of examination of mean of 19 months) for tertile 3 (relative to tertile 1), but the opposite was seen for tertile 2. Despite their limited exposure contrast, (Lee et al., 2018) reported a non-significant mean weight decrease at 2 years for each ln-unit PFDA increase (β= −140 g; 95%CI: −310, 30) in the overall population. They detected lower mean weight at 2 years across PFDA quartiles in a monotonic fashion (e.g., β range: −200 to −390 g). For example, they detected a statistically significant weight reduction (β= −390 g; 95%CI: −770, −10) in quartile 4 (relative to quartile 1). Both studies were based on measurements in children around 2 years of age.

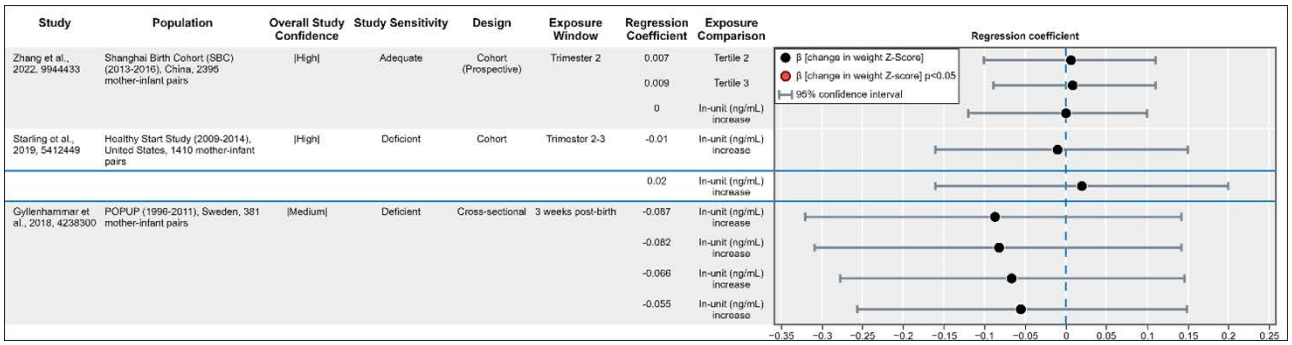


Figure 3-39. PFDA and postnatal growth-standardized weight measures (overall population)[a-c]. Refer to the HAWC link.

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b] Age at Outcome Measurement: Gyllenhammar et al. (2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom); Starling et al. (2019) at 5 months; Zhang et al. (2022) between 42 days and 12 months.
[c] Above the first blue line is Weight-for-Age Z-Score; between the two blue lines is Weight-for-Length Z-Score; below the last blue line is standardized PNG weight.

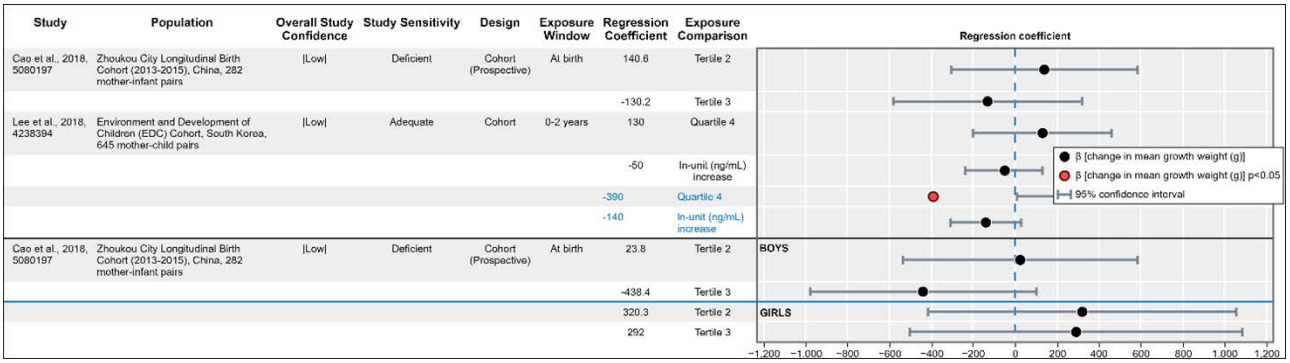


Figure 3-40. PFDA and postnatal growth mean weight (in grams)[a-d]. Refer to the HAWC link.

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b] Age at Outcome Measurement: Cao et al. (2018) at 19 months; Lee et al. (2018) data measurements taken from 0–2 years is in black and at 2 years is in blue.
[c] Overall population data above the black reference line; sex-stratified data below.
[d] Sex-stratified: male infant data above the blue line; females below.

Postnatal Weight: Sex-specific

Four studies (three *high* and one *low* confidence) included PFDA sex-specific analyses with one (Cao et al., 2018) reporting mean weight changes and three reporting standardized weight measures (Zhang et al., 2022; Starling et al., 2019; Wang et al., 2016) (Figure 3-41). Two of the four studies showed detected deficits in relation to PFDA albeit not consistent across sexes. The *low* confidence (Cao et al., 2018) study detected imprecise contrasting changes in postnatal weight, with non-significant decreases in the highest tertile for boys (β = −438 g; 95% CI: −980, 103) but increases among girls (β = 292 g; 95% CI: −501, 1,085). Two of the sex-standardized weight studies

reported null results for boys and girls based either on weight-for-age and weight-for-length standardized measures (Figure 3-41). Starling et al. (2019) reported no difference in either sex at 5 months for weight-for-age and weight-for-length z-scores across PFDA tertiles or for each ln-unit increase, as did Zhang et al. (2022) across PFDA tertiles for postnatal growth up to 12 months. In contrast, Wang et al. (2016) detected statistically significant reductions among females only for average childhood weight z-scores (β = −0.32; 95% CI: −0.63, 0). No relationship was seen for age 2 for weight z-score in either sex, and the largest weight z-scores were among females detected at birth and at age 11.

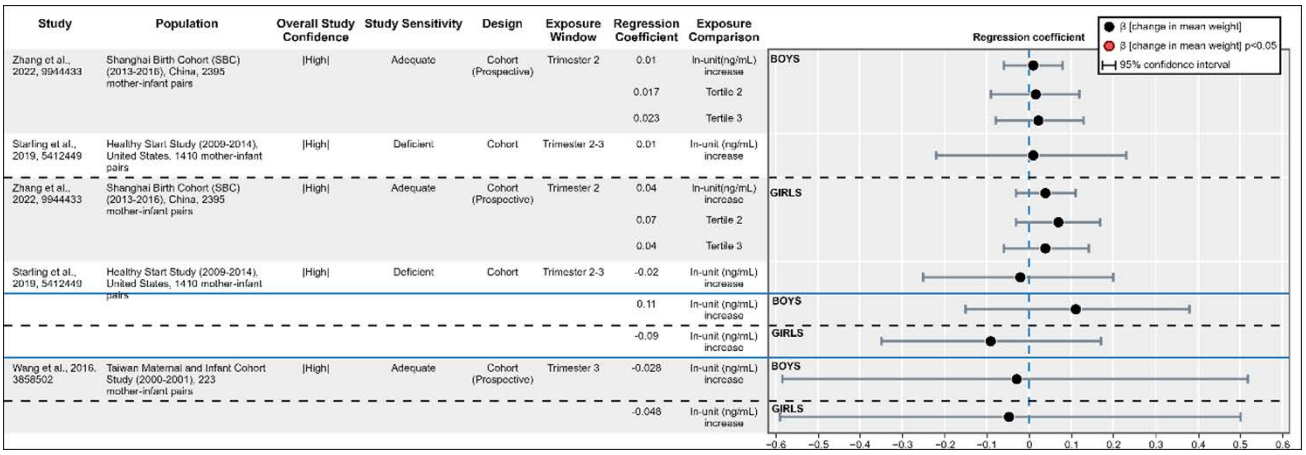


Figure 3-41. PFDA and postnatal standardized growth weight (sex-stratified; boys above dashed line, girls below)[a-d]. Refer to the HAWC link.

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b] Age at Outcome Measurement: Starling et al. (2019) at 5 months; Wang et al. (2016) between 2–11 years; Zhang et al. (2022) between 42 days and 12 months.
[c] Weight-for-age z-score above the blue line; weight-for-length z-score between the two blue lines; weight z-score below the last blue line.
[d] Boys above dashed line: girls below dashed line.

Postnatal Height

Postnatal Height: Overall Population

Four studies (one *high,* one *medium,* and two *low* confidence) examined mean or standardized postnatal height in the overall population (Zhang et al., 2022; Cao et al., 2018; Gyllenhammar et al., 2018; Lee et al., 2018) with only one study reporting height reductions in relation to PFDA. The *medium* confidence by (Gyllenhammar et al., 2018) and the *high* confidence study by (Zhang et al., 2022) were null for standardized height measures in the overall population (Figure 3-42). The *low* confidence study by (Cao et al., 2018) reported larger mean postnatal height increases across higher PFDA tertiles (β range: 1.27 to 1.56 cm) in the overall population (Figure 3-43). Despite a limited exposure contrast, the *low* confidence study by Lee et al. (2018) reported lower mean height at 2 years (β = −0.44 cm; 95%CI: −0.77, −0.10). They reported lower mean

height in a monotonic fashion with the largest statistically significant weight difference detected in quartile 4 (β = −1.11 cm; 95%CI: −1.86, −0.36).

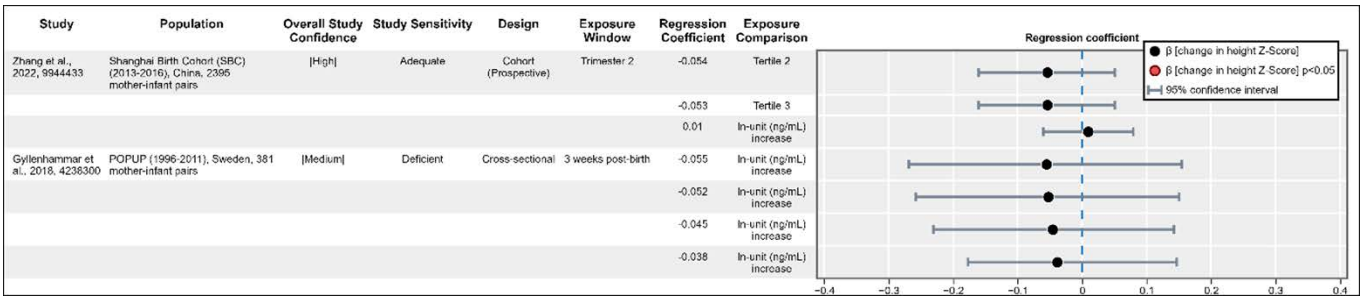


Figure 3-42. PFDA and postnatal growth – standardized height measures (overall population)[a,b]. Refer to the HAWC link.

[a] Studies are sorted first by overall study confidence level then by exposure window examined.
[b] Age at Outcome Measurement: Gyllenhammar et al. (2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom); Zhang et al. (2022) between 42 days and 12 months.

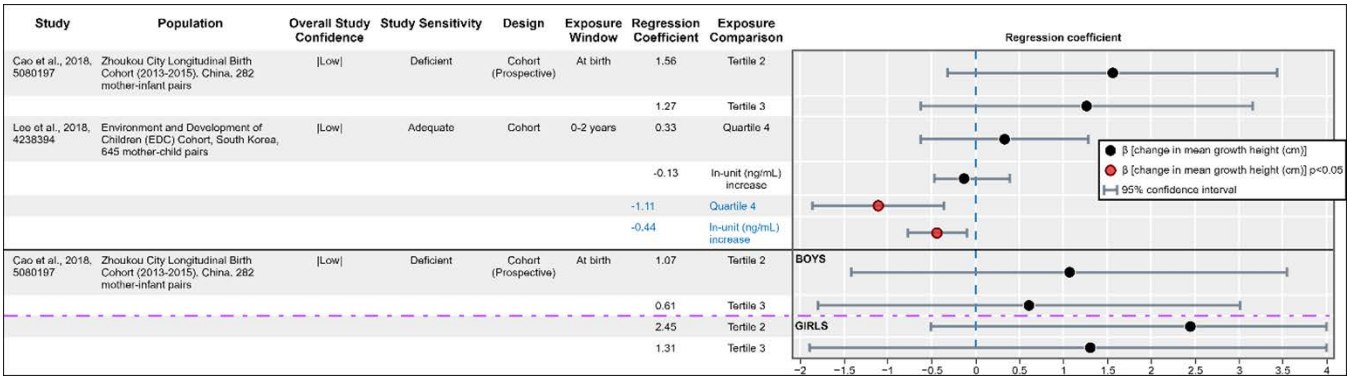


Figure 3-43. PFDA and postnatal growth mean height (in centimeters)[a,b,c,d] Refer to the HAWC link.

[a] Age at Outcome Measurement: Cao et al. (2018) at 19 months; Lee et al. (2018) data measurements taken from 0–2 years is in black and at 2 years is in blue.
[b] Sex-stratified data is located below the solid black line; boys are above the purple dotted line and girls are below.
[c] Cao et al. (2018) female results have upper bounds that have been truncated; the upper bounds are 5.41 for Tertile 2 and 4.5 for Tertile 3.

Postnatal Height: Sex-specific

Three studies (two *high* and one *low* confidence) examined height in relation to PFDA across sexes including one (Cao et al., 2018) examining mean and two studies examining standardized measures (Zhang et al., 2022; Wang et al., 2016). The *low* confidence study by (Cao et al., 2018) reported larger postnatal mean height increases among females (β range: 1.31 to 2.45 cm) than males (β range: 0.61 to 1.07 cm) across PFDA tertiles. The *high* confidence study by Zhang et al. (2022) reported null associations for both sexes based on continuous and categorical PFDA exposures (Figure 3-44). In contrast, the *high* confidence study by Wang et al. (2016) detected

statistically significant reductions among females only for childhood height z-scores averaged from birth, 2, 5, 8, and 11 years (β = −0.52; 95% CI: −0.80, −0.24). Smaller height z-scores were found for all time periods for both male and females but was only statistically significant for females at ages 2 and 11. For example, they reported height z-score reductions (β = −0.61; 95%CI: −1.02, −0.23 per each ln-unit PFDA increase) at age 2 among females and was much smaller among males (β= −0.17; 95%CI: −0.63, 0.30).

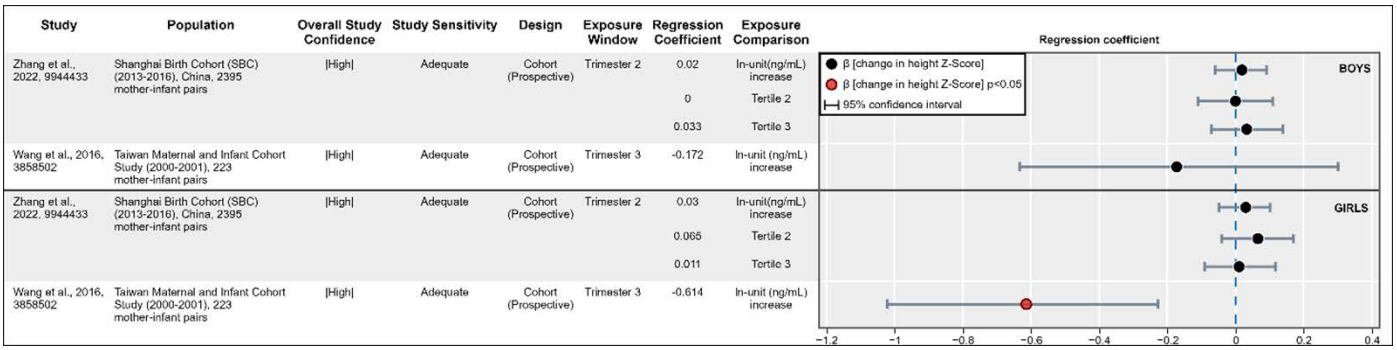


Figure 3-44. PFDA and postnatal growth – standardized height measures (sex-stratified; boys above reference line, girls below)[a-c]. Refer to the HAWC link.

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.
[b.] Age at Outcome Measurement: Dong et al. (2019) between 2–11 years; Zhang et al. (2022) between 42 days and 12 months.
[c.] Boys above reference line, girls below.

Postnatal Head Circumference

Three studies (one *high, medium,* and *low* confidence study each) examined post-natal standardized head circumference including two studies (Zhang et al., 2022; Gyllenhammar et al., 2018) that reported standardized results only in the overall population (Figure 3-45) and one (Cao et al., 2018) that examined mean head circumference data in the overall population as well as across sexes (Figure 3-46). None of the three studies examining head circumference showed much evidence of decreases in head circumference with increasing PFDA exposures. Null results were detected in Zhang et al. (2022) for postnatal head circumference-for-age Z score up to 12 months of age per each ln-unit increase and across PFDA tertiles and for Gyllenhammar et al. (2018) head circumference SDS measures were based on four different time points (3, 6, 12 and 18 months). In the overall population, Cao et al. (2018) detected a sizeable mean head circumference increase in PFDA tertile 2 (0.50 cm; 95%CI: −0.44, 1.44) but was null in tertile 3. Results were null for boys, while contrasting results were seen for tertile 3 (β= −0.69 cm; 95% CI: −2.26, 0.88) and tertile 2 (β= 0.67 cm; 95% CI: −0.79, 2.13) among girls.

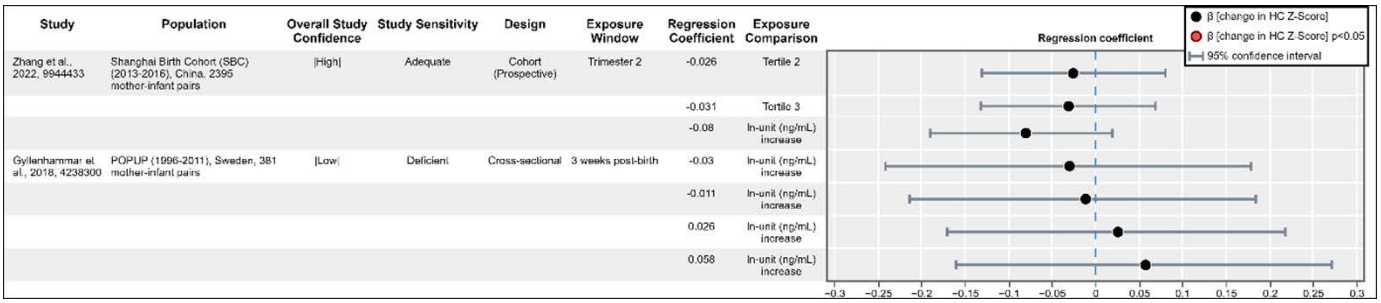


Figure 3-45. PFDA and postnatal growth standardized head circumference (overall population)[a,b]. Refer to the HAWC link.

Abbreviation: HC = Head Circumference

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Age at Outcome Measurement: Zhang et al. (2022) between 42 days and 12 months; Gyllenhammar et al. (2018) at 3 months, 6 months, 12 months, and 18 months (ordered top to bottom).

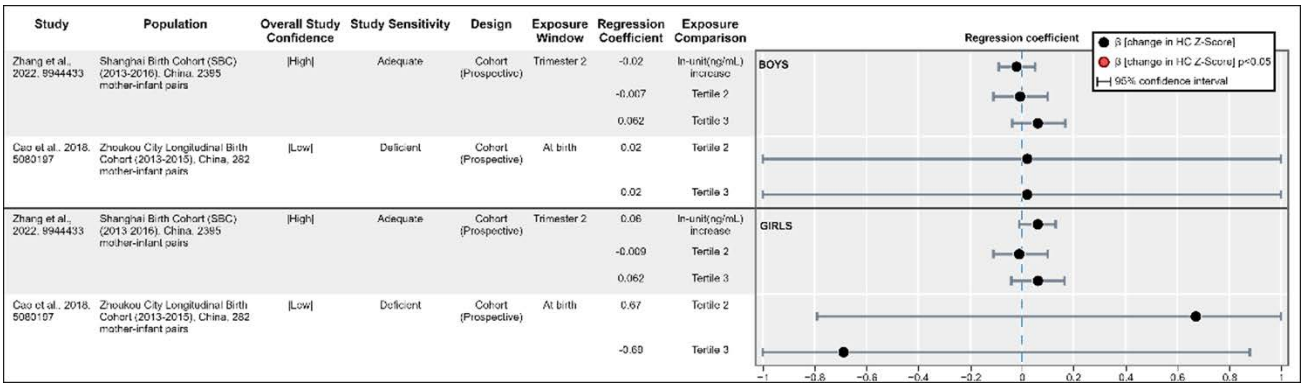


Figure 3-46. PFDA and postnatal growth head circumference (sex-stratified; boys above reference line, girls below)[a-d]. Refer to the HAWC link.

Abbreviation: HC = Head Circumference

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Age at Outcome Measurement: Cao et al. (2018) at 19 months (averaged); Zhang et al. (2022) between 42 days and 12 months.

[c.] Zhang et al. (2022) reports standardized results based on head circumference z-scores, while Cao et al. (2018) reports mean head circumference data (in cm).

[d.] Cao et al. (2018) upper and lower bounds have been truncated. For boys, for Tertile 2 the bounds are [-1.23, 1.27] and for Tertile 3 the bounds are [-1.19, 1.24]. For girls, for Tertile 2 the bounds are [-0.79, 2.13] and for Tertile 3 the bounds are [-2.26, 0.88].

Adiposity Measures (Waist Circumference/ Body Mass Index/Ponderal Index)

Three studies (two *high* and one *low* confidence) examined post-natal adiposity measures including % fat mass increase as well as standardized waist circumference, BMI, and ponderal index measures. Two studies detected increased adiposity relative to PFDA exposures, while one study (Zhang et al., 2022) reported null associations for BMI-for-age Z score per each ln-unit PFDA

increase in the overall population and across sexes (Figure 3-47). Jensen et al. (2020a) showed null associations for PFDA and waist circumference SDS in the overall population and across both sexes. However, they did report increased adiposity measures in the overall population including body mass index SDS (β = 0.42; 95%CI: 0.01, 0.84 per each ln-unit increase) with stronger associations among females (β = 0.58; 95%CI; -0.03, 1.19 per each ln-unit increase). Similarly, a statistically significant association with larger ponderal index SDS (β = 0.60; 95%CI: 0.18, 1.02 per each ln-unit increase) was detected in the overall population and was driven by associations in females (β = 1.02; 95%CI: 0.40, 1.64 per each ln-unit increase). Starling et al. (2019) reported a slight non-significant increase in infant adiposity at 5 months of age for each ln-unit increase in PFDA (β = 0.59% fat mass increase; 95%CI: −0.27, 1.44) with larger increases among males (β = 0.79% fat mass increase; 95%CI: −0.46, 2.04) compared to females (β = 0.44% fat mass increase; 95%CI: −0.82, 1.69). The opposite was seen in their categorical analyses dichotomized at the median with more adiposity in females (β = 0.70% fat mass increase; 95%CI: -0.78, 2.17) and males (β = 0.23% fat mass increase; 95%CI: -1.39, 1.85).

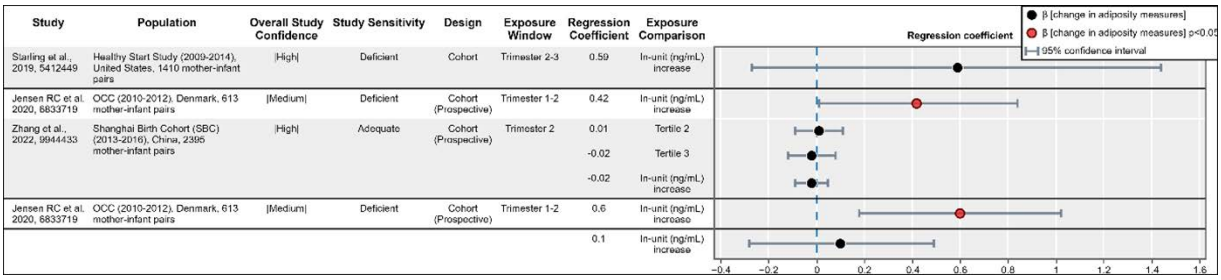


Figure 3-47. PFDA and postnatal growth measures-body mass index, adiposity, ponderal index, waist circumference (overall population)[a-e]. Refer to the HAWC link.

[a] Studies are sorted first by overall study confidence level then by exposure window examined.
[b] Age at Outcome Measurement: Jensen et al. (2020a) at 3 months (median); Starling et al. (2019) at 5 months; Zhang et al. (2022) between 42 days and 12 months.
[c] Solid black lines divide the outcomes examined here: adiposity, body mass index, ponderal index, and waist circumference (ordered top to bottom).
[d] Zhang et al. (2022) reports standardized body mass index data.
[e] Units: Fat mass increase % for Starling et al. (2019); not applicable for unitless standardized measures depicted for Jensen et al. (2020a) and Zhang et al. (2022).

Rapid Weight Gain

Two *high* confidence studies (Gao et al., 2022; Starling et al., 2019) examined different rapid weight gain measures in relation to PFDA. In the Healthy Start study, Starling et al. (2019) examined different rapid weight gain measures in relation to PFDA for the overall population and both sexes. In the Shanghai Birth Cohort, Gao et al. (2022) examined various measures of growth trajectories in the overall population as well as some sex-specific analyses.

Starling et al. (2019) reported null associations for rapid weight gain measures based on weight-for-age z-score (OR = 0.87; 95%CI: 0.50, 1.52 per each ln-unit PFDA increase) and a *weight-for-age* standard deviation growth rate between birth and 5-month follow-up (Figure 3-48). They did, however, report a non-significant increase in rapid weight gain derived from *weight-for-length z-score* (OR = 1.50; 95%CI: 0.84, 2.70) for categorical exposures above the median (0.2–3.5 ng/mL relative to the referent up to 0.1 ng/mL). In the overall population, Gao et al. (2022) reported null associations between PFDA and their *weight-for-age* and *weight-for-length z-score* endpoints across all trajectory designations. Based on the *weight-for-length z-score*, the low-rising participants (e.g., growth trajectory starts with a low value and followed by an increased trend afterward) vs. moderate-stable referent group (e.g., growth trajectory starts with a moderate value and followed by stable growth afterward) had a non-significant OR for the overall population (0.78; 95%CI: 0.53, 1.16 ln-unit PFDA increase). Results were contrasting in females (OR = 0.48; 95% CI: 0.27, 0.8 per each ln-unit PFDA increase) and males (OR = 1.30; 95%CI: 0.71, 2.37 per each ln-unit PFDA increase).

Gao et al. (2022) reported a non-significant increased risk (OR = 1.54; 95%CI: 0.85, 2.82 per each ln-unit PFDA increase) for *length-for-age z-score* among those participants that were considered high-rising vs. the moderate-stable group with comparable risks detected amongst male and females (OR range: 1.73-1.83). In a weighted quantile sum mixture model, they also detected higher odds (OR = 1.59; 95% CI: 0.90, 2.82 per each ln-unit PFDA increase) among the high-rising group (vs. moderate-stable) based on *length-for-age z-scores*, with PFDA having the highest weight among the PFAS mixtures. Gao et al. (2022) reported non-significant inverse associations comparable in magnitude based on *head-circumference-for-age* z-score for high-rising vs. moderate-stable (OR = 0.66; 95%CI: 0.38, 1.12 per each ln-unit PFDA increase) and low-stable vs. moderate-stable participants (OR = 0.67; 95%CI: 0.49, 0.93 per each ln-unit PFDA increase). They reported a statistically significant inverse association (OR = 0.51; 95%CI: 0.27, 0.99 per each ln-unit PFDA increase) for low-rising vs. moderate-stable groups in the single PFAS model. They reported a lower risk in the weighted quantile sum model (OR = 0.37; 95%CI: 0.18, 0.72), with PFDA having the highest weight among the PFAS mixtures. In general, the low- and high-rising groups examined by Gao et al. (2022) may be at most risk for metabolic syndrome, as evidenced by changes in obesity and other health effects later in life. However, results were not consistent in the overall population or across sexes for these different rapid growth measures. Therefore, there is no compelling evidence of increased postnatal weight gain among those that may represent low birth weight individuals with rapid weight gain trajectories (i.e., low-rising group).

Based on mixed results within and across these two studies, there is limited support that accelerated growth in infancy is related to PFDA. Although there was some evidence of increased risks occurring in the high-rising trajectory group which may be indicative of rapid weight gain for those that experienced fetal growth restriction, however, the evidence is scant and inconsistent to draw many conclusions in the overall population or across sexes.

Study	Population	Overall Study Confidence	Study Sensitivity	Design	Exposure Window	Regression Coefficient	Exposure Comparison	Population Description
Starling et al., 2019, 5412449	Healthy Start Study (2009-2014), United States, 1410 mother-infant pairs	|High|	Deficient	Cohort	Trimester 2-3	0.87	ln-unit (ng/mL) increase	Rapid Growth vs. Non-Rapid Growth
Gao et al., 2022, 10412913	Shanghai Birth Cohort (2013-2016), China, 3426 pregnant women	|High|	Adequate	Cohort (Prospective)	Trimester 3	1.18	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.96	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.07	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.01	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						1.36	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						1.04	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.32	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.38	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						1.1	ln-unit(ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.9	ln-unit(ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						0.94	ln-unit(ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						0.72	ln-unit(ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
Starling et al., 2019, 5412449	Healthy Start Study (2009-2014), United States, 1410 mother-infant pairs	|High|	Deficient	Cohort	Trimester 2-3	1.5	Quantile 2	Rapid Growth vs. Non-Rapid Growth
Gao et al., 2022, 10412913	Shanghai Birth Cohort (2013-2016), China, 3426 pregnant women	|High|	Adequate	Cohort (Prospective)	Trimester 3	0.78	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.85	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.19	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.13	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						1.3	ln-unit (ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.89	ln-unit (ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						1.56	ln-unit (ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.27	ln-unit (ng/mL) increase	Low-Stable vs Moderate Stable Trajectory
						0.48	ln-unit(ng/mL) increase	Low-Rising vs Moderate Stable Trajectory
						0.83	ln-unit(ng/mL) increase	High-Rising vs Moderate Stable Trajectory
						0.9	ln-unit(ng/mL) increase	High-Stable vs Moderate Stable Trajectory
						1.12	ln-unit(ng/mL) increase	Low-Stable vs Moderate Stable Trajectory

Figure 3-48. PFDA and postnatal growth rapid growth (overall population) and sex-specific (in grams)[a-f]. Refer to the HAWC link.

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Age at Outcome Measurement: Starling et al. (2019) at 5 months, Gao et al. (2022) modeled data (collected at 42 days, 6 months, 12 months, and 24 months).

[c.] Weight-for-Age Z-Score data above the black reference line; weight-for-length below.

[d.] Overall population data above the blue line; Sex-stratified data below.

[e.] Sex-Stratified data: male infants above the blue dash-dotted line; females below.

[f.] Quantile 2 in Starling et al. (2019) represents dichotomized exposure at median (quantile 1 referent: LOD-0.1 ng/mL; quantile 2: 0.2–3.5 ng/mL).

Postnatal Growth Summary

Overall, there were mixed results within and across the eight available postnatal PFDA studies of early childhood. For example, two (one *high* and one *low* confidence) out of five different studies measuring height and three (one *high* and two *low* confidence) out of six different studies measuring weight reported some deficits in relation to PFDA. Interestingly, there were more consistent results seen in three studies (Cao et al., 2018; Lee et al., 2018; Wang et al., 2016) that examined postnatal growth measures at age 2. For example, both studies showing some postnatal height deficits in either the overall population or across sexes were based on participants examined at 2 years of age. There was no evidence of associations between PFDA exposures and early childhood head circumference, but two (one *high* and one *low* confidence) of three studies showed some suggestion of increased postnatal adiposity. Only two studies examined rapid weight gain in relation to PFDA and were fairly inconsistent within and across studies based on different weight and length measurements.

Only three of the eight total studies reported categorical data which may inform presence of non-linearity or exposure-response relationships. Only one of these three studies showed any evidence of any monotonic deficits across PFDA categories. There were a fairly small number of studies across each common endpoints; thus, a lack of patterns across study characteristics (except age at examination) was not unexpected. For example, although there were no studies with good ratings for study sensitivity, this did not appear to be an explanatory factor for the null studies. However, limited exposure contrasts and statistical power may have hampered the ability to detect associations small in magnitude especially among the sexes.

In summary, although the evidence was mixed across various postnatal measures and different examination windows, with only minimal evidence of exposure-response relationships to support the continuous exposure scaled results. One challenge in evaluating consistency across heterogeneous studies includes disparate periods of follow-up and assessment (e.g., childhood age at examination). Despite the mixed evidence shown here, there was some suggestion of more consistency in studies that examined postnatal growth measures around 2 years of age. This may reflect the challenges that exist to detect associations in children that experience fetal growth restriction and subsequent rapid growth periods. Although, there was limited information and evidence of rapid weight associations among the two studies that considered this. Overall, the evidence for postnatal associations is *slight* largely due to the early childhood weight and adiposity results along with inconsistency across the other measures.

Anogenital distance

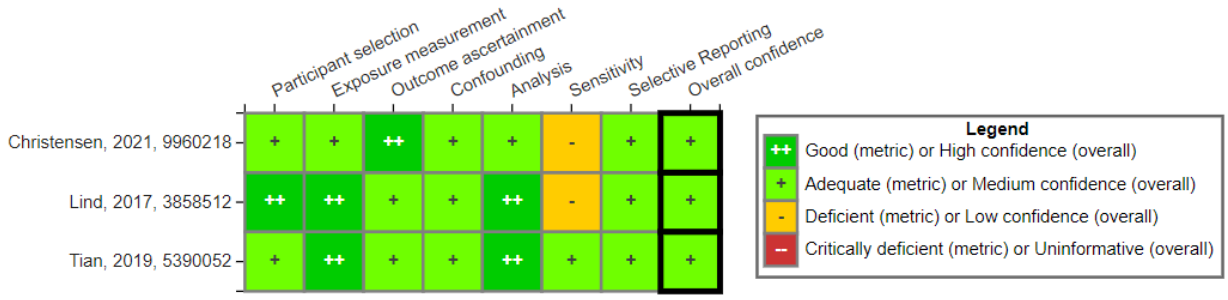


Figure 3-49. Study evaluation results for two epidemiological studies of anogenital distance and PFDA. Refer to the interactive HAWC link for additional details: **HAWC Human AGD**

Three *medium* confidence birth cohorts (Figure 3-49) in Denmark (Lind et al., 2017a), China (Tian et al., 2019), and the Faroe Islands (Christensen et al., 2021) examined the association between PFDA exposure and AGD at 3 months of age. All three studies examined boys while Lind et al. (2017a) and Christensen et al. (2021) also included girls.

Among boys, Tian et al. (2019) reported smaller AGD at birth with higher PFDA exposure (ASD β = −0.58, 95% CI: −1.11, −0.06; APD β = −0.63, 95% CI: −1.24, −0.01). Decrements were also observed at 6 months (p >0.05), but not at 12 months, which may be due to greater heterogeneity in size as children develop. A positive association was observed in Christensen et al. (2021) (Q2 β vs Q1 = 1.4; 95% CI: 0.4, 2.5; Q3 β = 1.0; 95%CI: 0.0, 2.1; Q4 β = 1.3;95%CI: 0.3, 2.4). No association was observed in Lind et al. (2017a). Exposure levels were considerably higher in Tian et al. (2019) (median 2.1 vs. 0.2 and 0.3 ng/mL), but this does not explain the inconsistent direction of association across studies.

For girls, there was an inverse association with PFDA for one of the two AGD measures (AGDAC, measured from the center of anus to the top of clitoris) reported in Lind et al. (2017a). They reported an association based on continuous exposure (β = −1.3, 95% CI: −2.8, 0.2), and across upper two PFDA exposure quartiles in non-monotonic fashion (Q2 vs. Q1: β = 0.4; 95% CI: −1.3, 2.0; Q3: β = −0.7; 95% CI: −2.4, 0.9; Q4: −1.7; 95% CI: −3.6, 0.1, p trend= 0.04). An association was also observed in the fourth quartile in the other AGD measure (AGDAF, measured from center of anus to posterior fourchette), though it was not statistically significant (Q4 β = −1.0; 95% CI: −2.4; 0.4). No association was observed in Christensen et al. (2021).

AGD is a marker of androgen exposure, and thus an inverse association in AGD would be expected to correspond with a decrease in testosterone. This was not observed in the single *low* confidence study of testosterone in neonates (see Male and Female Reproductive Effects); however, there is considerable uncertainty in the reproductive hormones evidence base. Thus, this lack of coherence does not reduce confidence in the AGD findings. Reduced AGD is associated with clinically relevant outcomes in males, including cryptorchidism, hypospadias, and lower semen

quality and testosterone levels (Thankamony et al., 2016), but adversity of reduced AGD is less established in females. As noted above few studies of birth defects, US EPA did not identify any epidemiological studies that examined PFDA in relation to congenital genitourinary defects, such as cryptorchidism and hypospadias. Overall, the evidence for AGD is indeterminate given the mixed results for various AGD measures across the sexes.

Gestational Duration Endpoints

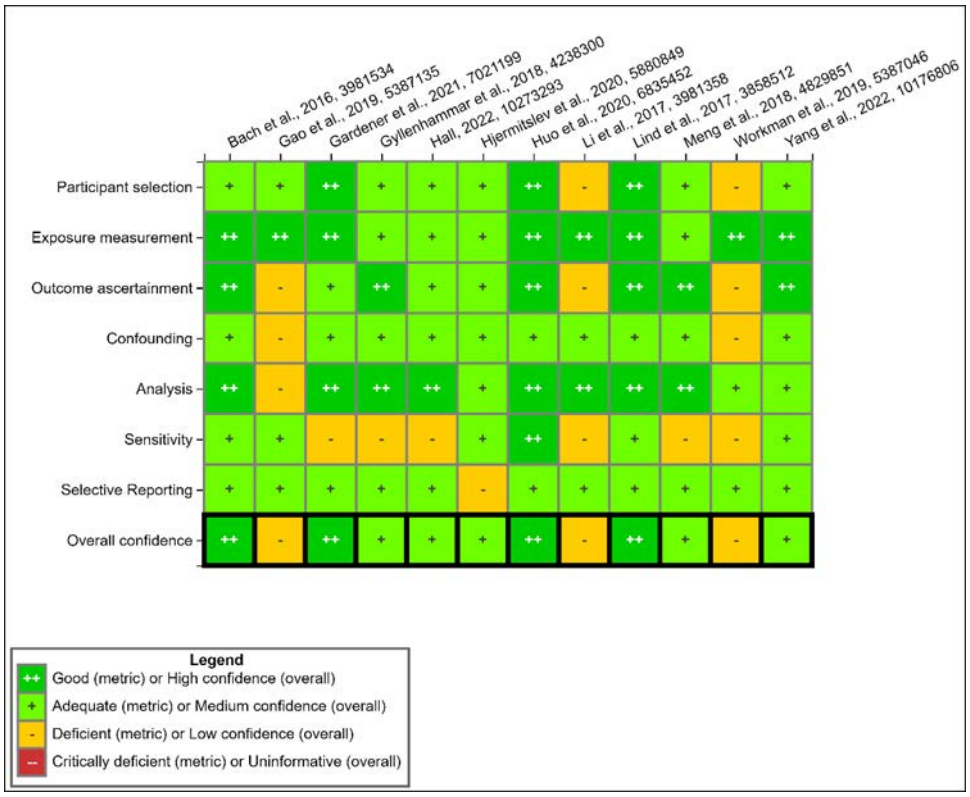


Figure 3-50. Study evaluation results for five epidemiological studies of gestational duration and PFDA. Refer to HAWC for details on the study evaluation review: HAWC Human Gestational Duration.

As shown in Figure 3-50, 12 informative epidemiological studies examined PFDA in relation to changes in gestational duration measures (i.e., gestational age or PTB). All 12 examined gestational age measures, while 6 included preterm birth. Four studies were *high* confidence (Gardener et al., 2021; Huo et al., 2020; Lind et al., 2017a; Bach et al., 2016), five were *medium* (Hall et al., 2022; Yang et al., 2022a; Hjermitslev et al., 2020; Gyllenhammar et al., 2018; Meng et al., 2018), and three studies were *low* owing largely to very limited exposure contrasts (Gao et al., 2019; Workman et al., 2019; Li et al., 2017). One study had good sensitivity (Huo et al., 2020), while five were adequate (Yang et al., 2022a; Hjermitslev et al., 2020; Gao et al., 2019; Lind et al., 2017a; Bach et al., 2016) and six were deficient (Hall et al., 2022; Gardener et al., 2021; Workman et al., 2019; Gyllenhammar et al., 2018; Meng et al., 2018; Li et al., 2017). Ten of the 12 studies were

prospective cohort or nested case-control studies (Hall et al., 2022; Yang et al., 2022a; Gardener et al., 2021; Hjermitslev et al., 2020; Huo et al., 2020; Gao et al., 2019; Workman et al., 2019; Meng et al., 2018; Lind et al., 2017a; Bach et al., 2016), and two were cross-sectional (Gyllenhammar et al., 2018; Li et al., 2017). For examination of consistency and between-study heterogeneity, we examined the type of statistical analyses in addition to the type of study design. As part of this, cross-sectional analyses are considered for any study that used maternal serum/plasma, umbilical cord, or placental post-partum PFDA measures in relation to gestational duration even if the data were derived from prospective cohort or nested case-control studies (Hall et al., 2022; Yang et al., 2022a).

The epidemiological studies had maternal exposure measures that were sampled either during trimester one (Lind et al., 2017a), two (Huo et al., 2020), three (Gardener et al., 2021; Gao et al., 2019) across multiple trimesters (Hjermitslev et al., 2020; Meng et al., 2018; Bach et al., 2016), or had post-partum maternal or infant samples (Hall et al., 2022; Yang et al., 2022a; Gyllenhammar et al., 2018; Li et al., 2017). All five of the cross-sectional studies/analyses had late sampling (defined here as trimester 2 exclusive onward). Four (Hjermitslev et al., 2020; Meng et al., 2018; Lind et al., 2017a; Bach et al., 2016) of the prospective cohort studies had early sampling (defined here as having at least some trimester 1 maternal sampling), while the remaining two (Gardener et al., 2021; Workman et al., 2019) relied on late biomarker sampling.

Preterm Birth

Six studies examined PFDA and preterm birth including three studies each being *high* (Gardener et al., 2021; Huo et al., 2020; Bach et al., 2016) and *medium* confidence (Yang et al., 2022a; Hjermitslev et al., 2020; Meng et al., 2018) (Figure 3-51). Three studies showed some evidence of increased risk of PTB with increasing PFDA exposures including two studies with early biomarker sampling. Null associations for PTB were reported in the *medium* confidence study by Yang et al. (2022a) and *high* confidence study by Bach et al. (2016), while a non-significant inverse association (OR = 0.65; 95%CI: 0.24, 1.79 per each PFDA ln-unit increase) was reported in the *medium* confidence study by Hjermitslev et al. (2020).

Although there was no evidence of an exposure-response relationship, the *high* confidence study by Gardener et al. (2021) reported that participants in PFDA exposure quartile 4 had a greater odd of PTB (OR = 1.82; 95%CI: 0.54, 6.19) relative to quartile 1. The *medium* confidence study by Meng et al. (2018) reported an increased OR of 1.6 for PTB (95% CI: 0.8, 3.0) in the PFDA quartile 4, but no evidence of monotonicity or increased risk in the other quartiles. A larger statistically significant result was detected for each ln-unit increase (OR = 2.2; 95%CI: 1.3, 3.8). Associations between PFDA and different PTB measures (including overall and different sub-types) were at or just below the null value based on continuous exposures in the *high* confidence study by Huo et al. (2020). Similar patterns emerged across PFDA exposure tertiles, albeit non-significant ORs with an exposure-response relationship was suggested for clinically indicated PTBs (T2 OR = 1.11; 95%CI: 0.50, 2.48; T3 OR= 1.30; 95%CI: 0.59, 2.89). This result seemed to be largely driven by

results in female neonates (OR = 1.38; 95%CI: 0.61, 3.11 per each ln-unit PFDA increase) (sex-specific data not shown on forest plots below).

PTB Summary

Three (two *high* and one *medium* confidence) of six studies showed increased odds of PTB with increasing PFDA exposures with risks ranging from 1.3 to 2.2. Although the number of studies was small, two of these three studies showing increased risks were based on late biomarker samples. No other patterns were evident by study confidence or other characteristics. For example, study sensitivity did not seem to be an explanatory factor among the null studies. One of the four studies with categorical data showed evidence of exposure-response relationships.

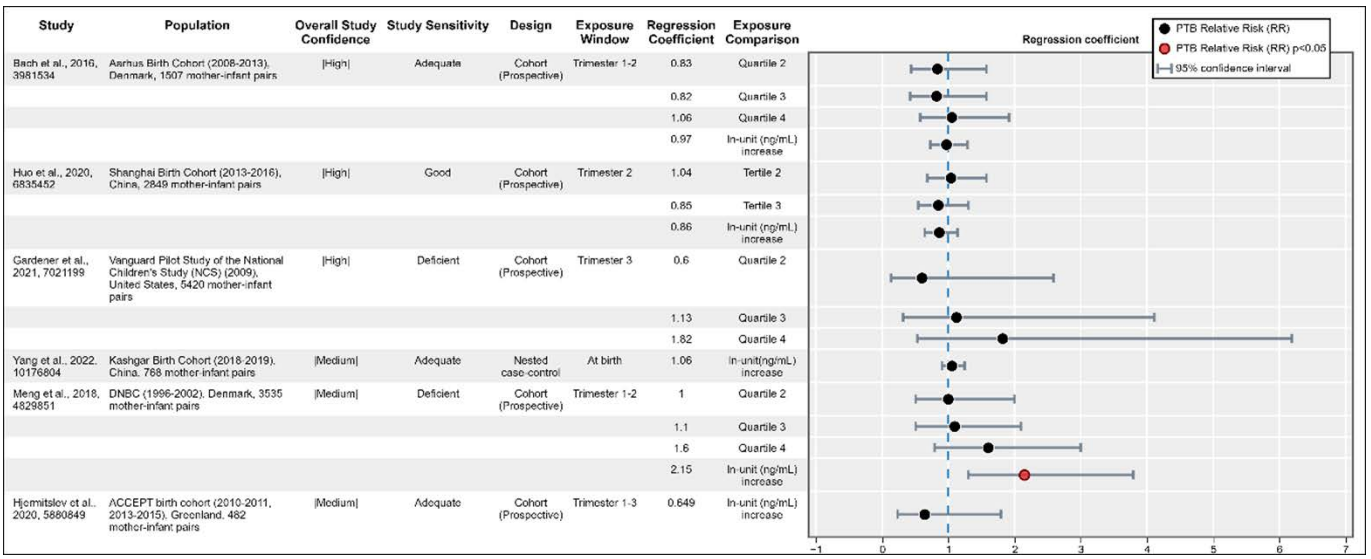


Figure 3-51. Preterm birth forest plot-six studies based on the overall population[a,b]. Refer to the HAWC link.

Abbreviation: PTB = Preterm Birth

[a] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b] For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses [e.g., (Yang et al., 2022a)].

Gestational Age

Twelve studies examined PFDA in relation to changes in gestational age. Two of these studies reported only sex-specific data (Hall et al., 2022; Lind et al., 2017a) with three studies reporting both sex-specific and overall population results (Hjermitslev et al., 2020; Meng et al., 2018; Li et al., 2017).

Gestational Age-Overall Population

Six of the ten studies based on the overall population were null including the *high* confidence studies by Bach et al. (2016) and Huo et al. (2020), the *medium* confidence studies by

Hjermitslev et al. (2020), and the *low* confidence studies by Gao et al. (2019); Workman et al. (2019); Li et al. (2017) (Figure 3-52). No patterns were seen by study sensitivity amongst these null studies but four of the six had adequate or good domain ratings.

Four studies in the overall population (one *high* and three *medium* confidence) showed some evidence of lower gestational age relative to PFDA in the overall population. The *high* confidence study by Gardener et al. (2021) showed decreased gestational age in only PFDA quartile 4 with no exposure-response relationship evident (Q4 β = −0.26 weeks vs. Q1). Although it was null for term births, there was an inverse association between gestational age and each PFDA (β = −0.72 weeks; 95%CI: −3.39, 1.97 per each ln-unit increase) among preterm births in the *medium* confidence study by Yang et al. (2022a). Two other *medium* confidence studies reported only slight non-significant deficits (-0.12) per each ln-unit increase (Gyllenhammar et al., 2018; Meng et al., 2018), but the latter showed larger deficits in both exposure quartile 3 and 4 (β range: -0.20 to -0.50 weeks, respectively). Three of these four studies reporting lower gestational age were based on later biomarker sampling.

Gestational Age-Sex Specific

Two of the five studies in male neonates reported some gestational age deficits compared to just one study in girls. The *medium* confidence study by Hjermitslev et al. (2020) and the *low* confidence study by Li et al. (2017) reported null findings for both boys and girls. The *high* confidence study by Lind et al. (2017a) showed minimal evidence of associations in the upper quartiles for either sex, although they reported an imprecise gestational age reduction of −0.21 weeks (95%CI: −0.66, 0.24) among girls that was incongruous with their categorical data. The *medium* confidence study by Hall et al. (2022) reported non-significant deficits in the upper tertile for boys (β = −0.26 weeks; 95% CI: −0.77, 0.27). The *medium* confidence study by Meng et al. (2018) detected a statistically significant decrease for boys per each ln-unit increase (β = −0.25 weeks; 95% CI: −0.43, −0.04) that was similar in magnitude.

Gestational Age Summary

Overall, there was mixed evidence of associations between PFDA and gestational duration endpoints. Only six of the twelve PFDA (two *high* and four *medium* confidence) studies showed some evidence of associations with gestational age in either the overall population, term/pre-term subsets, or either sex. Four of the six studies that showed some gestational age deficits were based on later biomarker sampling which might be indicative of an impact of pregnancy hemodynamics. Four of these studies had deficient study sensitivity ratings which may explain why some results were not statistically significant especially among the sex-specific analyses. No patterns were seen by study sensitivity among the six different null studies. There was limited evidence to draw any conclusions from the three sex-specific findings given that only two of five studies among boys and one study in girls detected any evidence of gestational age differences in relation to PFDA.

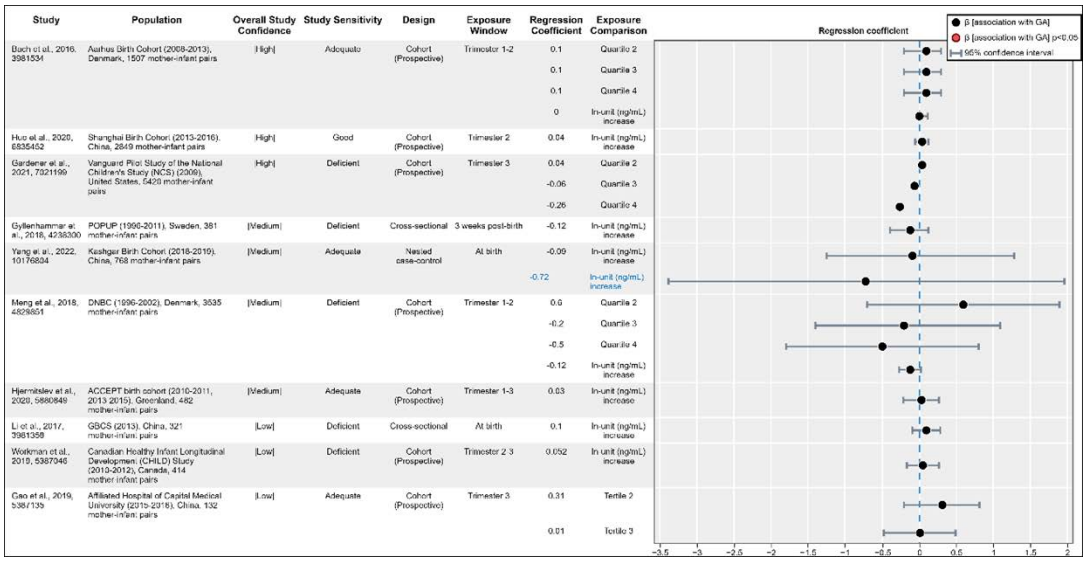


Figure 3-52. Overall population forest plot of 10 gestational age studies[a-d]. Refer to the HAWC link.

Abbreviation: GA = Gestational Age

[a.] Studies are sorted first by overall study confidence level then by Exposure Window examined.

[b.] Yang et al. (2022a), -0.7 per IQR Increase value is reported in the preterm birth population; the -0.08 per IQR increase value is in the term birth population.

[c.] Gardener gestational age differences estimated from digitization of their Figure 4; 95%CIs were not estimable.

[d.] For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses [e.g., (Yang et al., 2022a)].

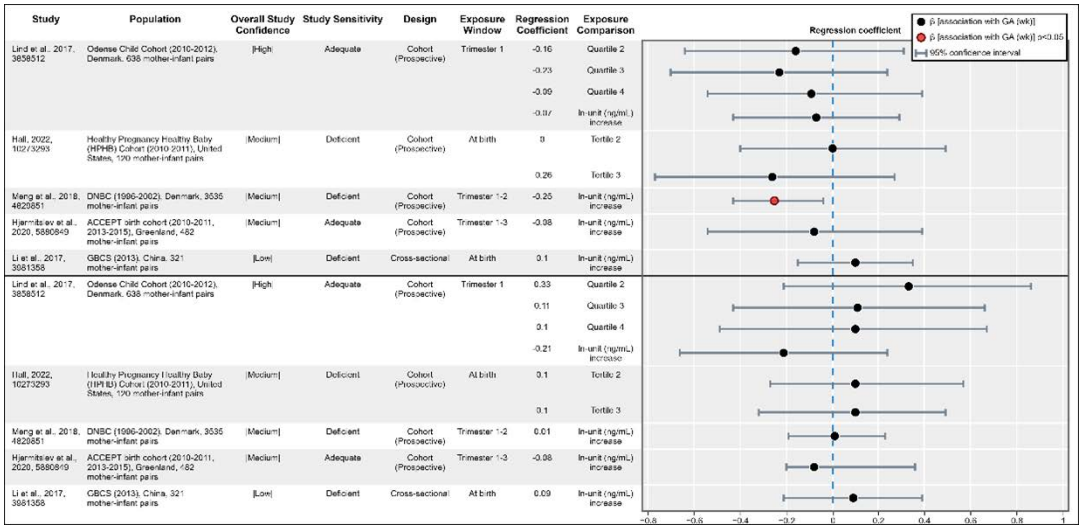


Figure 3-53. Sex stratified forest plot of five gestational age studies[a,b]. Refer to the HAWC link.

Abbreviation: GA = Gestational Age

[a.] Studies are sorted first by overall study confidence level then by exposure window examined.

[b.] For evaluation of patterns of results, we considered studies that collected biomarker samples concurrently or after birth to be cross-sectional analyses [e.g., Hall et al. (2022)].

Gestational Duration Summary

Seven different studies out of 12 showed some associations between PFDA exposures and different gestational duration measures with comparable levels of evidence in preterm birth and gestational age. Five of these seven studies were based on later biomarker sampling which might be indicative of an impact of pregnancy hemodynamics. Study sensitivity was limited in some studies and could explain some of the null results and lack of statistical significance especially in the sex-stratified analyses. Few other patterns were evident across sex or different study characteristics.

Table 3-21.Summary of 12 studies of PFDA exposure and gestational duration measures

Author	Study location/Years	n	Exposure median/IQR (range) in ng/mL	Study sensitivity domain judgment	PTB	GA
High Confidence Studies						
Bach et al. (2016)	Denmark, 2008–2013	1,507	0.30/0.20 (LOD–2.87)	Adequate	Ø All	Ø All
Gardener et al. (2021)	USA, 2009–2013	354	0.2/0.2 (LOD-2.6)	Deficient	-↑ All	– All
Huo et al. (2020)	China, 2013–2016	2,849	1.69/1.38 (N/A)	Good	↑ All ↑ Girls Ø Boys	Ø All
Lind et al. (2017a)	Denmark, 2010–2012	636	0.30/0.10 (0.1–1.8)	Adequate		Ø Girls Ø Boys
Medium Confidence Studies						
Gyllenhammar et al. (2018); Swedish Environmental Protection Agency (2017)[a]	Sweden, 1996–2001	381	0.24/0.14 (LOD–1.1)	Deficient		– All
Hall et al. (2022)	USA, 2010–2011	120	0.06/N/A (LOD-0.3)	Deficient		– Boys Girls
Hjermitslev et al. (2020)	Greenland, 2010–2011; 2013–2015	266	0.71/N/A (0.12–7.84)	Adequate	↓ All	Ø All Ø Girls Ø Boys
Meng et al. (2018)	Denmark, 1996–2002	2,132	0.20/0.10 (N/A)	Deficient	↑ **All***	– All **– Boys*** Ø Girls
Yang et al. (2022a)	China, 2018–2019	768	0.035-cases; 0.027- controls (range: 0.003-0.359)	Adequate	Ø All	– All
Low Confidence Studies						
Li et al. (2017)	China, 2013	321	0.15/0.16 (ND–2.12)	Deficient		Ø All Ø Girls Ø Boys
Gao et al. (2019)	China, 2015–2016	132	0.47 (LOD -3.15)	Adequate		Ø All
Workman et al. (2019)	Canada, 2010–2011	414	0.13/N/A (LOD-1.4)	Deficient		Ø All

Abbreviations: PTB = preterm birth; GA = gestational age.

***p < 0.05;** Ø: no association; +: positive association; −: negative association; ↑: increased odds ratio; ↓: decreased odds ratio.

Note: "Adverse effects" are indicated by both Increased ORs (↑) for dichotomous outcomes and negative associations (−) for the other outcomes.

[a]Swedish Environmental Protection Agency (2017) and Gyllenhammar et al. (2018) results are included here (both analyzed the POPUP cohort).

Birth Defects

Two studies examined birth defects in relation to PFDA exposures (Figure 3-53) with one each having adequate and deficient study sensitivity. The *medium* confidence congenital heart defect study by (Ou et al., 2021) showed increased risks for PFDA ≥0.53 ng/mL (vs. <0.53 ng/mL) for all defect groups examined including septal defects (OR = 2.33; 95%CI: 1.00, 5.45), conotruncal defects (OR = 2.58; 95%CI: 0.92, 7.25), and total heart defects (OR=1.83; 95%CI: 1.07, 3.12). The *low* confidence Cao et al. (2018) study showed minimal evidence of associations between PFDA exposures and all birth defects (OR = 1.37; 95%CI: 0.60, 3.08). There is considerable uncertainty in interpreting results for broad all birth defect groupings which decreases study sensitivity given the etiological heterogeneity across different birth defects. Overall, there was *limited* evidence of associations between PFDA exposures and birth defects in the two available epidemiological studies. However, there is insufficient data for any specific birth defects to draw further conclusions.

Fetal Loss-Spontaneous Abortion

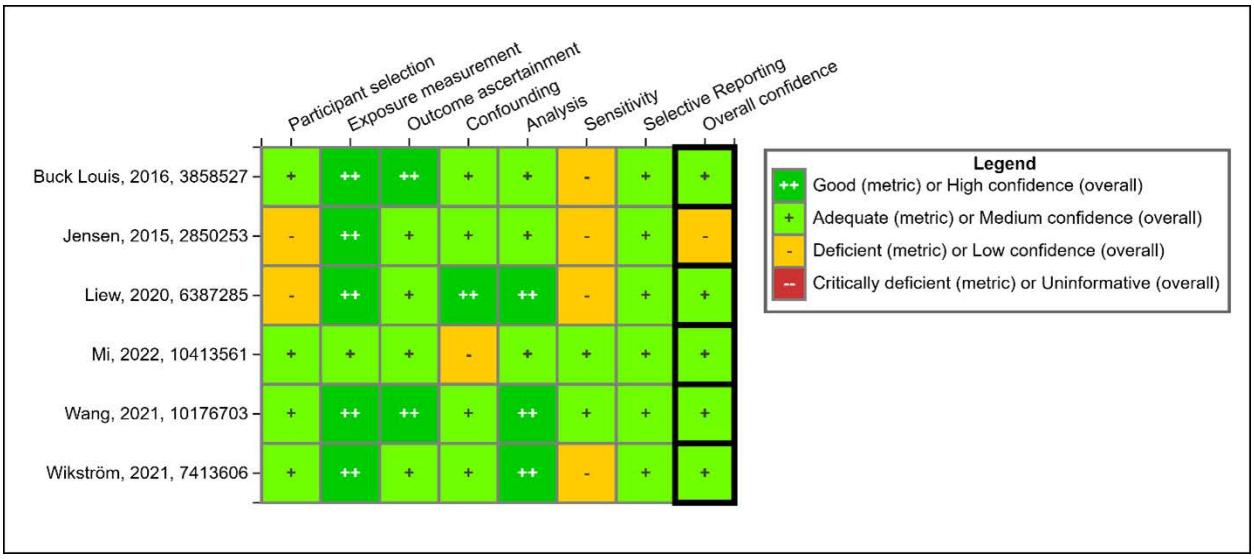


Figure 3-54. Study evaluation results for two epidemiological studies of spontaneous abortion and PFDA. Refer to HAWC for details on the study evaluation review: HAWC Human Spontaneous Abortion

Six (five *medium* and one *low* confidence) epidemiological studies (Mi et al., 2022; Wang et al., 2021; Wikström et al., 2021; Liew et al., 2020; Louis et al., 2016; Jensen et al., 2015) reported on the association between PFDA exposure and spontaneous abortion, which is defined as pregnancy loss occurring before approximately 20–22 weeks gestation. This period can be further divided into preclinical/early loss (occurring before implantation or before a pregnancy is clinically recognized) and clinical loss (occurring at 5–28 weeks gestation). The study evaluations of the

available studies are summarized in Figure 3-54. Two medium confidence studies were prospective cohorts with high ascertainment of early losses, one of couples trying to conceive, followed through delivery (Louis et al., 2016) and one of women undergoing in vitro fertilization (Wang et al., 2021). Three additional medium confidence studies assigned pregnant women from existing cohorts as controls and enrolled cases with first trimester losses (Wikström et al., 2021), throughout pregnancy (Mi et al., 2022), or identified cases via medical registry (Liew et al., 2020). One study considered *low* confidence. Jensen et al. (2015) is a cohort of pregnant women enrolled at 8–16 weeks gestation and was deficient for participant selection due to the high risk of incomplete case ascertainment (i.e., due to not including early losses and potential for loss to follow-up). Missing early losses has the potential to bias the results towards the null or even in a protective direction if there is a true effect but is unlikely to result in a spurious positive association. This potential also existed in Liew et al. (2020), but this study was not downgraded to low confidence as loss to follow-up was not a concern.

The results of the studies on spontaneous abortion are summarized in Table 3-22. Three of six studies showed some evidence of increased risk of spontaneous abortion. This included two studies (one *medium* and one *low* confidence) that reported strong positive associations between PFDA exposure and spontaneous abortion, with large effect sizes and statistical significance (Mi et al., 2022; Jensen et al., 2015). In addition, another *medium* confidence study by (Liew et al., 2020) reported a smaller (OR = 1.3; 95% CI: 0.7, 2.2) but not statistically significant positive association, while another medium confidence study (Wikström et al., 2021) was largely null. Two *medium* confidence studies, which were the only studies able to consider preclinical losses, reported inverse (nonsignificant) associations (Wang et al., 2021; Louis et al., 2016). It is unlikely that the limitations identified in the *low* confidence study would explain the observed positive associations, as bias in Jensen et al. (2015) is expected to be towards or past the null. Thus, while there is some evidence of an association with spontaneous abortion, there is considerable uncertainty due to inconsistency across *medium* confidence studies. It is possible that this is related to the inclusion of preclinical loss, but this is not clear based on available evidence.

Table 3-22. Associations between PFDA and spontaneous abortion in epidemiology studies

Reference, study confidence	Population	Median exposure (I25th, 75th) in ng/mL or as specified	Spontaneous abortion types included	Effect estimate description	Effect estimate (95% CI)
Liew et al. (2020), *medium*	Case-control nested within pregnancy cohort, Denmark; 438 women	0.2 (0.1-0.2)	Clinical, 12-22 weeks	OR (95% CI) for quartiles vs. Q1	Q2: 1.0 (0.6, 1.7) Q3: 1.1 (0.7, 1.9) Q4: 1.3 (0.7, 2.2)
Wikström et al. (2021), *medium*	Case-control nested within pregnancy cohort, Sweden; 1,529 women	0.3 (0.2-0.3)	Clinical, first trimester	OR (95% CI) for doubling of exposure	1.10 (0.81, 1.53)
Jensen et al. (2015), *low*	Pregnancy cohort, Denmark; 392 women	0.3 (0.2-0.6)	Clinical, post enrollment at 8-16 weeks	OR (95% CI) for tertiles vs. T1	T2: 1.9 (0.9, 3.8) **T3: 2.7 (1.3, 5.4)***
Louis et al. (2016), *medium*	Preconception cohort, U.S.; 344 women	0.4 (0.2-0.6)	Total	HR (95% CI) for tertiles vs. T1	T2: 0.83 (0.49, 1.40) T3: 0.68 (0.41, 1.14)
Wang et al. (2021), *medium*	Preconception cohort of women undergoing first IVF cycle, China, 305 women	0.5 (0.3-0.7)	Preclinical	RR (95% CI) for log-unit increase	0.67 (0.16, 2.73)
Mi et al. (2022), *medium*	Case-control nested within pregnancy cohort, China; 88 women	0.8	Clinical (9-12 weeks)	OR (95% CI) for above vs. below median	**5.00 (1.53, 16.33)***

Abbreviations: OR: odds ratio; HR: hazard ratio; RR: relative risk; T1: Tertile 1; T2: Tertile 2; T3: Tertile 3: IVF: in vitro fertilization.
*Denotes statistical significance at $p < 0.05$.

Animal studies

One toxicity study evaluated effects of PFDA on offspring (Harris and Birnbaum, 1989). This gavage study in mice examined maternal health, fetal survival, growth, and morphological development in two experiments covering different developmental windows. The two respective experiments consisted of gavage administration of 0–32.0 mg/kg-day on GD 10–13 to examine the developmental window related to cleft palate and hydronephrosis and gavage administration of 0–12.8 mg/kg-day on GD 6–15 to examine the entire developmental window related to the major period of organogenesis. The dams were necropsied on GD 18; the fetuses were removed from the uterus and examined. The Harris and Birnbaum (1989) study was evaluated as *high confidence* for most endpoints examined in both experiments (see Figure 3-55). Concerns were noted for fetal body weight measures as the study failed to report fetal body weights by sex, which impacted the results presentation domain and lowered the overall confidence of this endpoint to *medium*.

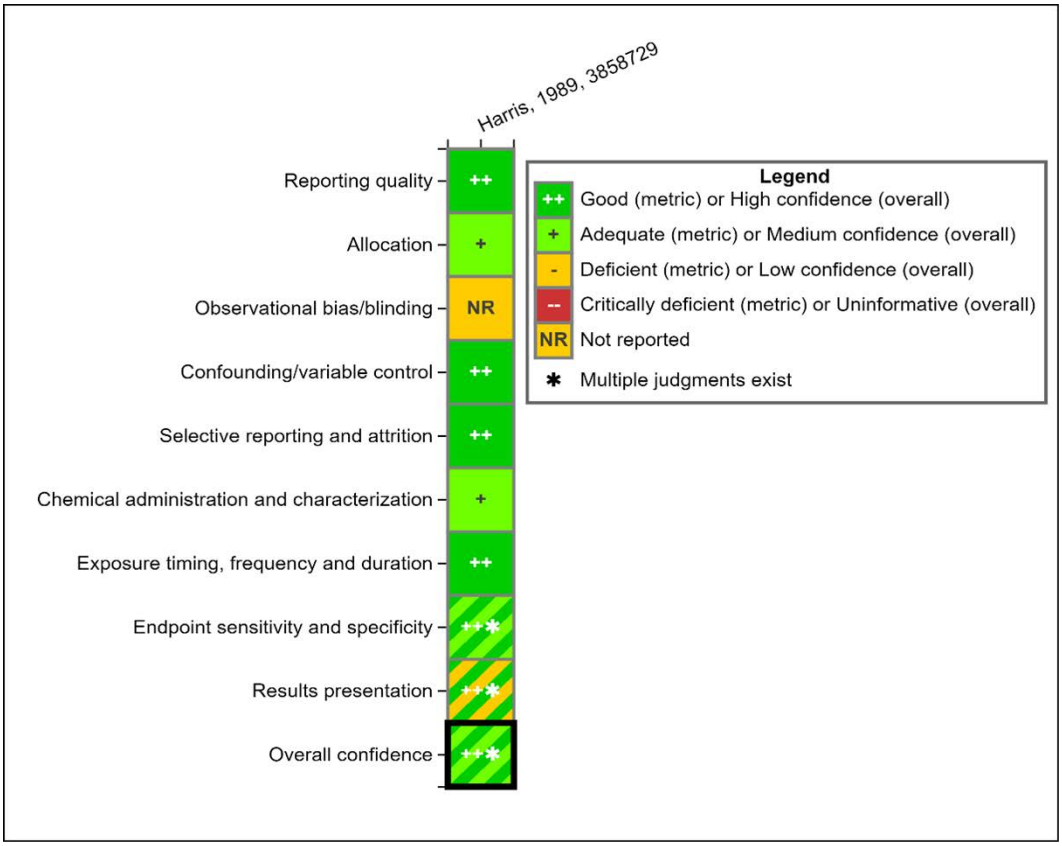


Figure 3-55. Developmental animal study evaluation heatmap. Refer to HAWC for details on the study evaluation.

Fetal growth

Fetal body weights were measured at GD 18 for each experiment (GD 10–13 or GD 6–15). Both experiments reported a significant trend in fetal body weight with decreases ≥5% being observed at ≥0.5 mg/kg-day (9.6–44%) for the GD 10–13 experiment and ≥3 mg/kg-day (6–50%) for the GD 6–15 experiment (see Figure 3-56 and Table 3-23). The changes in fetal body weight were of large magnitude and occurred at doses not associated with maternal toxicity. In the GD 10–13 experiment, changes in fetal body weight were ~10% at doses ranging from 0.5–4 mg/kg-day and were >40% at the highest dose (32 mg/kg-day). In the GD 6–15 experiment, changes in fetal body weight were 23% at 6.4 mg/kg-day and as large as 50% at the highest dose (12.8 mg/kg-day).

Endpoint Name	Study Name	Outcome Confidence	Study Design	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
Fetal Body Weight	Harris, 1989, 3858729	High confidence	Gestational Oral (GD 10-13)	F1 Mouse, C57BL/6n (♂♀)	significant	litter	0
							0.25
							0.5
							1
							2
							4
							8
							16
							32
			Gestational Oral (GD 6-15)	F1 Mouse, C57BL/6n (♂♀)	significant	litter	0
							0.03
							0.1
							0.3
							1
							3
							6.4
							12.8

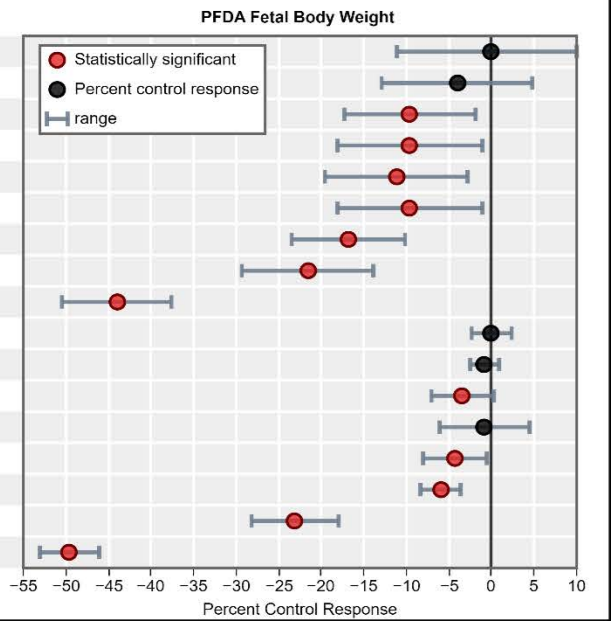


Figure 3-56. PFDA fetal body weight after gestational exposure (results can be viewed by clicking the HAWC link).

Table 3-23. Percent changes relative to controls in fetal body weight in a developmental mouse study after PFDA exposure (Harris and Birnbaum, 1989)

Endpoint	Dose (mg/kg-d)							
	0.25	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**
Decreased fetal body weight for the GD 10–13 experiment	–4	**–10**	**–10**	**–11**	**–10**	**–17**	**–22**	**–44**
	Dose (mg/kg-d)							
Endpoint	**0.03**	**0.1**	**0.3**	**1**	**3**	**6.4**	**12.8**	
Decreased fetal body weight for the GD 6–15 experiment	–1	**–3**	–1	**–4**	**–6**	**–23**	**–50**	

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

Maternal health

In the Harris and Birnbaum (1989) study, the health of the dams was assessed during both experiments through examination of body weight, liver weight and survival. Both exposure durations resulted in a significant trend in body weight change (defined as final body weight – gravid uterus weight + empty uterus weight – initial body weight) for the dams with statistically significant decreases in the two highest dose groups of both experiments. Body weight gain was markedly decreased (−149% change from controls) in the 12.8 mg/kg-day group of the GD 6–15 experiment (see Figure 3-57). A significant trend was also reported for increased liver weight in both the GD 10–13 and GD 6–15 experiments; refer to Section 3.2.1 for more detail on this effect. Maternal deaths were not observed in the GD 10–13 experiment, but 3 dams died in the high dose group (12.8 mg/kg-day) of the GD 6–15 experiment. This result is consistent with the overt toxicity of PFDA at high doses (refer to Section 3.2.10 on General toxicity effects for more details).

Fetal viability

In the Harris and Birnbaum (1989) study, endpoints related to fetal viability were measured at GD 18 for each experiment (i.e., groups dosed on GD 10–13 or GD 6–15). In both experiments, there was no difference in total implantations per litter between the control and treated groups indicating that the pregnancy rate was similar prior to exposure. However, following exposure, an increase in percent resorptions per litter (defined as [total number of resorptions and dead fetuses/number of total implantation sites] × 100) was observed in the high dose groups of both experiments (170% and 344% for the GD 10–13 and GD 6–15 experiments, respectively) with statistical significance reported for the GD 6–15 experiment (see Figure 3-33). A reduction in the number of live fetuses per litter was also reported in high dose groups of both experiments (32% and 36% for the GD 10–13 and GD 6–15 experiments, respectively) with statistical significance reported for the GD 6–15 experiment. Additionally, there was an increase in the number of dams

that experienced total resorption in the high dose groups of both experiments (4/12 dams vs. 0/13 in controls for the GD 10–13 experiment; 3/10 dams vs. 0/12 in controls for the GD 6–15 experiment) though the number of litters with resorptions were not different between control and treated groups (see Figure 333). Although these data might suggest an effect of maternal exposure on fetal viability as increased resorptions and decreased number of live fetuses are indicative of developmental toxicity per the U.S. EPA's Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991), effects on these endpoints were observed at doses that were also associated with significant maternal toxicity.

Morphological development

In the Harris and Birnbaum (1989) study, morphological development was examined in GD 18 fetuses for both the GD 10–13 and GD 6–15 experiments. This included external evaluation of all fetuses, soft tissue evaluation of 50% of the litters in each dose group (using Bouin's fixation and Wilson's free-hand sectioning technique), and skeletal evaluation of the remaining 50% of the litters in each dose group (using alizarin red S staining of ossified bone). In the GD 6–15 experiment, PFDA exposure caused significant dose-related trends for multiple skeletal variations (i.e., absence of fifth sternebrae, delay in braincase ossification, and delay in phalanges ossification) (see Figure 3-57). The fetal incidence of delayed braincase ossification was significantly increased at ≥0.03 mg/kg-day with the incidence rates ranging from 26 to 100%; it is unclear exactly which cranial bones are included in "braincase ossification." The number of fetuses with absence of the fifth sternebrae and delayed phalanges ossification was significantly increased at ≥6.4 mg/kg-day ranging from 15 to 35%. The statistical analyses of the skeletal variations data were performed independently by the U.S. EPA and not included in the original study. Litter incidence and individual fetus per litter data were not reported for these effects. Data for skeletal variations were reported as fetal incidence while data for individual fetus per litter is the preferred unit of analysis for these effects. Absence of the fifth sternebrae and delayed phalanges ossific- and mortality at 12.8 mg/kg-day). Whereas skeletal variations were significantly increased, the GD 6–15 experiment reported no soft tissue or skeletal malformations. Per the U.S. EPA's Guidelines for Developmental Toxicity Risk Assessment, a malformation is defined as "as a permanent structural change that may adversely affect survival, development, or function" while a variation "is used to indicate a divergence beyond the usual range of structural constitution that may not adversely affect survival or health." Furthermore, skeletal variations are commonly associated with maternal toxicity (Carney and Kimmel, 2007) as was observed for the absence of the fifth sternebrae and delayed phalanges ossification in mice exposed to PFDA. Based on the considerations above, including a lack of malformations and/or that some skeletal variations were observed at the same doses as maternal toxicity, the biological adversity for PFDA-induced skeletal variations is considered unlikely. Thus, the greatest level of concern is interpreted for the delayed brain ossification, although the significance of this variation (in terms of later biological consequences) is unclear.

Effect	Outcome Confidence	Experiment Name	Endpoint Name	Animal Description	Trend Test Result
Maternal Body Weight	High confidence	Gestational Oral (GD 10-13)	Maternal Body Weight Gain, Corrected	P0 Mouse, C57BL/6n (♀)	significant
		Gestational Oral (GD 6-15)	Maternal Body Weight Gain, Corrected	P0 Mouse, C57BL/6n (♀)	significant
Fetal survival	High confidence	Gestational Oral (GD 10-13)	Live Fetuses per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percent Resorptions per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with 100% Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Implantations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percentage Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
		Gestational Oral (GD 6-15)	Percent Resorptions per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with 100% Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
			Live Fetuses per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Implantations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Percentage Litters with Resorptions	F1 Mouse, C57BL/6n (♂♀)	not reported
Fetal growth	Medium confidence	Gestational Oral (GD 10-13)	Fetal Body Weight	F1 Mouse, C57BL/6n (♂♀)	significant
		Gestational Oral (GD 6-15)	Fetal Body Weight	F1 Mouse, C57BL/6n (♂♀)	significant
Morphological Development	Medium confidence	Gestational Oral (GD 10-13)	External Malformations per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Fetuses with Cleft Palate	F1 Mouse, C57BL/6n (♂♀)	not reported
			Fetuses with Hydronephrotic Kidneys	F1 Mouse, C57BL/6n (♂♀)	not reported
		Gestational Oral (GD 6-15)	Delay in Braincase Ossification	F1 Mouse, C57BL/6n (♂♀)	significant
			Absence of Fifth Sternabrae	F1 Mouse, C57BL/6n (♂♀)	significant
			Delay in Phalanges Ossification	F1 Mouse, C57BL/6n (♂♀)	significant
	High confidence	Gestational Oral (GD 6-15)	Number of Fetuses with Skeletal Defects	F1 Mouse, C57BL/6n (♂♀)	not reported
	Medium confidence	Gestational Oral (GD 6-15)	Misaligned Sternabrae	F1 Mouse, C57BL/6n (♂♀)	not reported
			Delay in Sternal Ossification	F1 Mouse, C57BL/6n (♂♀)	not reported
			Delay in Supraoccipital Ossification	F1 Mouse, C57BL/6n (♂♀)	not reported
			External Malformations, Variations or Cleft Palates per Litter	F1 Mouse, C57BL/6n (♂♀)	not reported
			Visceral Malformations or Variation	F1 Mouse, C57BL/6n (♂♀)	not reported

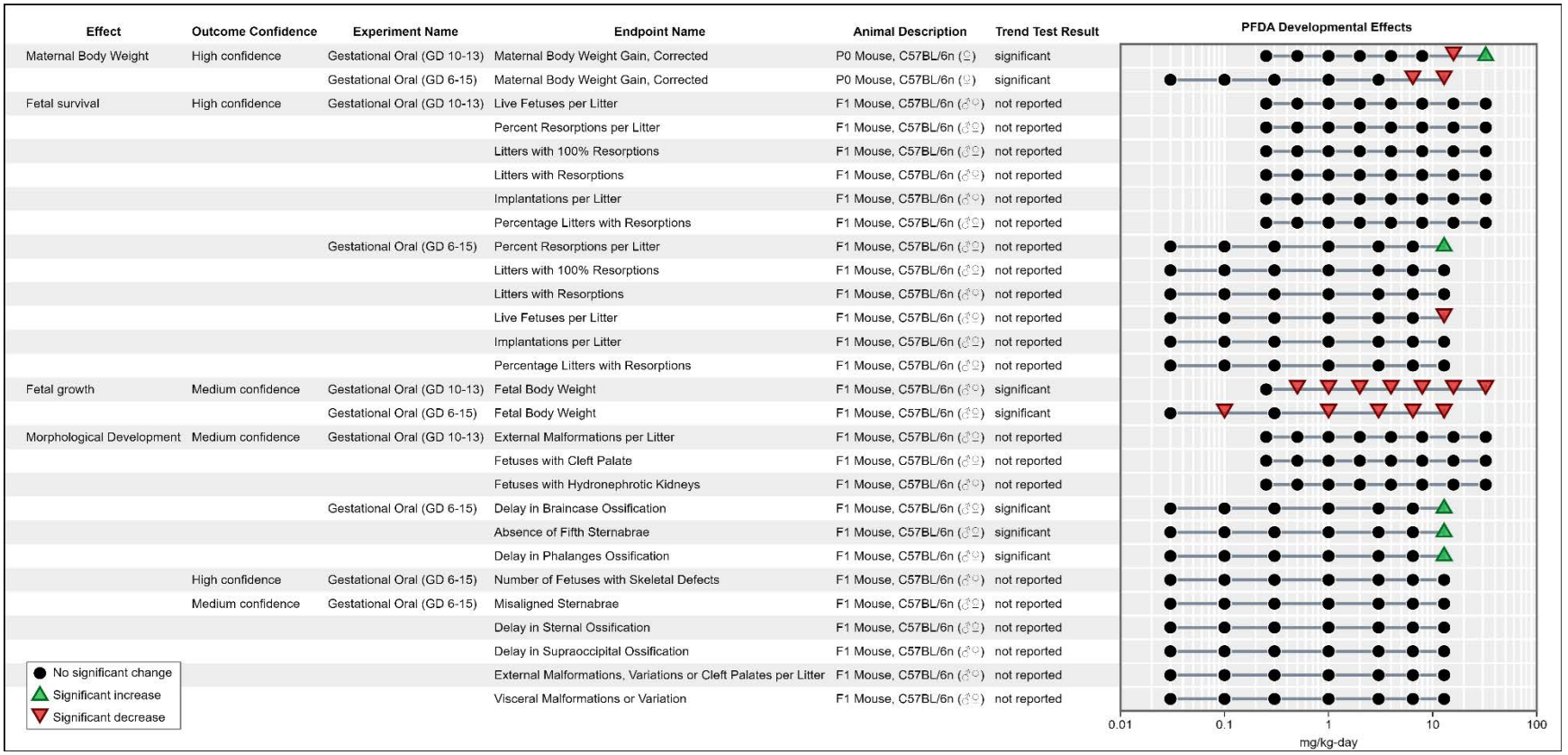


Figure 3-57. PFDA developmental effects (results can be viewed by clicking the HAWC link).

Mechanistic studies and supplemental information

In support for PFDA-induced developmental effects in humans and mice, (Truong et al., 2022) reported that PFDA caused morphological effects in embryonic zebrafish from a developmental toxicity screening study. Of the 139 PFAS tested, PFDA was determined to be the most potent for the induction of teratogenic effects. Similar results were reported in an additional study using zebrafish (Ulhaq et al., 2013). Ulhaq et al. (2013) reported that spinal curvature was a common malformation observed in zebrafish embryos exposed to PFDA and of the seven PFAS tested, PFDA was the second most potent for the induction of developmental toxicity.

Evidence Integration

Based on over 45 different epidemiological studies included here the evidence of an association between PFDA exposure and developmental effects in humans is considered *slight* but was supported by the *moderate* evidence in animals. The epidemiological evidence was strongest and most consistent for fetal growth restriction and in particular for birth-weight related measures, which were assessed by the most accurate growth restriction measures available. Out of 28 in total, 18 different studies showed some deficits for the overall population or for both/either sex across various birth weight measures. For example, 11 out of 22 PFDA studies in the overall population reported some birth weight deficits; this included 9 out of 14 *medium* and *high* confidence studies. Although data were more mixed, there appeared to be some coherence across these and other pre-natal growth measures with different postnatal growth parameters. For example, there was some consistency across 2 (one *high* and one *low* confidence) of the 3 postnatal weight studies with a common examination window (~2 years of age). The evidence for other endpoints was not as strong or consistent, including 10 of 17 birth length studies that showed some associations. Although the consistency varied somewhat across the developmental endpoints, the dearth of birth weight and birth length results in the overall study populations based on early or prepregnancy measures might be indicative of potential bias due to the impact due to pregnancy hemodynamics on PFDA levels. Despite fairly consistent evidence of an association between PFDA and different BWT-related measures, and more mixed for other endpoints, there is considerable uncertainty given that some sample timing differences may explain some of the reported fetal growth restriction deficits examined here.

Across the outcomes, this set of developmental studies was of good quality and generally had a low risk of bias, as 34 out of the 45 studies across the six primary endpoints [fetal growth restriction (including both birth weight and length measures), gestational duration, postnatal growth, anogenital distance, birth defects, and spontaneous abortions] were either *medium* or *high* overall confidence. Several studies demonstrated sufficient sensitivity to detect associations in the overall population and across sub-groups. However, many studies lacked power to detect statistical interactions or differences across populations especially those based on stratified analyses. This often results from low exposure levels with limited contrasts in many of the study

populations, which may have diminished the sensitivity of some studies to detect associations. As such, any null findings for studies with endpoints which lacked sensitivity should not be interpreted as supporting a lack of effect. In addition to the outcomes discussed in this section, pubertal development is discussed in the reproductive Sections (3.2.4 and 3.2.5) but could also be a developmental effect. The evidence for both males and females was based on one *medium* confidence study and was weak, but study sensitivity was again a concern.

As noted above, fetal growth restriction endpoints provided the strongest evidence for adverse developmental effects among the available studies. In considering the dose-dependence of the birth weight decreases, only one out of four *medium* or *high* confidence studies with categorical PFDA exposure data showed an exposure-response relationship. In addition, 9 of 14 *medium* or *high* confidence studies of the overall population as well as 9 of 14 sex-specific results showing adverse results based on continuous exposure also offer support for a biological gradient. Exposure-response relationships were less evident for other endpoints that were examined.

It can be challenging to identify patterns across heterogenous epidemiologic studies and study populations in the current database given the low exposure levels and/or limited and variable exposure contrasts. Examining birth weight differences in human populations is also challenging, since it can be difficult to differentiate pathological deficits versus natural biological variation. There was considerable variability in BWT deficits (β range: -29 to -101 g per ln-unit increases) in the overall population, with seven studies ranging from 31 to 59 g deficits per each ln-unit increase. The clinical significance of these changes may not be immediately evident, but effects of this magnitude can increase the number of infants at higher risk for other co-morbidities and mortality especially during the first year of life. These population-level changes may have a large public health impact when these mean birth weight deficits shift the birth weight distribution to include more infants in the low-birth-weight category. Additionally, decreased birth weight has been associated with long-term adverse health outcomes (Osmond and Barker, 2000).

Supporting the human evidence, the large and dose-dependent effects on fetal body weight observed across two independent experiments reported in the lone mouse study by Harris and Birnbaum (1989) (medium confidence for this endpoint) are without evidence to the contrary and thus provide moderate evidence coherent with the findings in humans. Following gestational PFDA exposure, decreases in fetal body weight with a significant trend were consistently observed in both experiments at ≥0.5 mg/kg-day, including doses (0.5–4 mg/kg-day) well below those that produced maternal toxicity. The changes in fetal body weight were also large in magnitude with the percent changes of up to 10% at the lower doses and ranging as high as 44–50% at the highest doses tested in both experiments. The rodent data for decreased fetal body weight are coherent with data from the human studies in which the strongest and most consistent evidence was for fetal growth restriction. Although an increased fetal incidence of several skeletal variations (i.e., delayed braincase and phalanges ossification and absence of fifth sternebrae) was observed, the delays in brain ossification, which started at ≥0.03 mg/kg-day, well below doses eliciting maternal toxicity,

were most notable. This change is potentially indicative of delayed development (which would be coherent with the PFDA-induced changes on fetal body weight); however, the significance of this variation (in terms of future adverse consequences), is unknown, and malformations, which are known to be adverse, were not observed. On a related note, PFDA was reported to be teratogenic in embryonic zebrafish (Truong et al., 2022; Ulhaq et al., 2013). There were also statistically significant changes reported for fetal viability in mice (i.e., increased % of resorptions per litter and reduced number of live fetuses per litter) at the highest dose tested in the GD 6–15 experiment (Harris and Birnbaum, 1989); however, effects on fetal viability were observed at the same doses as significant maternal toxicity, preventing the ability to draw conclusions at these doses.

A notable data gap exists, as animal studies evaluating the effect of PFDA on postnatal development were not identified. Although data were limited and not entirely consistent, some effects of PFDA on postnatal growth were observed in humans. Additionally, effects on postnatal development (e.g., delayed eye opening; reduced postnatal growth) have been observed in rodents exposed to other PFAS such as PFOA, PFBS, PFBA. Overall, the information for postnatal developmental effects is limited, introducing uncertainty on whether more sensitive developmental effects of PFDA might occur. An additional data gap is the lack of data to inform the potential mechanisms for PFDA-induced fetal growth restriction effects.

Taken together, the available ***evidence indicates*** that PFDA exposure is likely to cause developmental toxicity in humans given sufficient exposure conditions[10] (see Table 3-24). This conclusion is based primarily on findings of dose-dependent decreases in fetal weight in the only available toxicology study, with mice gestationally exposed to PFDA doses ≥0.5 mg/kg-day and supported by evidence of decreased birth weight from studies of exposed humans in which PFDA was measured during pregnancy, primarily with median PFDA values ranging from 0.11 to 0.46 ng/mL. The conclusion is further supported by coherent epidemiological evidence for biologically related effects (e.g., decreased postnatal growth and birth length).

[10] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-24. Evidence profile table for PFDA exposure and developmental effects

Evidence stream summary and interpretation					Inferences and summary judgment
Evidence from studies of exposed humans-fetal growth restriction (see Section 3.2.3: Human studies)					⊕⊕⊙ ***Evidence indicates (likely)***
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
<u>Fetal growth restriction</u> (Mean birth weight/ z-scores; Small for gestational age/low birth weight) 8 *high*, 10 *medium*, and 10 *low* confidence studies	• 18 of the 28 studies reported some inverse associations between PFDA exposures and standardized or mean birth weight measures including 17 of 26 studies of mean birth weight • 11 of 22 studies showed evidence of mean birth weight deficits in the overall population, including 9 out of 14 medium or high confidence studies • 9 of 14 studies in boys and girls reported some birth weight deficits including 8 out of 11 *medium* and *high* confidence studies in girls and 7 out of 11 in boys; 4 studies reported deficits in both sexes. • 3 of 5 studies of small for gestational age or *low* birth weight reported increased risks in the	• Consistent decreases across different populations and with variable study sensitivity • Most of the evidence among *high* and *medium* confidence studies (e.g., 9 out of 14 medium or high confidence studies showed BWT deficits) • Dose-dependent (evidence of linear relationships) in many studies examining continuous measures • Moderate or large magnitude of effect in many studies (typically > -30 grams per each ln-unit) • Although some variability is anticipated for observational studies of heterogenous	• Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth weight deficits, especially based on late biomarker sampling defined at trimester 2 or later, e.g., 9 of 11 studies in the overall population and 6 of 9 studies in girls and 5 of 9 in boys • Uncertainty of potential confounding in some studies due to some highly correlated PFAS like PFNA, although an evaluation of this possibility concludes that it would not fully explain the observed PFDA associations (see Appendix F)	⊕⊙⊙ *Slight* Based on consistent evidence for birth weight reductions, the most sensitive endpoint, with coherence across some other developmental endpoints (e.g., preterm birth, post-natal growth, and other fetal growth measures such as birth length, small for gestational age and low birth weight); more mixed for other endpoints like head circumference and gestational duration.	*Primary basis*: Slight human evidence for fetal and postnatal growth restriction supported by coherent moderate evidence in animals and for some other developmental endpoints in humans. *Human relevance*: Evidence in animals is presumed relevant to humans. *Cross-stream coherence*: Impaired fetal growth was observed in both humans and mice. *Susceptible populations and lifestages*: Based on evidence of impaired fetal growth from human and animal studies, early lifestages may be at higher risk. *Other inferences*: No specific factors are noted.

Evidence stream summary and interpretation					Inferences and summary judgment
	overall population; fairly consistent in magnitude (OR range: 1.2–1.8)	populations, exposure levels/sources, and design/analysis elements, coherence with findings for related outcomes, most notably for birth length and postnatal growth measures	• 1 out of 4 medium or high confidence studies with categorical data showed exposure-response relationships in overall population as well as in girls for standardized and mean BWT measures • Imprecision of some effect estimates		
Fetal growth restriction (Birth length) 6 *high*, 4 *medium,* and 7 *low* confidence studies	• 10 of 17 studies in total including 5 (2 *high*, 1 *medium* and 2 *low* confidence) of 15 examining the overall population reported some birth length deficits (including 3 of the 10 total *medium* or *high* confidence studies) • 7 (4 *high* and 3 *medium* confidence) of 10 sex-specific studies reported some birth length deficits; 4 studies each in boys and girls	• Overall population results were similar in magnitude despite between-study sources of heterogeneity including different exposure contrasts • Sex-specific deficits were often larger and more variable than the overall population	• Substantial uncertainty due to the potential impact of hemodynamic changes among studies showing birth length deficits based on later biomarker sampling, e.g., 4 of 5 studies in overall population and 4 of 7 sex-specific studies		
Fetal growth restriction (Head circumference) 5 *high*, 5 *medium*, and 4 *low* confidence studies (9 adequate and 5	• 5 (2 *high*; 3 *medium* confidence) of 14 studies reported smaller head circumference including 2 of 11 in overall	• Five of the 10 *high* and medium confidence studies reported smaller head circumference	• Limited evidence of associations especially in the overall population where five of the six		

Evidence stream summary and interpretation					Inferences and summary judgment
deficient study sensitivity)	population; and 3 of 7 sex-specific studies	in the overall population or either sex • Two of the 6 studies with adequate sensitivity reported some head circumference deficits across sexes	null studies had deficient study sensitivity		
Evidence from studies of exposed humans-anogenital distance (see Section 3.2.3: Human studies)					
Anogenital distance 3 *medium* confidence studies	• Inverse association between PFDA exposure and anogenital distance (AGD) in one of three medium confidence studies in boys and one of two studies in girls	• Adverse association in boys observed in 1 medium confidence study	• Unclear adversity of AGD decreases in girls • Although some variability is anticipated for observational studies of heterogenous populations, exposure levels/sources, and design/analysis elements, unexplained inconsistency	⊙⊙⊙ *Indeterminate* Based on inconsistent results across *medium* confidence studies	
Evidence from studies of exposed humans-gestational duration (see Section 3.2.3: Human studies)					
Gestational Duration (Preterm birth) 3 high and 3 medium confidence studies	• 3 (2 high and 1 medium confidence) of 6 preterm birth studies reported increased risk; 6 studies had deficient study	• Risks fairly consistent in magnitude (OR range: 1.3 to 2.2).	• Some uncertainty due to potential impact of pregnancy hemodynamics as 2 of 3 studies based on	⊕⊙⊙ *Slight* Mixed evidence and uncertainty due to the potential impact of hemodynamic changes among	

Evidence stream summary and interpretation					Inferences and summary judgment
	sensitivity; 5 adequate, and 1 good		later biomarker sampling • Potential confounding by PFAS including highly correlated PFNA; limited evidence for PFNA suggests would not likely fully explain PFDA associations	studies with gestational duration deficits	
Gestational Duration (Gestational age) 4 *high*, 5 *medium*, and 3 *low* confidence studies	• 6 of 12 studies reported lower gestational age; 4 of these 6 had deficient study sensitivity	• No factors noted	• Unexplained inconsistency, although this may be partially due to poor sensitivity • Substantial uncertainty due to the potential impact of hemodynamic changes among 4 of 6 studies showing gestational age deficits, especially based on late sampling (defined as trimester 2 or later) • Outcome may be prone to some measurement error		
Evidence from studies of exposed humans-postnatal growth (see Section 3.2.3: Human studies)					
Postnatal growth	• 3 (1 *high* and 2 *low* confidence) of 6 studies	• *Consistency* across 2 of the 3 weight	• Potential confounding across	⊕⊙⊙ *Slight*	

Evidence stream summary and interpretation					Inferences and summary judgment
4 high, 1 medium and 3 low confidence studies	showed postnatal weight deficits; with limited sensitivity in some studies (3 adequate; 3 deficient) • 2 (1 *high* and 1 *low* confidence) of 5 studies showed postnatal height deficits; with limited sensitivity in some studies (3 adequate; 2 deficient) • 2 (1 *high* and 1 *low* confidence) of 3 studies showed increased adiposity; with limited sensitivity in some studies (1 adequate; 2 deficient) • Both *high* confidence studies showed minimal and mixed rapid weight gain results; with limited sensitivity in some studies (1 adequate; 1 deficient)	studies with a common examination window (2 years of age), including one *high* and one *low* confidence study	PFAS for some endpoints • Unknown critical window(s) for childhood growth endpoints; assumption was in utero period is most relevant	Mixed results across different measures, with limited study sensitivity in some studies. Results were more consistent when a homogenous population considered (~2 years of age).	
Evidence from studies of exposed humans-spontaneous abortion (see Section 3.2.3: Human studies)					
<u>Spontaneous abortion</u> 5 medium and 1 low confidence studies	• Two *medium* and one *low* confidence studies reported increased odds of spontaneous abortion while 2 medium confidence study	• Large effect size in two studies (OR>2)	• Unexplained inconsistency across medium confidence studies	⊕⊙⊙ *Slight* Based on inconsistent evidence across studies	

Evidence stream summary and interpretation					Inferences and summary judgment
	reported an inverse association.		• Potential confounding across PFAS		
Evidence from in vivo animal studies (see Section 3.2.3: Animal studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
<u>Fetal growth</u> 1 *medium* confidence study (2 independent experiments)	• Fetal body weight was reduced at ≥0.5 mg/kgday in the GD 10–13 experiment (maternal body weight decreased at ≥16.0 mg/kg-d). • Fetal body weight was reduced at ≥1.0 mg/kg-d in the GD 6–15 experiment (maternal body weight decreased at ≥6.4 mg/kg-d, with mortality at higher doses).	• *Consistency* across the *medium* confidence GD 10–13 and GD 6–15 experiments. • *Dose-response* gradient observed within experiments and exposure duration gradient observed across experiments • Large magnitude of effects (up to 50%).	• No factors noted	⊕⊕⊙ *Moderate* Based primarily on decreased fetal growth at ≥0.5 mg/kg-d in two independent experiments from a single study in mice. The reliability and biological significance of other, potentially related, findings from this study are unclear.	
<u>Fetal viability</u> 1 *high* confidence study	• A treatment-related increase in the percentage of resorptions per litter was reported at 12.8 mg/kg-d in dams treated from GD 6–15. • The number of live fetuses per litter was reduced at 12.8 mg/kg-d	• Coherence of effects on percentage of resorptions and number of live fetuses in a *high* confidence study.	• Substantial concern for potential confounding as decreased fetal viability occurred at the same dose as maternal mortality.		

Evidence stream summary and interpretation					Inferences and summary judgment
	in dams treated from GD 6–15.				
Morphological development 1 *medium* confidence study (two independent experiments)	• Increased fetal incidences of skeletal variations (i.e., absence of fifth sternebrae at ≥6.4 mg/kg-d • Delayed ossification of the phalanges at ≥6.4 mg/kg-d • Delayed braincase ossification at ≥0.03 mg/kg-d).	• Dose-response gradient for skeletal and braincase ossification variations. • Consistent increase in variations across two *medium* confidence experiments	• Unclear biological relevance of variations as no malformations were reported. • Potential confounding of skeletal and phalanges ossification variations at doses causing overt toxicity.		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated** • **Key findings, interpretation, and limitations**			**Evidence stream judgment**	
Other evidence	*Interpretation*I: PFDA causes developmental toxicity in embryonic zebrafish. Key findings: • Of the 139 PFAS chemicals tested, PFDA was the most potent for the induction of teratogenic effects in zebrafish (Truong et al., 2022) • Of the 7 PFAS chemicals tested, PFDA was the second most potent for the induction of developmental effects in zebrafish. Spinal curvature, a malformation, was commonly reported in zebrafish embryos exposed to PFDA (Ulhaq et al., 2013). *Limitations*: A comprehensive list of tested/observed developmental endpoints was not provided.			The findings in zebrafish provide some support for the biological plausibility of the developmental effects in humans and animals.	

1

3.2.4. MALE REPRODUCTIVE EFFECTS

Human studies

There are nine epidemiology studies that examined the association between PFDA exposure and male reproductive effects. The outcomes included in these studies were semen parameters, reproductive hormones, timing of pubertal development, and anogenital distance. The studies are described below.

Semen evaluations

Semen concentration and sperm motility and morphology were considered the core endpoints for the assessment of semen parameters. Key issues for the assessment of semen parameters involve sample collection and sample analysis. Samples should be collected after an abstinence period of 2–7 days, and analysis should take place within two hours of collection and follow guidelines established by the World Health Organization (WHO, 2010). While exposure would ideally be measured during the period of spermatogenesis rather than concurrent with the outcome, a cross-sectional design is considered adequate because the period of spermatogenesis in humans is fairly short (74 days plus 12 days of maturation) (Sigman et al., 1997), the half-life of PFDA is long, and there is no concern for reverse causality with this outcome because it is not expected the semen quality would influence PFDA concentrations in blood.

Four cross-sectional studies examined the relationship between PFDA and semen quality. Based on the above considerations, three were evaluated as medium confidence overall (Figure 3-34), though one of these was considered uninformative for the core endpoint sperm motility due to the overnight delay between collection and analysis (Buck Louis et al., 2015). One study analyzed male partners from a preconception cohort in the U.S. (Buck Louis et al., 2015), one study enrolled young adult men whose mothers were enrolled in a national pregnancy cohort (Petersen et al., 2022), and one enrolled healthy young man being considered for military service (Joensen et al., 2013). The remaining study was low confidence due to multiple identified deficiencies and was focused on men seeking infertility assessment (Huang et al., 2019a). All four studies analyzed PFDA in serum used appropriate methods, and, thus, exposure misclassification is expected to be minimal.

Figure 3-58. Semen parameters epidemiology study evaluation heatmap.
Refer to HAWC for details on the study evaluation review: HAWC Human Semen Parameters.

The results for the association between PFDA exposure and semen quality are presented in Table 3-25. The studies analyzed the outcomes differently, so the effect estimates are not directly comparable. None of the results were statistically significant, but there was a suggestion of a decrease in motility with increased exposure in Joensen et al. (2013) and in concentration in Huang et al. (2019a), but not in Petersen et al. (2022). Because the methods used to assess motility was considered critically deficient in Buck Louis et al. (2015), it was not possible to evaluate its consistency with the other medium confidence studies. For concentration and morphology, there was no clear decrease in the medium confidence studies. However, PFDA levels in both studies were lower than levels of other measured PFAS (≤0.5 ng/mL) and the exposure contrasts were narrow, which introduces concerns regarding sensitivity, i.e., lack of ability to detect and association if present.

Table 3-25. Associations between serum PFDA and semen parameters in epidemiology studies

Reference; study confidence	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Concentration (× 106/mL)	Motility (% motile)	Morphology (% normal)
Huang et al. (2019a); *low*	Cross-sectional study of men seeking infertility assessment (2009–2010); 57 men	0.0 (range 0.0–1.2)	β (95% CI) for 1 ln-unit increase in serum PFDA	−21.59 (−77.91, 34.73)	5.96 (−11.58, 23.50)	−0.02 (−0.10, 0.07)

Reference; study confidence	Population	Median exposure (IQR) (ng/mL)	Effect estimate	Concentration (× 106/mL)	Motility (% motile)	Morphology (% normal)
Petersen et al. (2022), *medium*	Cross-sectional analysis within cohort of general population men (2017-2019), Denmark; 1,041 men (18–20 yr)	0.2 (5^{th}-95^{th}: 0.1-0.3)	% difference (95% CI) for tertiles of PFDA vs T1	T2: 3 (-9, 17) T3: -3 (-15, 11)	T2: -1 (-6, 6) T3: -3 (-9, 3)	T2: -1 (-11,10) T3: 2 (-8,13)
Joensen et al. (2013); *medium*	Cross-sectional study of men evaluated for military service (2008–2009), Denmark; 247 men (18–22 yr)	0.4 (0.3–0.5)	β (95% CI) for 1-unit increase in serum PFDA	Cubic root transformed 0.22 (−0.76, 1.19)	Square transformed −1343 (−2759, 73.69)	Square root transformed −0.097 (−0.88, 0.69)
Buck Louis et al. (2015); *medium*	Cross-sectional analysis within preconception cohort (2005–2009), U.S.; 462 men	0.5 (0.3–0.6)	β (95% CI) for 1 ln-unit increase in serum PFDA	−1.06 (−30.5, 28.3)	Uninformative	5.80 (−1.31, 12.9)

*$p < 0.05$.

Reproductive hormones

Testosterone and estradiol were considered the primary endpoints for male reproductive hormones. Progesterone, LH, FSH, and SHBG were also reviewed where available. Key issues for the evaluation of these studies were sample collection and processing (see Figure 3-59). For testosterone, LH, and FSH, due to diurnal variation, blood sample collection should be in the morning, and if not, time of collection should be accounted for in the analysis. If there is no consideration of time of collection for these hormones, the study is classified as *deficient* for outcome ascertainment and *low* confidence overall. A cross-sectional design was considered appropriate for this outcome since levels of these hormones are capable of being rapidly upregulated or downregulated and they are not expected to directly bind to or otherwise interact with circulating PFAS.

Seven studies (eight publications) examined the relationship between PFDA and reproductive hormones. Three studies were *medium* confidence cross-sectional studies in adults, including Joensen et al. (2013) and Petersen et al. (2022), cross-sectional studies of young adult men described above. An analysis of NHANES data in adult men (Xie et al., 2021) was also *medium* confidence for estradiol but *low* confidence for testosterone due to potential outcome misclassification as previously described. A cross-sectional study in adolescents (aged 13–15 years) (reported in Zhou et al. (2016) and Zhou et al. (2017b)) was *low* confidence due to concerns

for confounding (e.g., pubertal indicators were not considered). Three studies, one a birth cohort in Denmark (Jensen et al., 2020b) and two cross-sectional studies in China (Liu et al., 2020b; Yao et al., 2019) examined associations in infants. Yao et al. (2019) and Yao et al. (2019) were low confidence due to not accounting for time of day of sample collections (both studies) and potential concerns for confounding (Yao et al., 2019). Liu et al. (2020b) was *medium* confidence due to less concern for diurnal variation of the included hormone (progesterone).

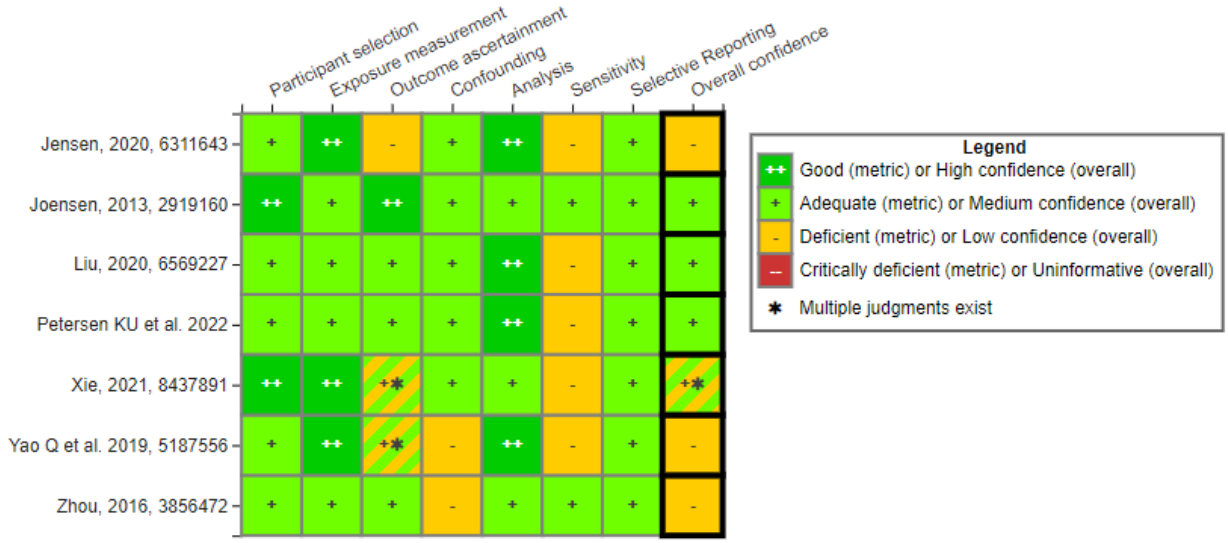


Figure 3-59. Male reproductive hormones epidemiology study evaluation heatmap. Refer to HAWC for details on the study evaluation review: HAWC Human Male Reproductive Hormones.

*Outcome-specific ratings differed for this domain.

Given the differences in populations (adults, adolescents, newborns), evaluation of consistency across studies is not straightforward. For testosterone, inverse associations between PFDA exposure and testosterone levels were observed in two studies. Among the two *medium* confidence studies for this outcome, Joensen et al. (2013) observed a decrease in log-transformed testosterone with higher PFDA exposure in adult men, though this was not statistically significant (β (95% CI) = −0.17 (−0.41, 0.07)). Petersen et al. (2022) reported no association with association. Also in adults, but *low* confidence for testosterone, Xie et al. (2021) found positive associations between PFDA exposure and free and total testosterone (statistically significant for free testosterone, with exposure gradient observed across quartiles). In adolescent boys, the *low* confidence study by Zhou et al. (2016) reported an inverse association (β (95% CI) = −0.26 (−0.41, −0.10)). In infants, one study (Jensen et al., 2020b) reported a positive association between PFDA exposure and testosterone (β=0.37, 95% CI: −0.11, 0.84, p=0.1) while no association was observed in Yao et al. (2019).

For estradiol, in adults in Joensen et al. (2013), there was also a decrease with higher PFDA exposure (β (95% CI) = −0.22 (−0.48, 0.002)), but this was not observed in the other two studies in adults (Petersen et al., 2022; Xie et al., 2021) or in adolescents in Zhou et al. (2016) or infants in Yao et al. (2019). Joensen et al. (2013) also examined several other reproductive hormones and sex hormone binding globulin (SHBG) in young men and found no evidence of association with PFDA exposure for SHBG, luteinizing hormone, or inhibin-B, but did report a positive association with follicle stimulating hormone (FSH) (β (95% CI) = 0.42 (−0.005, 0.85)). The increase in FSH would be consistent with an increase in gonadotropin production as a compensatory response to a decrease in testosterone. However, Petersen et al. (2022) found no association with FSH, LH, or SHBG. In (Jensen et al., 2020b), inverse associations, though not statistically significant were observed with DHEA, DHEAS, and Androstenedione. Liu et al. (2020b) found no association with progesterone.

Pubertal development

Pubertal development is primarily assessed using established criteria, such as Tanner stage ratings. For boys, Tanner staging involves evaluation of the development of genitalia (scrotum appearance, testes, and penile size) and pubic hair. Stage 1 represents prepubertal development; Stage 2, the onset of pubertal development, and Stage 5 represents full sexual maturity. Two medium confidence birth cohorts in Denmark (Ernst et al., 2019) and the U.S. (Carwile et al., 2021) examined timing of pubertal development with PFDA exposure. Ernst et al. (2019) used maternal exposure measured in blood and prospectively identified pubertal onset with follow-up checks every six months. In boys, they reported that there was no clear pattern of association between PFDA exposure and Tanner stages of genital development or pubic hair, or other markers of pubertal development such as axillary hair, acne, voice break, or first nocturnal ejaculation when exposure was analyzed in tertiles. For each outcome, the mean age of onset was later in the middle (0.16–0.21 ng/mL) vs. the lowest (0.08–0.15 ng/mL) tertile, but earlier in the highest tertile (0.22–0.9 ng/mL). This pattern was also observed with a combined puberty indicator outcome, with boys in the middle tertile reaching the indicator 4.59 months later (95% CI: −0.93, 10.11) and the highest tertile 2.83 months earlier (95% CI: −8.43, 2.77) than the lowest tertile. Carwile et al. (2021) used exposure measured during mid-childhood (median 8 years) with follow-up to early adolescence (median 13 years). Using a pubertal development score based on parental responses to scales of multiple pubertal markers (voice deepening, body hair growth, facial hair growth, acne, and growth spurt), they reported no association with PFDA exposure. This was consistent with their findings for older age at peak heigh velocity (used as a proxy for pubertal development). Exposure contrast was narrow in both studies (median 0.2 ng/mL, 10th–90th percentile 0.1–0.3 in Ernst et al. (2019), 0.3, 25th–75th percentile 0.2–0.5 in Carwile et al. (2021)), which may have reduced study sensitivity.

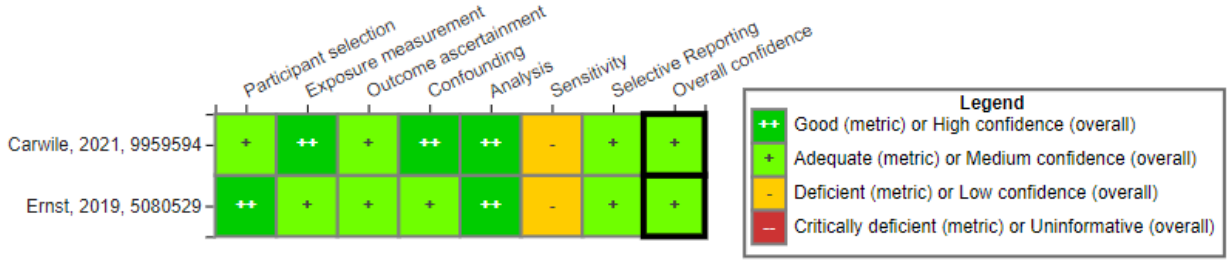


Figure 3-60. Male pubertal development epidemiology study evaluation heatmap. Refer to HAWC for details on the study evaluation review: HAWC Human Male Pubertal Development.

Summary of human studies

Overall, there is inconsistent evidence for male reproductive effects of PFDA exposure. One medium confidence study in adult men found reduced sperm motility and testosterone (Joensen et al., 2013) and one low confidence study also found an inverse association in adolescents (Zhou et al., 2016). This is coherent with an inverse association with anogenital distance in one medium confidence study (Tian et al., 2019) (see Section 3.2.3 on Developmental Effects). However, the other available studies did not report consistent findings for semen parameters and reproductive hormones. No clear association was observed with estradiol or pubertal development.

Animal studies

Only one animal toxicity study evaluated male reproductive effects after PFDA exposure (NTP, 2018). This study examined the following endpoints after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) in 7- to 8-week-old male Sprague-Dawley rats: sperm evaluations, histopathology, hormone levels, and organ weights. The endpoints evaluated by NTP (2018) are considered to be reliable measures for assessing male reproductive toxicity (Creasy and Chapin, 2018; Creasy et al., 2012; Sellers et al., 2007; U.S. EPA, 1996b). The NTP (2018) study was evaluated as high confidence for most endpoints examined with no notable concerns in any of the study evaluation domains (see Figure 3-61). Concerns for potential insensitivity were identified for sperm measures as the exposure duration (28 days) used for this experiment was insufficient to fully detect potential effects on sperm development, resulting in a low confidence rating; this potential bias is towards the null. In rats, spermatogenesis takes approximately 8 weeks for germ cells to mature from spermatogonia to spermatozoa (Creasy and Chapin, 2018).

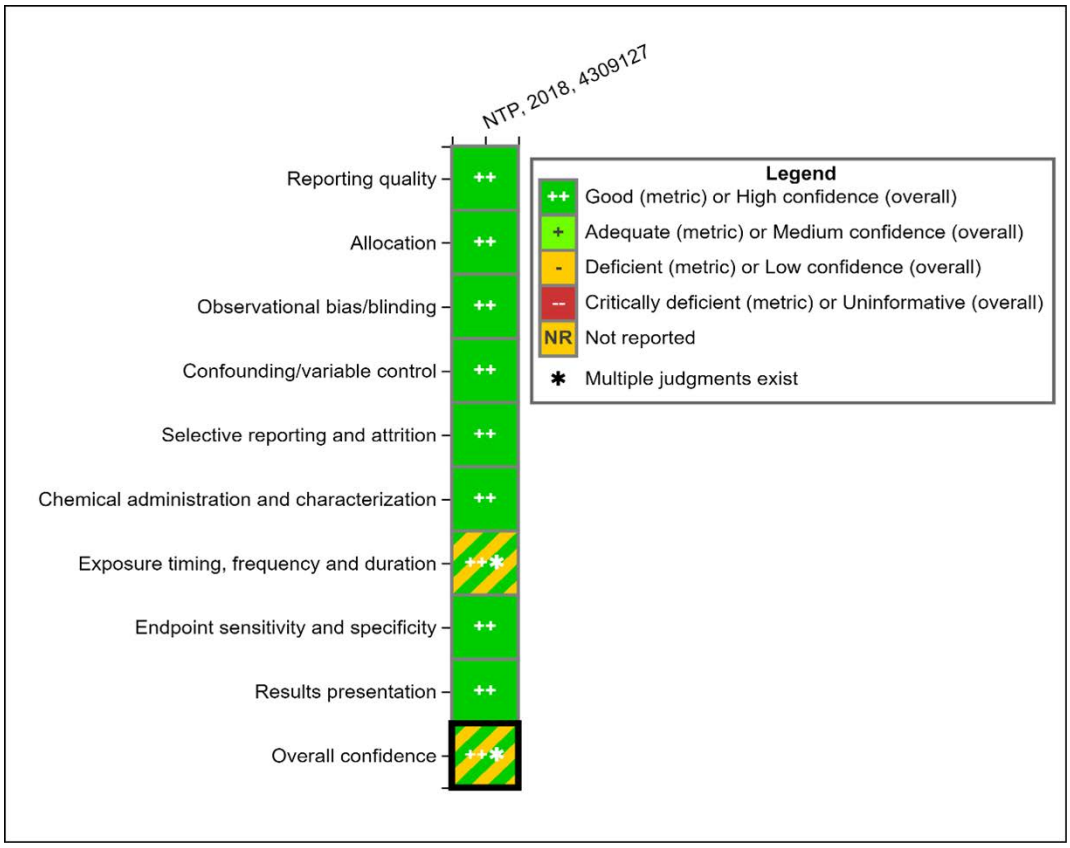


Figure 3-61. Evaluation results for animal study assessing effects of PFDA exposure on male reproductions. Refer to HAWC for details on the study evaluation review: HAWC NTP (2018).

Sperm evaluations

Testicular and epididymal sperm counts and testicular sperm motility were only measured for the three highest dose groups (0.625, 1.25 and 2.5 mg/kg-day) (see Figure 3-62). Testicular sperm counts are indicative of changes in sperm production in the testis, while epididymal counts indicate both changes in testicular sperm production and storage of sperm in the epididymis; therefore, both measures are considered informative for evaluating effects on sperm parameters (Creasy and Chapin, 2018; Creasy et al., 2012). Testicular sperm counts (absolute and relative to organ weight) decreased dose- dependently at 0.625 and 1.25 mg/kg-day (−10% and −19–21% change compared to controls, respectively) but not at the highest dose group (2.5 mg/kg-day). As such, a clear trend for testicular sperm counts could not be established. A significant trend was reported for cauda epididymal sperm counts with decreases of 11–30% compared to controls across 0.625–2.5 mg/kg-day. NTP (2018) also reported sperm counts normalized to cauda epididymis weight and observed no treatment-related effects (data not shown in Figure 3-62). However, this is not considered a sensitive measure as sperm contributes to epididymal weight and reporting findings as a ratio may mask reductions in sperm number (U.S. EPA, 1996b). A non-statistically significant decrease in testicular sperm motility of 11% compared to controls was

reported at 2.5 mg/kg-day, but there was no clear dose-response effect. In summary, the dose-related decreases in sperm counts in the epididymis suggest that PFDA can affect sperm parameters at doses ≥ 0.625 mg/kg-day after 28-day exposure.

The findings on sperm measures from NTP (2018) are interpreted with caution as sensitivity concerns for these outcomes are based on the exposure duration used in this study which did not capture the entire process of spermatogenesis (approximately 8 weeks in rats) (Creasy and Chapin, 2018).

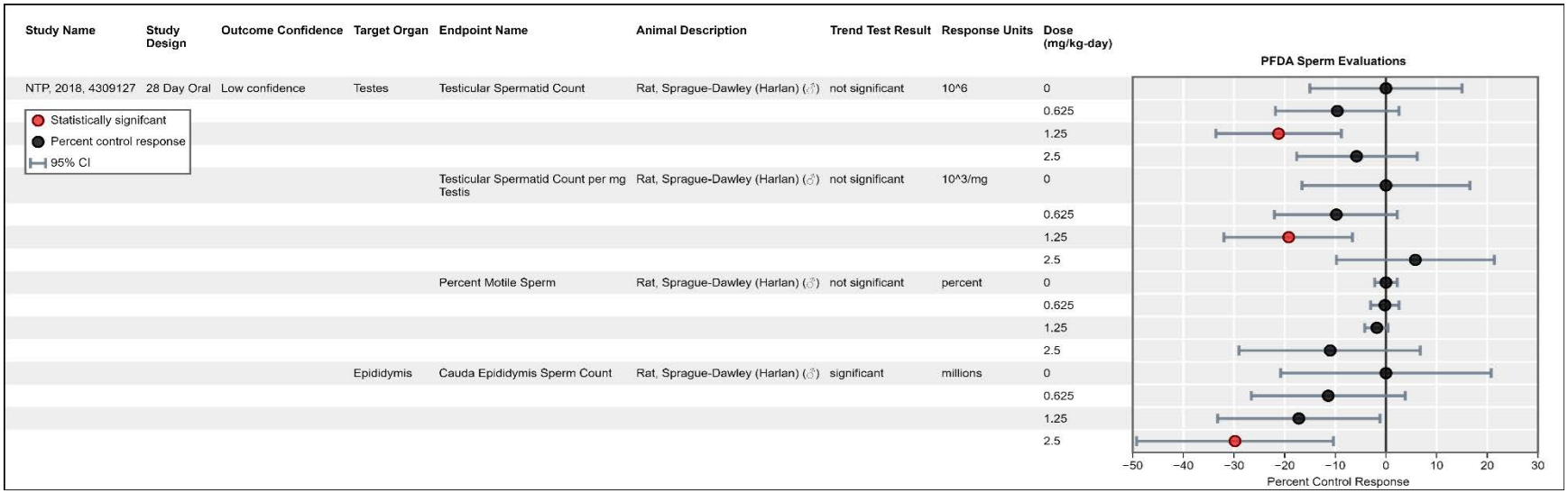


Figure 3-62. Effects on sperm evaluations following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Histopathology

Testicular and epididymal lesions were reported in the 28-day rat study by NTP (2018). The testes were examined in all dose groups for histopathologic responses (see Figure 3-63). Minimal to mild atrophy of the interstitial (Leydig) cells was observed in nearly all the rats exposed to the two highest PFDA dose groups (8/10 and 10/10 for 1.25 and 2.5 mg/kg-day, respectively) but not in the controls. Leydig cell atrophy is a response coherent with reduced sperm production (Creasy and Chapin, 2018; Creasy et al., 2012) and indicative of reduced androgen levels, which were also observed in this study (see synthesis of Reproductive hormones in this Section). Mild degeneration of the germinal epithelium and spermatid retention within the seminiferous tubules was also increased in 4/10 rats from the high dose group; control group incidence was 1/10 and 0/10, respectively. The epididymis was examined in the three highest dose groups (0.625, 12.5 and 2.5 mg/kg-day) (see Figure 3-63). Only the highest dose group (2.5 mg/kg-day) displayed mild duct germ cell exfoliation in 4/10 rats examined compared to 1/10 rats in the control group and a single marked case of hypospermia (1/10 rats) not observed in the controls. Sperm granuloma was found in 1/10 rats in the controls but not in the exposed animals (data not shown in Figure 3-63). NTP (2018) did not observe any histopathological effects on the preputial gland, seminal vesicle, and prostate when examining animals in the control and high dose groups. In summary, there is consistent evidence of histopathological observations indicative of mild degenerative changes in the testes and epididymis at doses ≥ 1.25 mg/kg-day after 28-day exposure. Note that these doses are associated with significant body weight changes (see Evidence Integration section below for a discussion on potential confounding due to co-occurring systemic toxicity at doses causing some PFDA-induced male reproductive effects).

Study Name	Study Design	Outcome Confidence	Target Organ	Endpoint Name	Animal Description	Trend Test Result	Incidence	Dose (mg/kg-day)
NTP, 2018, 4309127	28 Day Oral	High confidence	Testes	Germinal Epithelium Degeneration	Rat, Sprague-Dawley (Harlan) (♂)	significant	1/10 (10.0%)	0
							0/10 (0.0%)	0.156
								0.312
								0.625
								1.25
							4/10 (40.0%)	2.5
				Interstitial Cell Atrophy	Rat, Sprague-Dawley (Harlan) (♂)	significant	0/10 (0.0%)	0
								0.156
								0.312
								0.625
							8/10 (80.0%)	1.25
							10/10 (100.0%)	2.5
				Seminiferous Tubule Spermatid Retention	Rat, Sprague-Dawley (Harlan) (♂)	significant	0/10 (0.0%)	0
								0.156
								0.312
								0.625
								1.25
							4/10 (40.0%)	2.5
			Epididymis	Exfoliated Germ Cell, Epididymal Duct	Rat, Sprague-Dawley (Harlan) (♂)	significant	1/10 (10.0%)	0
							0/10 (0.0%)	0.625
								1.25
							4/10 (40.0%)	2.5
		Low confidence	Epididymis	Hypospermia	Rat, Sprague-Dawley (Harlan) (♂)	not significant	0/10 (0.0%)	0
								0.625
								1.25
							1/10 (10.0%)	2.5

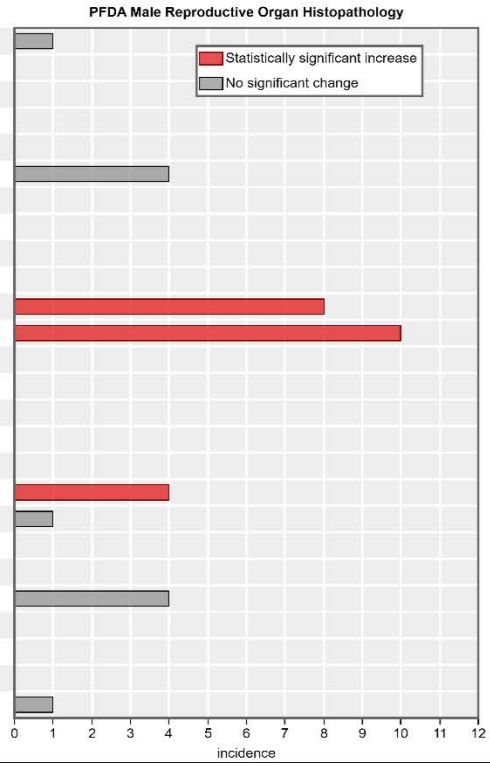


Figure 3-63. Effects on male reproductive organ histopathology following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Reproductive hormones

NTP (2018) evaluated serum testosterone in all dose groups at study termination (see Figure 3-64). A significant trend was reported with 25, 64, and 75% decreases in serum testosterone when compared to controls for the 0.625, 1.25, and 2.5 mg/kg-day dose groups, respectively. Testosterone is essential for the development and maturation of the male reproductive system, and it also plays a role in maintaining spermatogenesis and reproductive functions in adults (Toor and Sikka, 2017). The changes in serum testosterone levels at doses ≥ 0.625 mg/kg-day are concordant with the reductions in sperm counts and Leydig cell damage in adult male rats exposed to PFDA for 28 days (see synthesis on Sperm evaluations and Histopathology in this section).

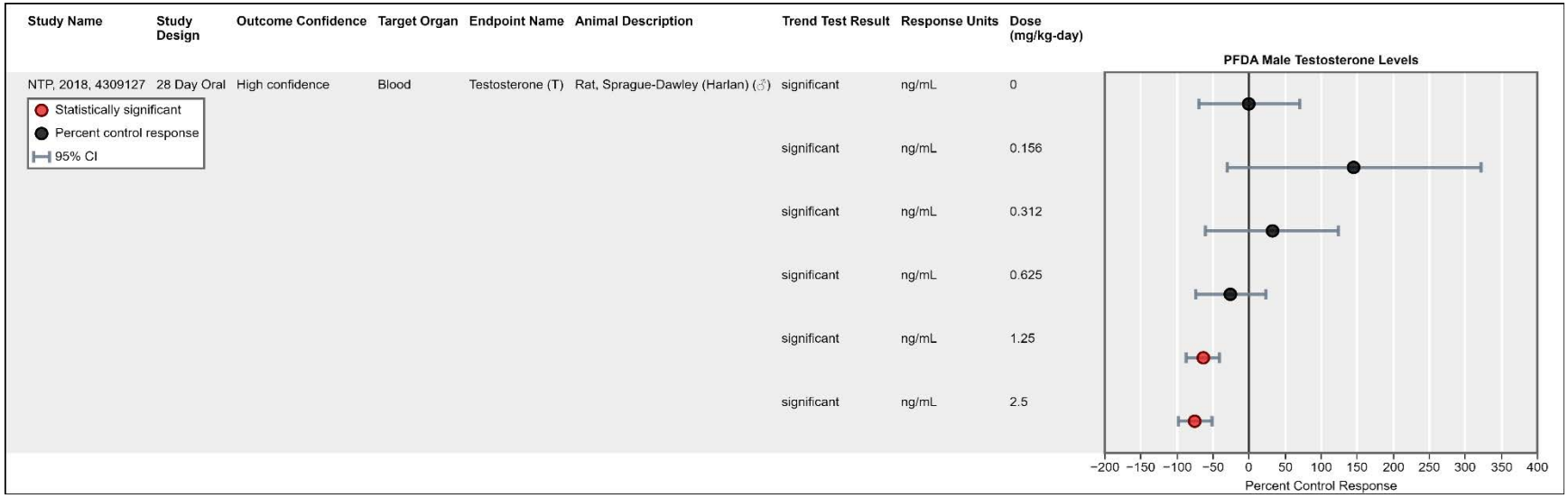


Figure 3-64. Effects on serum testosterone levels following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Organ weight

The right testis was measured at study termination in all dose groups, while epididymis weights (both whole and the cauda segments) were evaluated in the three highest dose groups (0.625, 12.5, and 2.5 mg/kg-day) (NTP, 2018) (see Figure 3-65). Absolute weights are the preferred measure for testis and epididymis as these organs appeared to be conserved even with body weight changes (Creasy and Chapin, 2018; U.S. EPA, 1996b). A decreasing trend ($p < 0.01$) in absolute testis weight was reported across the doses, reaching a −13% change compared to controls at 2.5 mg/kg-day. Absolute epididymis weights for whole and cauda segments also showed a decreasing trend ($p < 0.01$) and reported −10–11% and −23–25% change relative to controls for the 1.25 and 2.5 mg/kg-day dose groups, respectively. Decreases in epididymis weight, particularly in the cauda segment, may reflect reductions in sperm counts (Creasy and Chapin, 2018; Evans and Ganjam, 2011), which was observed to occur at similar doses (see synthesis on Sperm evaluations in this Section). Overall, the data shows consistent dose-related decreases in organ weights in the testis and epididymis at ≥ 0.625 mg/kg-day after short-term exposure to PFDA.

Study Name	Study Design	Outcome Confidence	Target Organ	Endpoint Name	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
NTP, 2018, 4309127	28 Day Oral	High confidence	Testes	Right Testis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.156
								0.312
								0.625
								1.25
								2.5
			Epididymis	Cauda Epididymis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.625
								1.25
								2.5
				Epididymis Weight, Absolute	Rat, Sprague-Dawley (Harlan) (♂)	significant	g	0
								0.625
								1.25
								2.5

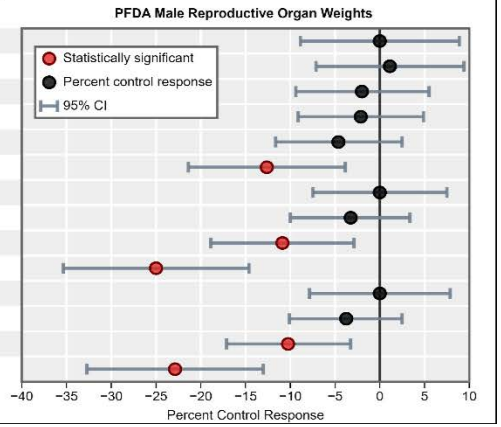


Figure 3-65. Effects on male reproductive organ weights following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link).

Mechanistic studies and supplemental information

Several studies have evaluated the potential mechanisms by which PFDA exposure may lead to male reproductive effects. Experimental studies have investigated PFDA-induced effects on Leydig cell steroidogenesis, androgen (AR) and estrogen (ER) receptor functions, aromatase activity and androgen metabolism and excretion and the potential impact of indirect systemic toxicity on the male reproductive effects of this chemical.

In vitro cell culture studies have evaluated PFDA-induced effects on Leydig cell functions and steroidogenesis. Leydig cells are the primary site of testosterone synthesis (Creasy and Chapin, 2018). Cholesterol uptake by the mitochondria in Leydig cells is a critical step in human chorionic gonadotropin (hCG)-induced testosterone production (Scott et al., 2009). In both immortalized mouse (MA-10) Leydig cells (LCs) and primary rat LCs, exposure to PFDA significantly decreased mitochondrial cholesterol uptake, and hCG-stimulated testosterone synthesis (Boujrad et al., 2000). The PFDA exposure levels affecting hormone synthesis in MA-10 cells did not lead to increased cytotoxicity measured as DNA damage, protein synthesis, and mitochondrial integrity (Boujrad et al., 2000). In contrast, PFDA showed a lack of activity in HTS assays from the EPA's ToxCast and Tox21 database evaluating steroid hormone biosynthesis, including glucocorticoids, androgens, estrogens, and progestogens in adrenal gland H295R cells (U.S. EPA (2019b); refer to Appendix E.2 for more details on the HTS results).

The in vitro observations of PFDA-induced effects on Leydig cell functions are consistent with both the 28-day gavage study in rats by NTP (2018) discussed above and high dose, i.p. injection studies that exposed rodents (predominantly rats) to single PFDA doses ranging from 20 to 400 mg/kg and evaluated effects on histopathology, androgen levels, and androgen-responsive reproductive organ weights after observational periods of 7 to 28 days (Bookstaff et al., 1990; Van Rafelghem et al., 1987b; Olson and Andersen, 1983). The i.p. injections studies report decreases in serum testosterone and 5-α-dihydrotestosterone levels (Bookstaff et al., 1990), altered testicular testosterone production (Bookstaff et al., 1990), and reduced androgen-responsive reproductive organ weights in rats (Bookstaff et al., 1990; Olson and Andersen, 1983). Furthermore, these studies report that PFDA exposure was associated with increased incidence of histopathological effects considered indicative of androgen disruption and spermatogenic disturbance (Creasy and Chapin, 2018; Creasy et al., 2012). Effects observed in rats include increased seminal vesicle and prostatic acini atrophy, and reduced seminal vesicle epithelial cell height, (Bookstaff et al., 1990), and while mice appeared to be resistant to seminiferous tubule degeneration, rats, hamsters, and guinea pigs were responsive to this PFDA-induced effect (Van Rafelghem et al., 1987b).

Another mechanism by which PFDA could alter male reproductive function is via increased hepatic metabolism and excretion of androgens or metabolic precursors such as cholesterol. Bookstaff et al. (1990) performed an experiment in which castrated Sprague-Dawley rats were supplemented with testosterone via sustained release capsules and then treated with vehicle or PFDA. They observed that acute PFDA exposure (20–80 mg/kg, i.p.) had no effect on serum

testosterone levels when the source of this hormone was the capsule rather than the testes. These findings suggest that PFDA does not impact hepatic androgen metabolism and excretion, and that decreases in serum testosterone levels observed after exposure are likely caused by a disruption in steroidogenesis in the testis. This argument is supported by the reductions in testosterone secretion in response to hCG stimulation in testicular tissue harvested from PFDA-exposed rats evaluated in the same study (Bookstaff et al., 1990) and inhibition of hGC-mediated steroidogenesis in cell culture rodent models using immortalized and primary Leydig cells described above (Boujrad et al., 2000).

Overall, the findings from available in vivo and cell culture studies provide support for an effect of PFDA exposure on Leydig cell functions ultimately resulting in reduced steroidogenesis.

Separately, PFDA-induced effects on AR and ER functions and aromatase activity have been evaluated in in vitro cell culture studies and HTS assays from the EPA's ToxCast and Tox21 platforms U.S. EPA (2019b); refer to Appendix E.2 for more details on the HTS results). AR and ER are known to regulate male reproductive functions (Wan et al., 2013; Wilson et al., 2008) and aromatase is a key enzyme in the conversion of androgens to estrogens, which is important for sexual development and differentiation (Sweeney et al., 2015; Hotchkiss et al., 2008; Jones et al., 2006). Disruption of AR transactivation has been demonstrated in Chinese hamster ovary cells (CHO-K1) (Kjeldsen and Bonefeld-Jørgensen, 2013) and androgen sensitive TARM-Luc cells (McComb et al., 2019) at PFDA concentrations that did not induce cytotoxicity. No significant effects on ER transactivation were observed in human breast adenocarcinoma MCF-7 cells with PFDA exposure alone (Li et al., 2020b; Kjeldsen and Bonefeld-Jørgensen, 2013) but in combination with 17β-estradiol, PFDA displayed antiestrogenic activity measured by inhibition of ER transactivation and downregulation of ER-responsive genes at non-cytotoxic concentrations (Li et al., 2020b). In HTS assays profiling AR and ER functions across multiple endpoints and in vitro test models, PFDA displayed low activity for these receptors at concentrations closely associated with cytotoxicity (Table E-3 in Appendix E.2). PFDA was active in 2 out of 17 AR assays (displaying binding activity in rat prostrate tissue and induction of cell proliferation in human prostate carcinoma 22Rv1 cells) and in 2 out of 21 assays profiling the ERα (1 out of 2 independent assays measuring transcriptional activity in HepG2 cells and an antagonist transactivation assays in human embryonic kidney HEK293T cells). Consistent with the HTS results, the ToxCast model predictions suggest that PFDA is inactive for both AR/ER agonist and antagonist activities (Table E-4 in Appendix E.2). Lastly, PFDA exposure decreased aromatase activity in the human choriocarcinoma JEG-3 cell line under conditions of cytotoxicity (Kjeldsen and Bonefeld-Jørgensen, 2013) but no activity in a HTS assay measuring aromatase inhibition in human breast cancer MCF-7 cells (Table E-5 in Appendix C). Taken together, findings from in vitro cell culture studies and HTS assays do not provide consistent and reliable evidence for potential effects of PFDA on AR or ER functions, or aromatase activity. However, for the most part, these in vitro cell models are not derived from the male reproductive

system and variability in the cellular/tissue environment may lead to differences in hormone receptor/enzyme functions (Leehy et al., 2016; Abdel-Hafiz and Horwitz, 2014).

In addition to the mechanisms described above, PFDA-induced wasting syndrome (see General toxicity effects; Section 3.2.10) may indirectly affect the male reproductive system. This is because severe decreases in body weight are known to alter reproductive functions (Creasy and Chapin, 2018; U.S. EPA, 1996b). Decreased body weight and food consumption were observed in acute, i.p. injection studies at doses ≥40 mg/kg and lethality were reported in some studies at doses ≥50 mg/kg (Bookstaff et al., 1990; Van Rafelghem et al., 1987b; Olson and Andersen, 1983). Bookstaff et al. (1990) addressed the impact of PFDA-induced changes in body weight on male reproductive endpoints by adding pair fed control rats that were weight-matched to each PFDA treatment groups. The authors observed that single exposure to 20, 40, or 80 mg/kg of PFDA via i.p. injection significantly decreased serum testosterone and DHT, testicular testosterone production, seminal vesicle and prostate weights, and seminal vesicle epithelial cell height. In pair fed control animals, there were no significant responses in the male reproductive system except in the group matched to the highest PFDA dose (80 mg/kg), which was associated with large reductions in food intake (44%) and body weight (72%) and observed responses were attenuated compared to PFDA exposure. These results indicate that PFDA-induced effects at the low and medium doses were direct reproductive system effects and not secondary to chemical-induced systemic effects. The body weight reductions in male rats observed in the 28-day gavage study at 1.25–2.5 mg/kg-day are consistent with moderate body weight changes (21–38%) that are not associated with confounding effects from overt systemic toxicity in supplemental studies tailored to examine that potential linkage.

Overall, the available evidence from in vivo and cell culture studies provides evidence of a biologically plausible mechanism for PFDA-induced adverse responses in the male reproductive system by disruption of steroidogenesis in Leydig cells, which in turn could impair reproductive functions and spermatogenesis. Specifically, it appears that PFDA exposure can disrupt androgen production in Leydig cells, which may lead to downstream histopathological effects, organ weight changes, and decreased spermatogenesis. Disruptions in androgen levels/production is a known pathway for chemical-induced alterations in spermatogenesis (Toor and Sikka, 2017; Sharpe, 2010). This support for biological plausibility is derived from studies in exposed animals and in vitro animal models; studies informing the relatability of these data to exposed humans are currently unavailable.

Evidence integration

The evidence of an association between PFDA exposure and male reproductive effects in humans is limited to two *medium* (Tian et al., 2019; Joensen et al., 2013) and one *low* confidence study (Zhou et al., 2016), with findings suggesting potential decreases in testosterone, decreased sperm motility, and anogenital distance (see Section 3.2.3 on Developmental Effects) with higher

PFDA exposure. There are concerns over inconsistency and imprecision, thus, the evidence is considered indeterminate.

The available evidence from a 28-day gavage study in rats and supportive data from i.p. injection and cell culture studies in rodents provide moderate evidence of male reproductive toxicity in animals with PFDA exposure. The 28-day rat study showed coherent effects across several relevant endpoints, including sperm evaluations, histopathology, hormone levels and organ weights (NTP, 2018), with most effects observed at doses below those shown to cause overt toxicity. Adverse histopathological changes were observed at doses associated with body weight decrements of potential concern. The study methods were considered *high* confidence for all endpoints other than sperm evaluations, which were considered potentially insensitive due to an inadequate exposure duration (i.e., biased towards the null; confidence is reduced specifically in the interpreted reliability of null findings [i.e., sperm motility]). A consistent pattern of decreased testicular and epididymal sperm counts occurred at ≥0.625 mg/kg-day, but only the effects in the epididymis were dose related. Dose-related decreases in serum testosterone levels and testicular and epididymal weights were also reported in rats at ≥0.625 mg/kg-day. The reductions in sperm counts, serum testosterone levels and organ weights are coherent with the mild degenerative changes found in testes and epididymis at similar doses, particularly Leydig cell atrophy, which is associated with androgen deficiency and decreased spermatogenesis (Creasy et al., 2012). Consistent effects on serum androgen levels, male reproductive organ weights, and histopathology were observed in rodents exposed to high doses of PFDA (≥20 mg/kg) in, single, i.p. injection studies. The adverse effects observed in the in vivo oral and i.p. exposure studies are biologically consistent with a potential mechanism for PFDA-induced reproductive effects in which alterations in Leydig cell functions result in decreased steroidogenesis and androgen levels (see synthesis on Mechanistic studies and supplemental information above for more details).

Limitations of the animal evidence base include the availability of only a single, short-term oral exposure study in a single species, and uncertainties regarding the potential impact of systemic toxicity, particularly with regard to the observed histopathological effects. Significant reductions in body weight were reported in the highest dose groups in the 28-day gavage study (21% at 1.25 mg/kg-day and 38% at 2.5 mg/kg-day; see Section 3.2.9 on General toxicity effects for more details) (NTP, 2018). However, concern for nonspecific effects on the male reproductive system is attenuated by the observed dose-related effects (i.e., sperm counts, testosterone levels and organ weights) at a lower PFDA dose, not associated with body weight changes (0.625 mg/kg-day). Likewise, an i.p. injection study that examined potential effects of PFDA-induced "wasting syndrome" using pair-fed control rats observed androgenic deficiency and male reproductive toxicity at 20 and 40 mg/kg that were independent from severe body weight depression at the highest dose (72% at 80 mg/kg) (Bookstaff et al., 1990). With respect to in vitro evidence, a general lack of in vitro models derived from the male reproductive system, and models restricted to rodents, limits the ability of the available evidence to inform potential pathways involved in PFDA-

induced male reproductive toxicity and to elucidate conserved mechanisms across species, including humans. Nonetheless, the mechanistic information from acute i.p. and in vitro animal studies is both consistent and coherent with the oral exposure study evidence, and therefore, provides support for the biological plausibility of the phenotypic responses. In the absence of information to the contrary and given the conserved role of androgen-dependent pathways in male reproductive functions across species (including humans), the available evidence is considered to be relevant to humans.

A potentially susceptible population for PFDA-induced male reproductive effects are young individuals exposed during critical developmental life stages (e.g. the masculinization programming, which occurs prior to the differentiation of androgen-sensitive tissues and determines penis size and anogenital distance (Dent et al., 2015), although no such studies were available in the current animal evidence base and few epidemiological studies examining pubertal development and anogenital distance were available. Androgens play a critical role in the normal development of the male reproductive system and disruptions caused by exposures to reproductive toxicants during gestation and early post-natal life stages can lead to agenesis of the male reproductive system and/or infertility (Foster and Gray, 2013; Sharpe, 2010; Scott et al., 2009).

Taken together, available ***evidence indicates*** that PFDA is likely to cause male reproductive effects in humans under sufficient exposure conditions (see Table 3-26). This conclusion is based primarily on a constellation of coherent evidence from a *high* confidence study in animals exposed to 0.625–2.5 mg/kg-day for 28 days, with some support for biological plausibility provided by mechanistic evidence from i.p. and cell culture models. Although no direct information on the human relevance of the animal evidence is available, many aspects of the male reproductive system are conserved across species, and the limited sensitivity in human studies may explain the lack of associations observed. Uncertainties in the database of PFDA-induced male reproductive toxicity includes the absence of subchronic, chronic, developmental, or multigenerational studies testing these outcomes in animals (which, overall, are anticipated to be more sensitive than the available short-term study design), and a general lack of adequate epidemiological or toxicological studies evaluating the potential for effects of early life PFDA exposure on male reproductive system development.

Table 3-26. Evidence profile table for PFDA exposure and male reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.4: Human studies)					⊕⊕⊙ ***Evidence indicates*** **(likely)**
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Semen evaluations 3 *medium* and 1 *low* confidence cross-sectional studies (1 is *uninformative* for motility)	• Decreased motility with increased exposure in Joensen et al. (2013). • No clear decrease in concentration or morphology in three medium confidence studies, but sensitivity is low.	• Large effect size for motility in medium confidence study	• Unexplained inconsistency in medium confidence studies for motility • Imprecision	⊕⊙⊙⊙ *Indeterminate* Coherent results in semen motility and testosterone across a *medium* and a *low confidence* study; inconsistency and imprecision add uncertainty.	*Primary basis:* Single, short-term study (*high* confidence) in rats, generally at ≥ 0.625 mg/kg-d PFDA *Human relevance:* Effects in rats are presumed relevant to humans based on the conserved role of androgen-dependent pathways in male reproductive functions across species. *Cross-stream coherence:* N/A, human evidence is indeterminate. *Susceptible populations and lifestages:*
Reproductive hormones For estradiol: 2 *medium* and 1 *low* confidence cross-sectional studies For testosterone: 1 *medium* and 3 *low* confidence studies	• Decreased testosterone in one of three studies of adults (one of two medium confidence) and one low confidence study of adolescents. No inverse association observed in two studies of infants.	• No factors noted	• Unexplained inconsistency in medium confidence studies • Imprecision		

Evidence stream summary and interpretation					Evidence integration summary judgment
Pubertal development 2 *medium* confidence cohort studies	• In one study, for several indicators of puberty, mean age of onset was later in middle vs. lowest tertile of exposure, but earlier in the highest tertile. The other study reported no association with timing of puberty.	• No factors noted	• Unexplained inconsistency		Based on the potential for exposure to cause impaired androgen function, males exposed during critical windows of androgen-dependent development may be susceptible. *Other inferences:* Mechanistic evidence from rodent i.p. studies and cell culture models suggest that male reproductive toxicity is a primary target for PFDA (likely through disruption of Leydig cells and steroidogenesis), even at doses associated with overt systemic toxicity (i.e., moderate body weight decreases).
Evidence from in vivo animal studies (see Section 3.2.4: Animal studies)					
Studies and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream judgment**	
Sperm evaluations 1 *low* confidence study (due to insensitivity) in rats exposed for 28 days	• Decreases in testicular and epididymal sperm counts at ≥0.625 mg/kg-d • No effects on sperm motility • Low confidence (due to the potential insensitivity of a short exposure duration) is mitigated by consistent effects	• *Consistent effects* for decreased sperm count across tissues • *Dose-response gradient* for epididymal sperm counts	• Lack of expected *dose-response* for testicular sperm counts	⊕⊕⊙ *Moderate* Coherent effects across sperm counts, serum testosterone levels and male reproductive histopathology and organ weights in a single, *high* confidence study; some concerns about insensitivity due to short-term exposure.	
Histopathology 1 *high* confidence study in rats exposed for 28 days	• Mild degenerative lesions in testes and epididymis at ≥1.25 mg/kg-d	• *Consistent* pattern of lesions across tissues • Leydig cell atrophy is *coherent* with decreased sperm	• Potential confounding by body weight decreases, although this concern is mitigated by findings from		

Evidence stream summary and interpretation					Evidence integration summary judgment
		counts and testosterone levels • *High* confidence study	supplemental mechanistic studies.		
Reproductive hormones 1 *high* confidence study in rats for 28 days	• Decreases in serum testosterone levels at ≥0.625 mg/kg-d	• Dose-response gradient • *High* confidence study	• No factors noted		
Organ weight 1 *high* confidence study in rats for 28 days	• Decreases in testis and epididymis weights at ≥0.625 mg/kg-d	• Consistent effects across tissues • *Coherence* with sperm counts histopathology and testosterone levels • Dose-response gradient • *High* confidence study	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways (or other information)	**Summary of key findings, interpretation, and limitations**			**Evidence stream judgment**	
Leydig cell androgen function	*Key findings and interpretation:* • Impaired Leydig cell mitochondrial cholesterol uptake and testosterone synthesis in two vitro rodent models. • Altered testosterone secretion in rat testes and altered androgen levels, reproductive organ weights and histopathology in rodent species after acute, i.p. injection consistent with evidence of reduced steroidogenesis. *Limitations*: few studies; in animal models only; acute, i.p. exposure at high doses associated with systemic toxicity			Evidence of altered Leydig cell function and decreased androgen production provide support for the biological plausibility of the male reproductive effects of PFDA.	

Evidence stream summary and interpretation			Evidence integration summary judgment
Reproductive hormone signaling	*Key findings and interpretation*: • Effects in a minority of in vitro studies/assays relating to the AR (receptor binding, transactivation and cell proliferation) and ER pathways (transactivation), and in one study on aromatase. • ToxCast model predictions suggests that PFDA is inactive for AR/ER agonist and antagonist activities. *Limitations*: Mixed results across studies; some effects at cytotoxic levels; models generally not in male reproductive tissues.		
Other mechanisms	*Key findings and interpretation*: • Generally, lack of support for potential role of hepatic androgen metabolism or indirect systemic toxicity in PFDA-induced male reproductive effects in rodent studies *Limitations*: acute i.p. exposure; high dose; few studies.		

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3.2.5. FEMALE REPRODUCTIVE EFFECTS

Human studies

Studies of possible female reproductive effects of PFDA are available for reproductive hormones, fecundity (i.e., time to pregnancy), menstrual cycle characteristics, and endometriosis. In addition, studies were available for spontaneous abortion and preterm birth which could be driven by either female reproductive or developmental toxicity. These outcomes are reviewed in Section 3.2.3 on Developmental effects in this assessment but are included in the consideration of coherence across outcomes for female reproductive effects. The study evaluations for these outcomes are summarized in Figure 3-66.

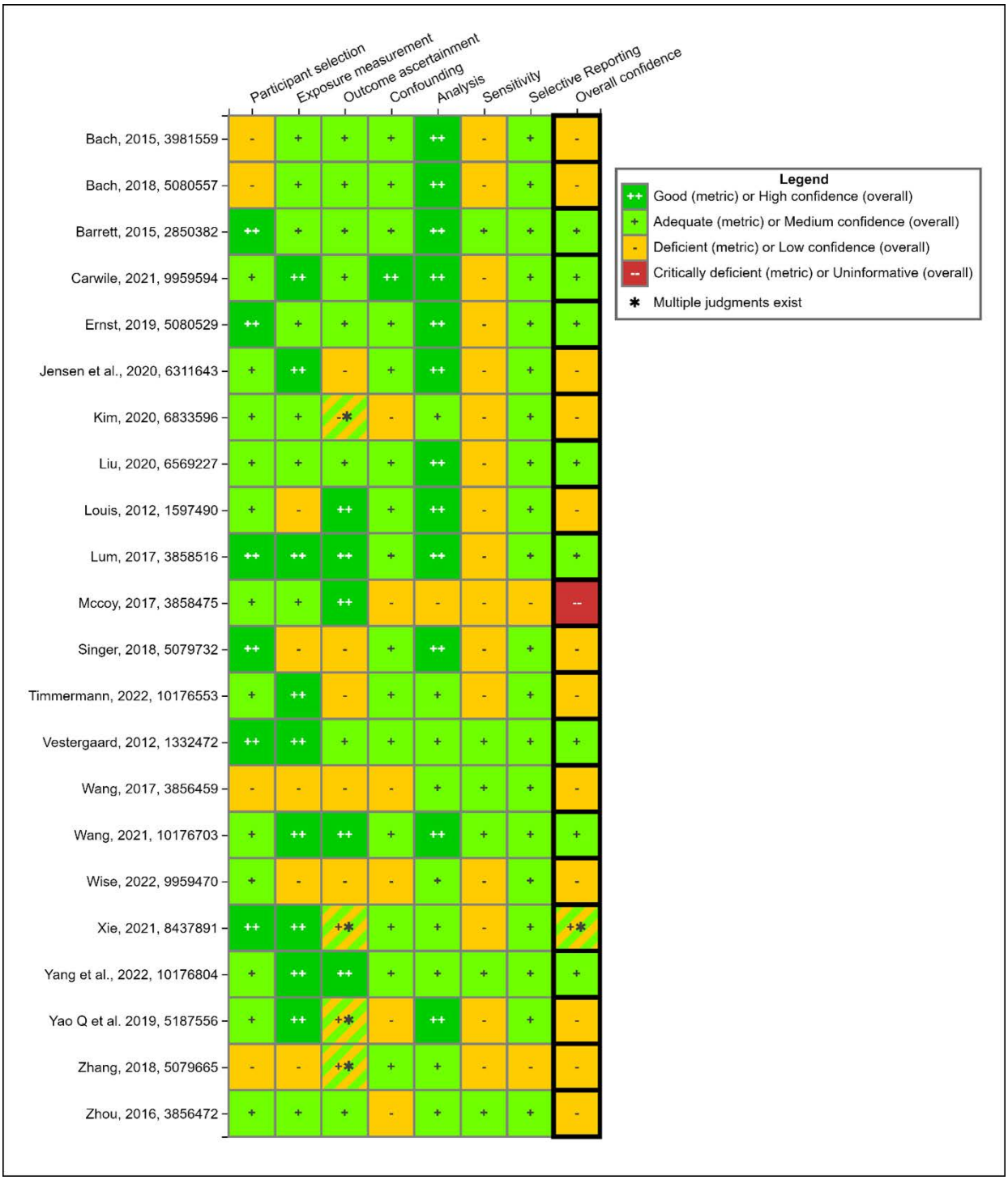


Figure 3-66. Study evaluations for epidemiology studies of PFDA and female reproductive effects. Refer to HAWC for details on the study evaluation review: HAWC Human Female Reproductive Effects.

Reproductive hormones

Reproductive hormones examined in the evaluated studies include testosterone, estradiol/estrogen, insulin like growth factor 1 (IGF-1), follicle stimulating hormone (FSH), luteinizing hormone (LH), progesterone, prolactin, and inhibin B, as well as sex hormone binding globulin (SHBG). Key issues for the evaluation of these studies were sample collection and processing. For testosterone, LH, FSH, and prolactin, due to diurnal variation, blood sample collection should be in the morning, and if not, time of collection must be accounted for in the analysis. If there is no consideration of time of collection for these hormones, the study is classified as deficient for outcome ascertainment and *low* confidence overall. The timing of PFDA exposure relevant for influencing reproductive hormones is unclear and dependent on several factors, and thus all exposure windows with available data were considered relevant for these endpoints of interest, particularly given the long half-life of PFDA. This includes cross-sectional studies since levels of these hormones are capable of being rapidly upregulated or downregulated and they are not expected to directly bind to or otherwise interact with circulating PFAS.

Ten studies (Timmermann et al., 2022; Yang et al., 2022b; Xie et al., 2021; Jensen et al., 2020b; Liu et al., 2020b; Yao et al., 2019; Zhang et al., 2018a; McCoy et al., 2017; Zhou et al., 2016; Barrett et al., 2015) reported on associations between PFDA exposure and female reproductive hormones. Four studies were *medium* confidence, including cross-sectional studies of healthy adults in Norway (Barrett et al., 2015) and the U.S. (Xie et al., 2021) (latter is low confidence for testosterone), a cross-sectional study of newborns in China (Liu et al., 2020b), and a pregnancy cohort in China (Yang et al., 2022b). Most of the remaining six studies were *low* confidence. In adults, this included an analysis of women with premature ovarian insufficiency in China (Zhang et al., 2018a) and a cohort of pregnant women in Denmark (Timmermann et al., 2022). In children and adolescents, there was a cohort of adolescents in Taiwan (Zhou et al., 2016) and two studies in infants, a cohort in Denmark (Jensen et al., 2020b) and a cross-sectional study in China (Yao et al., 2019). Lastly, McCoy et al. (2017) was considered *uninformative* due to multiple deficiencies in study evaluation.

For estrogen, one study, a cohort in pregnant women with follow-up across pregnancy (Yang et al., 2022b) examined estrone (E_1), estradiol (E_2), and estriol (E_3) and reported an inverse association between PFDA (median 0.8 ng/mL) and estrone (β [95% CI]: -0.12 (-0.24, -0.01)). Associations with estradiol and estriol were in the same direction but not statistically significant. The remaining studies examined only estradiol. In general population adults, an inverse, though non-monotonic, association (β [95% CI] vs Q1 for Q2: -78.64 [-310.37, 153.09]; Q3: -183.04 [-353.51,-12.56]; Q4: =117.92 [-285.64, 49.70]) was also reported in (Xie et al., 2021) (median 0.1 ng/mL). Associations varied by age group, with inverse associations in adolescents and 12–49 year olds, but a positive association in women 50 years of age and older. No association with PFDA was reported with follicular estradiol in Barrett et al. (2015) (mean PFDA 0.3 ng/mL), or with blood

estradiol in Zhang et al. (2018a) (median PFDA 0.4 ng/mL), Zhou et al. (2016) (median PFDA 1.0 ng/mL), or cord blood estradiol in Yao et al. (2019) (median PFDA 0.2 ng/mL).

For testosterone, since Barrett et al. (2015) did not examine associations with testosterone, all of the available evidence is *low* confidence. None of the four available studies reported a statistically significant association between PFDA and testosterone (Xie et al., 2021; Yao et al., 2019; Zhang et al., 2018a; Zhou et al., 2016), and the direction of association was not consistent across studies (positive association in Yao et al. (2019) and Xie et al. (2021), inverse association in the other two studies.

For other reproductive hormones, Barrett et al. (2015) also examined luteal phase progesterone, finding a positive association with PFDA (0.472 (−0.043, 0.987)). Liu et al. (2020b) examined progesterone in newborns and found no association with PFDA. Zhang et al. (2018a) examined FSH, LH, and prolactin and also found no association with PFDA. Jensen et al. (2020b) reported inverse associations between PFDA and DHEA ($p < 0.05$), DHEAS, Androstenedione, and 17-OHP ($p>0.05$). Lastly, Timmermann et al. (2022) found a positive, though imprecise association with prolactin during pregnancy (3.3% difference (95% CI -0.4, 7.2) per doubling of PFDA concentrations).

Overall, the findings in reproductive hormones are primarily null, with a few inconsistent associations observed. However, due to low exposure levels in most studies and the availability of a small number of studies per population type (adult women, adolescents, newborns) and reproductive hormones, the evidence is difficult to interpret.

Fecundity

There are six epidemiology studies that report on the association between PFDA exposure and fecundity. Fecundity is the biological capacity to reproduce. Time to pregnancy, defined as the number of calendar months or menstrual cycles from the time of cessation of contraception to detection of pregnancy, is a primary outcome measure used to study fecundity. There are challenges in studying this outcome as it is ideal to enroll women at the point when contraception is discontinued, but this is generally limited to women trying to get pregnant who may not be representative of the general population. An alternative approach is to enroll pregnant women and ask for their recall of time to pregnancy, but this is subject to selection bias due to excluding women who are unable to conceive, and thus are potentially most affected. Two studies were preconception cohorts and considered medium confidence (Lum et al., 2017; Vestergaard et al., 2012), and two were pregnancy cohorts and considered low confidence (Bach et al., 2018; Bach et al., 2015) due to the potential for selection bias described above. Another fecundity-specific consideration is the potential for confounding in parous women due to factors related to previous pregnancies (Bach et al., 2018). In addition to the studies of time to pregnancy, two studies examined women undergoing infertility treatment; one medium confidence cohort examined successful pregnancies using IVF (Wang et al., 2021) and one low confidence cross-sectional study compared PFAS concentrations in women with different types of infertility (with male factor

infertility as the control group) and associations with fertilization rate (Kim et al., 2020c). A summary of the study evaluations is presented in Figure 3-46, and additional details can be obtained from HAWC.

The results for the association between PFDA exposure and time to pregnancy are presented in Table 3-27. A fecundability ratio less than 1 indicates a decrease in fecundity/increase in time to pregnancy. One study (Bach et al., 2018) reported longer time to pregnancy with higher exposure in the fourth quartile, but only in parous women, which despite adjustment for interpregnancy interval, may be more likely to be confounded. None of the other available studies reported a decrease in fecundity/increase in time to pregnancy with higher exposure, though this observed lack of association could be due to poor study sensitivity resulting from low exposure levels. In addition to the time to pregnancy results, two studies (Bach et al., 2015; Vestergaard et al., 2012) also analyzed infertility as an outcome and found no increase with higher exposure. Similarly, Wang et al. (2021) reported no increase in negative hcG test or clinical pregnancy failure following IVF with higher PFDA exposure (associations indicated less pregnancy failure and test negativity with higher exposure). Kim et al. (2020c) found no association between different infertility factors (endometriosis, PCOS, genital tract infections, or idiopathic) compared to male factor infertility. However, Kim et al. (2020c) did report an inverse, though imprecise, association between PFDA exposure and fertilization rate (β=-60.83, 95% CI: -129.25, 7.59).

Table 3-27. Associations between PFDA and time to pregnancy in epidemiology studies

Reference, study confidence	Population	Median exposure (IQR) or as specified	Comparison for effect estimate	Fecundability ratio (FR) (95% CI)
Vestergaard et al. (2012), *medium*	Preconception cohort (1992–1995), Denmark; 222 nulliparous women	0.1 (0.1, 0.1) [a]	log-unit increase	1.15 (0.89, 1.49)
			Above median vs. below	1.40 (0.96, 2.03)
Bach et al. (2018), *low*	Danish National Birth Cohort sub-sample (1996–2002), Denmark, 638 nulliparous women and 613 parous women	0.2 (0.1–0.2)	Quartiles vs. Q1	Nulliparous Q2: 1.13 (0.89, 1.43) Q3: 1.02 (0.82, 1.28) Q4: 1.11 (0.89, 1.39) Parous[b] Q2: 0.92 (0.68, 1.26) Q3: 0.95 (0.71, 1.28) Q4: 0.86 (0.65, 1.15)
Bach et al. (2015), *low*	Aarhus pregnancy cohort (2008–13), Denmark; 1,372 nulliparous women	0.3 (0.2–0.4)	0.1 ng/mL increase	1.00 (0.97, 1.03)
			Quartiles vs. Q1	Q2: 1.08 (0.91, 1.28) Q3: 0.98 (0.83, 1.16) Q4: 1.08 (0.91, 1.28)

Reference, study confidence	Population	Median exposure (IQR) or as specified	Comparison for effect estimate	Fecundability ratio (FR) (95% CI)
Lum et al. (2017), *medium*	LIFE preconception cohort (2005–09), U.S.; 401 women	0.4 (0.2–0.6)	Tertiles vs. T1	T2: 0.7 (0.5, 1.1) T3: 0.9 (0.6, 1.3)

*$p < 0.05$.

[a]Participants with pregnancy.

[b]These results were based on a model that corrected PFAS exposure based on an interpregnancy interval of median length. An alternate model where interpregnancy interval was included as a covariate was statistically significant in Q4. A model with no adjustment for interpregnancy interval was not significant but had a monotonic decrease across quartiles (FRs of 0.92, 0.87, 0.78).

Pubertal development

Pubertal development is primarily assessed using established criteria, such as Tanner stage ratings. In girls, Tanner staging involves evaluation of the development of breasts and pubic hair. Stage 1 represents prepubertal development; Stage 2, the onset of pubertal development, and Stage 5 represents full sexual maturity. Age at menarche and age at peak height velocity (i.e., the age at which a child experiences the largest increase in height) can also be used as measures of pubertal development. Three studies, including two *medium* confidence cohorts in Denmark (Ernst et al., 2019) and the United States (Carwile et al., 2021) and one *low* confidence cross-sectional study (Wise et al., 2022), examined timing of pubertal development with PFDA exposure.

Carwile et al. (2021) used exposure measured during mid-childhood (median 8 years) with follow-up to early adolescence (median 13 years). Using a pubertal development score based on parental responses to scales of multiple pubertal markers (breast development, body hair growth, acne, growth spurt, and menarche), they reported less pubertal development in early adolescence with higher exposure (β (95%) per doubling of exposure: -0.11 (-0.18, -0.03)). This was consistent with their findings for older age at peak height velocity (0.23 (0.11, 0.35)) and older age at menarche (HR (95% CI) per doubling of exposure: 0.91 (0.77, 1.06)). Ernst et al. (2019) used maternal exposure measured in blood and prospectively identified pubertal onset with follow-up checks every six months. In girls, age at Tanner stages 2 and 3 for breast development were lower with higher exposure, consistent with Carwile et al. (2021), though not statistically significant. No association was observed for Tanner stages 4 and 5. No clear patterns for associations were observed with pubic hair development, axillary hair, or age at menarche. Results for the second and third tertiles were discordant for some outcomes (lower age at menarche and axillary hair development in second tertile, higher in third). Looking at a combined puberty indicator outcome, there was lower age at puberty (not significant) in the second tertile and no difference in the third tertile compared to the first. Wise et al. (2022) did not report a clear association with age at menarche (age was higher in both the first and third tertiles compared to the second), but this study was *low* confidence due to concerns for lack of temporality between exposure and outcome misclassification due to recall of age at menarche among adult women. Sensitivity was a concern for

all three studies, as exposure contrast was narrow. Exposure levels and contrast were *slightly* higher in Carwile et al. (2021) than the other studies (IQR 0.4 ng/mL vs 10th-90th percentile difference of 0.2 ng/mL in Ernst et al. (2019), so it is possible that this is a basis for the clearer associations in the former study.

Menstrual cycle characteristics

Four epidemiology studies report on the association between PFDA exposure and menstrual cycle characteristics. Two were cohorts, one a preconception cohort already described for fecundity (Lum et al., 2017) and one a pregnancy cohort (Singer et al., 2018). Two studies were cross-sectional, one of participants in a preconception cohort (Zhou et al., 2017a) and one of general population black women of reproductive age (Wise et al., 2022). For any outcome related to menstruation, there is potential for reverse causation because menstruation is one of the mechanisms by which PFAS are removed from the body (Wong et al., 2014; Zhang et al., 2013b). This potential bias could be away from the null with irregular and longer cycles. Thus, all four studies were considered low confidence. There were no associations reported between menstrual cycle length or irregularity and PFDA exposure, but due to limited sensitivity related to exposure contrasts and low confidence in the studies, these findings are difficult to interpret.

Endometriosis

Two epidemiology studies report on the association between PFDA exposure and endometriosis. Both studies were cross-sectional, which decreases confidence for this chronic outcome due to the inability to establish temporality and the likely lack of measurement in the relevant etiologic window. There is potential for reverse causality as described above since endometriosis can influence the menstrual cycle and it is possible that this would act in a protective direction since endometriosis can be associated with heavier and more frequent bleeding which could increase elimination of PFDA from the body. Parity and related factors such as time since last child have also been suggested as a source of reverse causality for this association as a longer inter-pregnancy interval could allow more accumulation of PFAS levels (Wang et al., 2017), but this was not a major concern in this set of studies as one study adjusted for parity and the other performed a sensitivity analysis with only women without a history of pregnancy. Still, because of the concern related to menstrual cycle irregularity association with endometriosis, all the studies were classified as *low* confidence, though one is considered higher quality within that classification; this study included two groups of women, one group scheduled for surgery (laparoscopy or laparotomy), and one group identified through a population database who underwent pelvic MRI to identify endometriosis (Louis et al., 2012). The remaining study was additionally deficient for outcome ascertainment, specifically a case definition including only endometriosis-related infertility among surgically confirmed cases (Wang et al., 2017), which is likely to include less severe or asymptomatic cases among the controls. The low confidence study with good outcome ascertainment (Louis et al., 2012) reported higher odds of endometriosis with higher exposure in

the operative sample (OR = 2.95, 95% CI: 0.72, 12.1), but lower odds in the population sample (OR = 0.06, 95% CI: 0.00, 12.3), though both estimates were imprecise. The low confidence study by Wang et al. (2017) reported lower odds of endometriosis-related infertility with higher exposure (OR vs. T1: T2: 0.93 (95% CI: 0.51, 1.70), T3: 0.74 (95% CI: 0.40, 1.35). It is difficult to reconcile the differing results considering the low number of studies, all of which were low confidence, and the potential for reverse causality for this outcome.

Premature Ovarian Insufficiency

One *low* confidence study, a case-control study in China, examined the association between PFDA exposure and premature ovarian insufficiency (POI) (Zhang et al., 2018b). In this study, POI was defined as an elevated FSH level greater than 25 IU/L on two occasions more than four weeks apart and oligo/amenorrhea for at least four months. Because this definition is closely tied to menstruation, there are concerns for reverse causality as with the previous two outcomes, which would be expected to be biased away from the null as there is reduced bleeding/elimination of PFDA from the body. The study reported higher odds of POI (not statistically significant) with higher PFDA exposure (OR (95% CI) for tertile 2 vs. 1: 1.03 (0.54,1.96), tertile 3 vs. 1: 1.36 (0.71,2.60), but given the lack of additional evidence and concerns for reverse causality, there is considerable uncertainty in these results.

Animal studies

A single study in the database of toxicity studies for PFDA evaluated female reproductive effects (NTP, 2018). The study examined the following endpoints after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) in adult female rats: organ weights, histopathology, hormone levels, and estrous cycles. The NTP (2018) study was evaluated as *high* confidence for all endpoints examined (see Figure 3-67). Although there is only a 28-day study available, the duration of the study is sufficient for assessing female reproductive toxicity given that significant effects on estrous cyclicity were observed as early as Day 21 of the 28-day study and the mean estrous cyclicity length is reported to be 4.4 days amongst multiple sub-strains of Sprague Dawley rats (Marty et al., 2009).

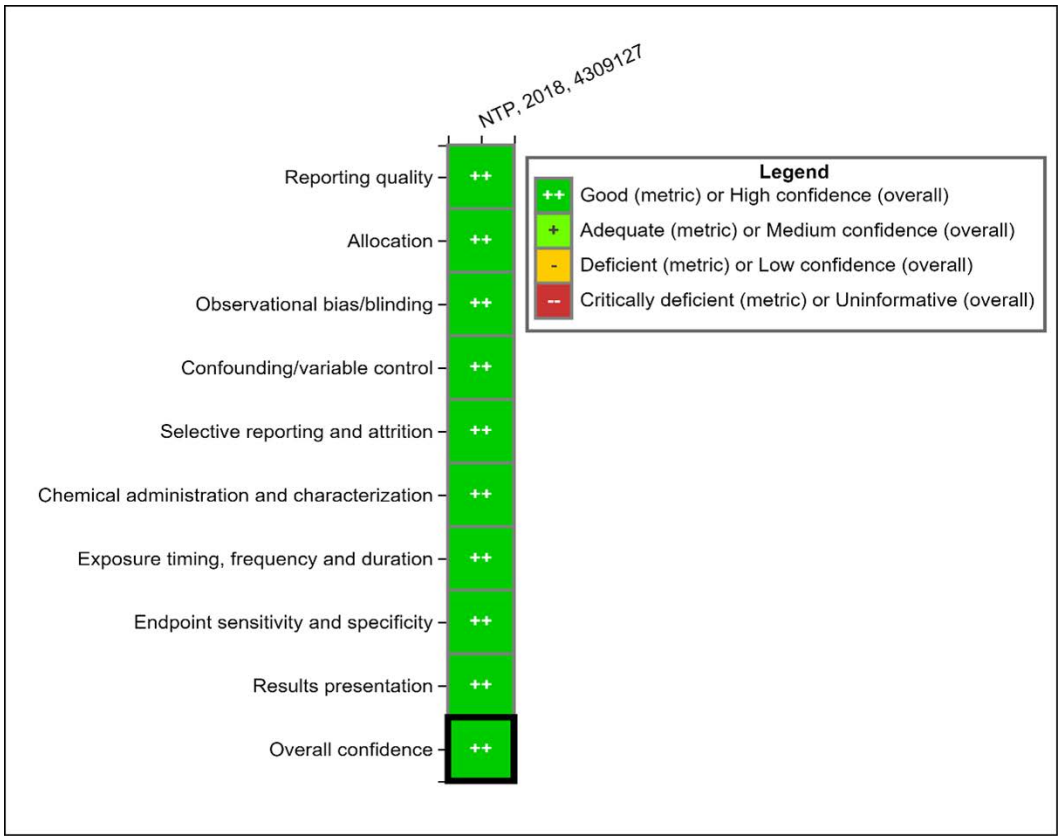


Figure 3-67. Female reproductive animal study evaluation heatmap. Refer to HAWC for details on the study evaluation review.

Estrous cycle

Female rats from the three highest dose groups (0.625, 1.25, and 2.5 mg/kg-day) were evaluated for changes in the estrous cycle due to PFDA exposure, as compared to controls. To examine this endpoint, vaginal smears were performed for sixteen consecutive days before animals were necropsied. Changes in the percent of time spent in each estrous stage (proestrus, estrus, metestrus, diestrus) were affected by exposure (see Figure 3-68 and Table 3-28). Specifically, for proestrus, the percentage of time spent was increased by 103 and 123% at 0.625 and 1.25 mg/kg-day, respectively but then decreased by 81% at 2.5 mg/kg-day. For metestrus, the percentage of time spent was increased by 23% at 0.625 mg/kg-day but then decreased by 100% at ≥1.25 mg/kg-day. A significant trend test was observed for the percentage of time spent in estrus with statistically significant decreases (42–84%) at ≥1.25 mg/kg-day (Figure 3-68 and Table 3-28). Correspondingly, a significant trend test was observed for the percentage of time spent in diestrus with statistically significant increases (27–63%) at ≥1.25 mg/kg-day (see Figure 3-68 and Table 3-28). Estrous cyclicity was disrupted and all female rats remained in a continuous state of diestrus at 2.5 mg/kg-day starting on Day 21 (Day 9 of the sixteen days in which vaginal cytology was assessed). The sustained state of diestrus suggests that these animals may have been infertile (U.S. EPA, 1996a), although this was not specifically evaluated. Although decreased body weight in

female rats was observed at the same doses (body weight decreases were 12–36% at ≥1.25 mg/kg-day; refer to Section 3.2.10 on General toxicity effects for more details) as effects on estrous cyclicity, it is unclear if these effects are related and the effect on female reproductive function is disproportionately more severe and concerning than the moderate changes in body weight. Although body weight has been shown to fluctuate during the different estrous stages and weight loss has been shown to correlate with disrupted estrous cyclicity in rats (Tropp and Markus, 2001), it is not possible to determine if the decreases in body weight in female rats might be responsible for the effects on estrous cyclicity observed in the NTP (2018) study. Furthermore, even though no changes were observed on other stages of the estrous cycle (i.e., proestrus and metestrus), the effects of PFDA on estrus and diestrus are still considered biologically relevant given the potential influence that the lack of cyclicity may have on fertility, regardless of whether the observed decrease in body weight may have partially contributed to these changes. Changes in cycle length and the number of cycles during the study were not affected in the 0.625 and 1.25 mg/kg-day groups. Data for cycle length and number of cycles could not be determined for the 2.5 mg/kg-day group because estrous cyclicity was disrupted in all female rats at this dose and all animals remained in a state of continuous diestrus starting at Day 21 until sacrifice.

Table 3-28. Percent changes relative to controls in time spent in each estrous stage (proestrus, estrus, metestrus, diestrus) in female S-D rats exposed to PFDA exposure for 28 days (NTP, 2018)

Endpoint	Dose (mg/kg-d)		
	0.625	1.25	2.5
% of Estrous cycle in diestrus	10	**27**	**63**
% of Estrous cycle in estrus	−22	**−42**	**−84**
% of Estrous cycle in metestrus	23	−100	−100
% of Estrous cycle in proestrus	103	123	−81

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

Hormone levels

Testosterone was measured in all dose groups at study termination; it is unclear from the study description if the study authors controlled for fasting or time of necropsy. A significant trend test was observed with statistically significant increases reported at ≥0.312 mg/kg-day (see Figure 3-68). Increases were monotonic and varied from 30% to 348% change from controls; levels of circulating testosterone were increased more than two-fold at 1.25 mg/kg-day. Other sex hormones (e.g., estradiol) were not measured in this study. The biological relevance of increased testosterone to the development of PFDA-induced female reproductive toxicity is unclear. Specifically, the association of increased testosterone and altered estrous cycling (e.g., prolonged diestrus) requires further investigation.

Histopathology

Histological examination of the clitoral gland, ovaries, uterus, and mammary glands were performed at study termination. Histopathology was examined for the ovaries at all doses; all other reproductive tissues were examined only in the control and high-dose groups. Histological changes due to PFDA treatment were not reported for any tissue examined including the uterus (see Figure 3-68) even though PFDA-effects on estrous cyclicity and uterine weight were reported.

Organ weights

Uterine weights were measured in all dose groups at study termination. A significant trend test was observed for both absolute and relative weights with the two highest dose groups reaching statistically significant decreases for both measures (see Figure 3-68). Decreases reached −64% and −44% change from controls for absolute and relative weights, respectively. Other organs related to the female reproductive system were not measured. It should be noted that comparisons of uterine weights were not made in rats that were in the same estrous stage. As noted below, many studies in rats have shown that uterus weight decreases during diestrus. Therefore, it is unclear if the reductions in uterus weight are a direct effect of PFDA or rather a secondary effect due to prolonged diestrus owing to PFDA exposure.

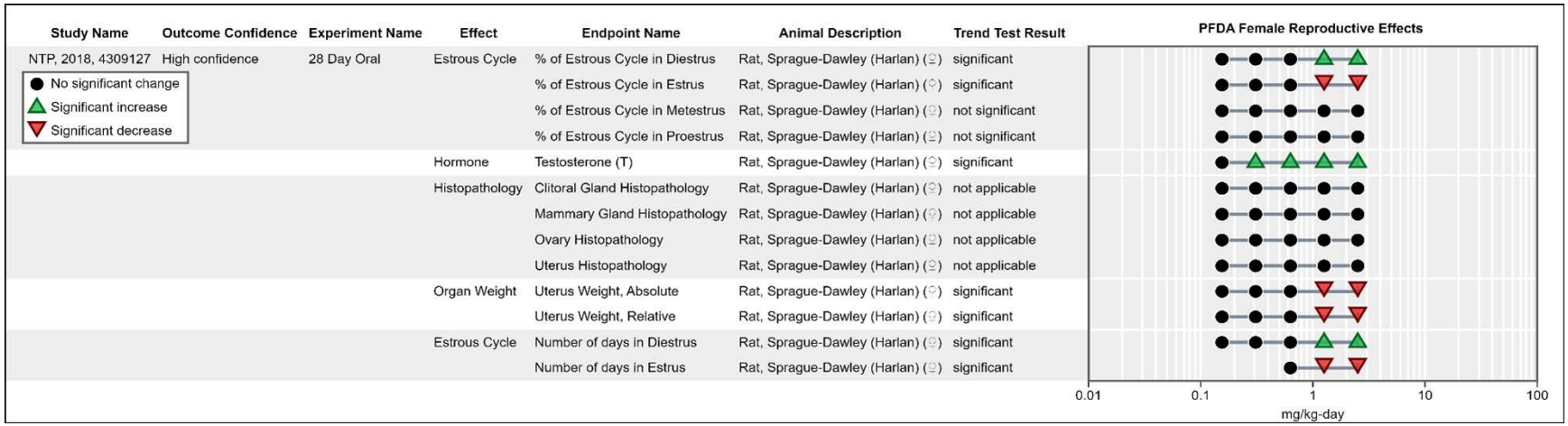


Figure 3-68. PFDA female reproductive effects (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/pfda-female-reproductive-animal/).

Mechanistic studies and supplemental information

As discussed in the male reproductive section (see Section 3.2.4), PFDA-induced effects on AR and ER functions and aromatase activity have been evaluated in in vitro cell culture studies and high throughput screening (HTS) assays from ToxCast and Tox21. Findings from in vitro cell culture studies and HTS assays do not provide consistent evidence for potential effects of PFDA on AR or ER functions, or aromatase activity. Additional in vivo and/or cell culture studies are necessary to address inconsistencies in the available in vitro data and determine whether these pathways might be disrupted by PFDA exposure. In an in vitro study, PFDA inhibited progesterone production in mouse Leydig tumor cells, which the study authors postulated was due to oxidative stress (Zhao et al., 2017). It is not possible to corroborate this effect with data from the lone reproductive study in rats (NTP, 2018) given that progesterone was not measured in the (NTP, 2018) study. In the NTP (2018) study, Wyeth-14,643 (a PPARα agonist) was shown to cause effects on estrous cyclicity similar to those reported for PFDA. However, mechanistic studies that investigate the role of PPARα in PFDA-altered estrous cyclicity are not available.

Evidence Integration

There is *indeterminate* evidence of an association between PFDA exposure and female reproductive effects in human studies, though the *low* confidence studies that were available had concerns for study sensitivity which reduces the ability to interpret the observed null findings. A significant inverse association between PFDA and anogenital distance in girls was observed in one study (see Developmental Effects), which is relevant to female reproductive toxicity. The biological relevance of this effect on anogenital distance is unclear given that an increase in this measure is considered adverse in girls rather than a decrease per the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment. Furthermore, the available reproductive hormone evidence for PFDA does not support an association. Previous studies have shown an association between increased testosterone and increased anogenital distance in women (Mira-Escolano et al., 2014), however the human evidence is inadequate for examining PFDA-induced effects on testosterone in women. Whereas increased testosterone was observed in female rats in the NTP (2018) study, the study authors did not measure anogenital distance given that there was no developmental exposure in the study. The increased testosterone observed in female rats is considered relevant to humans and given the known association between increased testosterone and anogenital distance in women, an increase in anogenital distance rather than a decrease would be expected in women exposed to PFDA. Overall, there is little biological understanding of how hormonal perturbation or other biological processes might result in a decrease in anogenital distance owing to PFDA exposure.

In addition to the outcomes described in this Section, there is potential for two of the outcomes described in the developmental section (refer to Section 3.2.3 for more details), preterm birth and spontaneous abortion, to be related to female reproductive toxicity. The evidence for

these outcomes was inconsistent. Given that most of the evidence for female reproductive effects was null or inconsistent, there is little clear indication of an association. However, the exposure levels in most of the study populations were low, which resulted in low sensitivity to detect an effect, and thus these findings should not be interpreted as supporting a lack of effect.

The available data from a 28-day gavage study in rats provide *moderate* evidence that PFDA exposure may cause female reproductive toxicity (see Table 3-29). The evidence is sparse. The data are from a single animal study that did not evaluate fertility, pregnancy outcomes, multiple hormone levels (only testosterone was measured), or markers of reproductive development. PFDA was observed to cause effects on the following female reproductive parameters: organ weight (i.e., decreased uterine weights at ≥1.25 mg/kg-day), hormone levels (i.e., increased testosterone levels at ≥0.312 mg/kg-day), and estrous cycle (i.e., percentage of time spent in estrus and diestrus at ≥1.25 mg/kg-day). One factor increasing the strength of the evidence is the severity of the effect on estrous cyclicity; specifically, that PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested (2.5 mg/kg-day), which could be indicative of reductions or delays in fertility. However, some caution in the interpretation of the higher dose effects is warranted given the significant decreases in body weight, particularly at 2.5 mg/kg-day (36% decrease). Support for the adversity and concerning nature of prolonged diestrus and its association with infertility is provided by the following text in the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment:

- "Persistent diestrus indicates temporary or permanent cessation of follicular development and ovulation, and thus at least temporary infertility,"
- "Pseudopregnancy is another altered endocrine state reflected by persistent diestrus."
- "Significant evidence that the estrous cycle (or menstrual cycle in primates) has been disrupted should be considered an adverse effect."
- "The greatest confidence for identification of a reproductive hazard should be placed on significant adverse effects on sexual behavior, fertility or development, or other endpoints that are directly related to reproductive function such as menstrual (estrous) cycle normality, sperm evaluations, reproductive histopathology, reproductive organ weights, and reproductive endocrinology."

Furthermore, prolonged diestrus is commonly reported in rodent models of impaired fertility (Li et al., 2017; Caldwell et al., 2014; Miller and Takahashi, 2014; Mayer and Boehm, 2011) and continuous diestrus is observed during reproductive senescence in aged female rats (Lefevre and Mcclintock, 1988). There was also coherence between decreased uterus weight and increased percentage of time spent in diestrus at ≥1.25 mg/kg-day. Previous studies have shown that decreased uterus weight in rats is commonly observed during diestrus (Westwood, 2008; Vasilenko et al., 1981; Walaas, 1952; Boettiger, 1946). In addition to prolonged diestrus, PFDA decreased the

percentage of time spent in estrus (NTP, 2018), which could indirectly cause infertility given that rodents are sexually receptive only during estrus (Goldman et al., 2007). The severe, PFDA-induced decreased time spent in estrus is expected to result in decreased opportunities for mating in the rats, and therefore reductions or delays in fertility. Unfortunately, no multi-generational studies of PFDA were available to inform this hypothesis.

In this study, PFDA did not cause histopathological changes in female reproductive tissues. Given the short-term duration of the lone animal study, it cannot be reasonably ruled out that detectable histopathological effects could have become apparent with a longer observation window. The short-term duration of the lone animal study does not reduce confidence in the database for PFDA-induced female reproductive effects given that biologically relevant effects (e.g., prolonged diestrus) were still observed.

Taken together, the available ***evidence indicates*** that PFDA is likely to cause female reproductive toxicity in humans under sufficient exposure conditions[11] (see Table 3-29). This conclusion is based primarily on evidence from a *high* confidence study in rats exposed to doses ranging from 1.25–2.5 mg/kg-day PFDA for 28 days. The PFDA-induced disruption of estrous cyclicity observed in female rats from the NTP study (NTP, 2018) and its implications for infertility can be considered relevant to humans given that the mechanisms responsible for regulating female reproductivity (e.g., estrous cyclicity in rats and menstrual cycling in humans) are similar between rats and humans (Goldman et al., 2007; Bretveld et al., 2006). Given the sparse evidence base (i.e., one short-term animal study and largely *low* confidence or null human studies) and the lack of understanding for how PFDA exposure causes the observed reproductive effects or whether they might progress with longer exposures, further studies that could inform this conclusion include those that examine the effect of PFDA on female fertility and pregnancy outcomes in exposed animals from subchronic, chronic, developmental, or multigenerational studies, as well as in vivo or cell culture mechanistic studies.

[11] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

Table 3-29. Evidence profile table for PFDA exposure and female reproductive effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.5: Human studies)					⊕⊕⊙ ***Evidence indicates*** **(likely)**
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
Reproductive hormones 4 *medium* and 5 *low* confidence studies	• Inverse association between PFDA exposure and estrogen observed in 2 studies. Most studies reported no association with female reproductive hormones, but sensitivity was limited in most studies	• No factors noted	• No factors noted	⊙⊙⊙ *Indeterminate* Within and across outcomes, findings were mixed, null, and/or of *low* confidence. Interpretation of the lack of an association for most outcomes in these studies is complicated by poor sensitivity for observing effects due to *low* exposure levels.	*Primary basis*: Evidence from a high confidence study in rats showing biologically coherent effects on uterus weight and the estrous cycle after oral exposure to PFDA at ≥1.25 mg/kg-d for 28 days. *Human relevance*: Evidence in animals is presumed relevant to humans given that mechanisms regulating female reproduction are similar between rats and humans. *Cross-stream coherence*: N/A, human evidence is indeterminate.
Fecundity 3 *medium* and 3 *low* confidence studies	• One study reported longer time to pregnancy with higher PFDA exposure, but only in parous women. No association observed in other studies, but sensitivity was limited.	• No factors noted	• Unexplained inconsistency, although a lack of association in some studies may be attributable to limited sensitivity		
Pubertal development 2 *medium* and 1 *low* confidence cohort studies	• One study reported later age at pubertal onset based on pubertal development score, age at peak height velocity, and age at menarche. Two other studies reported no clear association	• Coherence of related effects in one study	• Unexplained inconsistency		
Menstrual cycle 4 *low* confidence studies	• No association observed between PFDA exposure and menstrual cycle characteristics, but sensitivity was limited.	• No factors noted	• Potential for reverse causality		

Evidence stream summary and interpretation					Evidence integration summary judgment
Endometriosis 2 *low* confidence studies	• Higher odds of endometriosis with higher PFDA exposure in women scheduled for laparoscopy or laparotomy in one study, but lower odds of endometriosis in a population-based sample in the same study and a low confidence study.	• No factors noted	• Unexplained inconsistency across *low* confidence studies • Potential for reverse causality		*Susceptible populations and lifestages*: Based on altered estrous cyclicity data in animals, females of reproductive age may be at higher risk. *Other inferences*: No specific factors are noted.
Evidence from in vivo animal studies (see Section 3.2.5: Animal studies)					
Studies, outcomes, and confidence	**Summary and key findings**	**Factors that increase certainty**	**Factors that decrease certainty**	**Evidence stream summary**	
Estrous cycle 1 *high* confidence study	• The percentage of time spent in estrus was significantly decreased at ≥1.25 mg/kg-d. • The percentage of time spent in diestrus was significantly increased at ≥1.25 mg/kg-d. • Estrous cyclicity was disrupted at 2.5 mg/kg-d and all female rats in this dose group remained in a continuous state of diestrus by Day 21.	• Large magnitude of effect and concerning severity • In a *high* confidence study • Dose-response gradient for effects on the percentage of time spent in estrus and diestrus. • Coherence with reduced uterus weight.	• Lack of expected coherence for histopathology, although possibly explained by short exposure duration • Potential confounding by body weight decreases.	⊕⊕⊙ *Moderate* Based on multiple, coherent changes in female reproductive endpoints, most notably that PFDA induced a continuous phase of diestrus, which could be indicative of infertility, in 100% of rats at 2.5 mg/kg-d.	

Evidence stream summary and interpretation					Evidence integration summary judgment
Organ weight 1 *high* confidence study	• Decreased absolute and relative uterine weights at ≥1.25 mg/kg-d.	• Dose-response gradient in a high confidence study	• Potential confounding by body weight decreases (mitigated some by comparable effects on absolute and relative weights)		
Hormone levels 1 *high* confidence study	• Increased testosterone levels at ≥0.312 mg/kg-d.	• Dose-response gradient in a high confidence study	• Unclear biological relevance of increases		
Histopathology 1 *high* confidence study	• No PFDA-induced histopathological changes were observed for the clitoral gland, ovaries, uterus, and mammary glands.	• No factors noted	• No factors noted		
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways	**Primary evidence evaluated Key findings, interpretation, and limitations**	**Evidence stream judgment**			
Hormone levels	*Interpretation:* PFDA inhibits progesterone production. *Key findings:* • PFDA reduced progesterone production in mouse Leydig tumor cells. The study authors suggested that oxidative stress may be a possible mechanism. *Limitations:* Single study available, lack of evidence examining effects on other sex hormones.	• Evidence of decreased progesterone production provides limited support for the biological plausibility of the female reproductive effects of			

Evidence stream summary and interpretation					Evidence integration summary judgment
		PFDA. It is not possible to corroborate this effect with data from the lone reproductive study in rats (NTP, 2018) progesterone was not measured in the(NTP, 2018) study.			

1

3.2.6. CARDIOMETABOLIC EFFECTS

Methodological considerations

Cardiometabolic risk refers to the likelihood of developing diabetes, heart disease, or stroke. Contributors to this risk include a combination of metabolic dysfunctions mainly characterized by insulin resistance, dyslipidemia, hypertension, and adiposity.

Human studies

There are 22 epidemiology studies that report on the relationship between PFDA exposure and cardiometabolic effects, including serum lipids (12 studies), blood pressure (5 studies), atherosclerosis (2 studies), cardiovascular disease (2 studies), ventricular geometry (1 study), diabetes and insulin resistance (11 studies), adiposity and weight gain (6 studies), and metabolic syndrome (2 studies).

Serum lipids

Cholesterol as found in, low-density lipoprotein (LDL) is one of the major controllable risk factors for cardiovascular disease including coronary heart disease, myocardial infarction, and stroke. Cholesterol levels are typically measured in the blood. Twenty-three studies (28 publications) report on the association between PFDA exposure and serum lipids (e.g. total cholesterol, lipoprotein complexes, and triglycerides). There were multiple outcome-specific considerations for study evaluation that were influential on the ratings. First, for outcome ascertainment, collection of blood during a fasting state is preferred for all blood lipid measurements (NIH, 2020; Nigam, 2011) but lack of fasting was considered deficient for triglycerides and LDL-cholesterol (which is typically calculated using levels of triglycerides, as well as total cholesterol and HDL, using the Friedewald equation). This is because triglyceride levels remain elevated for several hours after a meal (Nigam, 2011). Self-reported high cholesterol was also considered deficient due to the high likelihood of misclassifying cases as controls (Natarajan et al., 2002). Both of these issues are likely to result in nondifferential outcome misclassification and to generally bias results towards the null. It was also considered important to account for factors that meaningfully influence serum lipids, most notably use of cholesterol lowering medications and pregnancy. Studies that did not consider these factors by exclusion, stratification, or adjustment were considered deficient for the participant selection domain. All the available studies analyzed PFDA in serum or plasma and serum lipids using standard, appropriate methods. As described in Section 3.2.8 on Endocrine effects, reverse causation was considered but is unlikely to significantly bias the results because PFAS, including PFDA, do not preferentially bind to serum lipids, so exposure measurement was adequate for this outcome across all studies.

A summary of the study evaluations is presented in Figure 3-69, and additional details can be obtained from HAWC. Three studies were excluded from further analysis due to critical

deficiencies in at least one domain. Most studies (14) were classified as medium confidence, though five of these were classified as low confidence for triglycerides and LDL cholesterol due to lack of fasting as described above (Blomberg et al., 2021; Jensen et al., 2020a; Yang et al., 2020; Zeng et al., 2015; Starling et al., 2014b). Six studies were classified as low confidence (Varshavsky et al., 2021; Khalil et al., 2020; Lin et al., 2020b; Koshy et al., 2017; Christensen et al., 2016; Fu et al., 2014) for all lipid endpoints. For the majority of studies, sensitivity to detect an effect was a concern due to limited exposure contrast, and thus null associations are interpreted with caution. Potential for confounding across PFAS was considered within individual study evaluations and synthesized across studies.

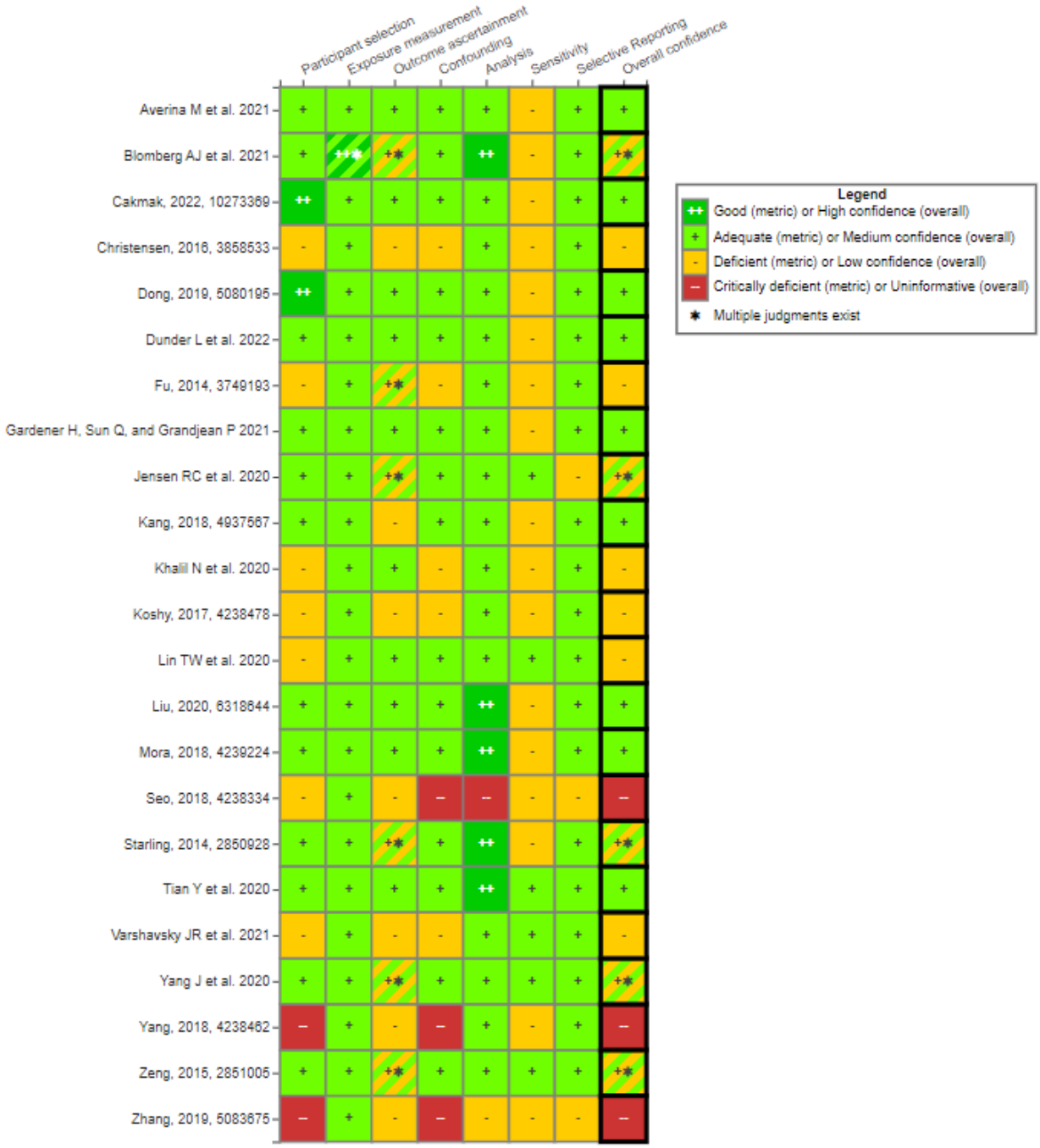


Figure 3-69. Study evaluation results for epidemiology studies of PFDA and serum lipids. Refer to HAWC for details on the study evaluation review: HAWC Human Serum Lipids.

Multiple publications of the same study: Dong et al. (2019) (on figure) includes Christensen et al. (2019) and Jain and Ducatman (2019a). Liu et al. (2020a) (on figure) includes Liu et al. (2020a)

The results for the association between PFDA exposure and blood lipids among the *medium* confidence studies are presented in Table 3-30. Of the 14 *medium* confidence studies, 4 were in general population adults, 3 were in pregnant women, and 7 were in adolescents and children. In adults, the majority of studies reported higher total cholesterol with higher exposure, including four in general population adults (Cakmak et al., 2022; Dunder et al., 2022; Liu et al., 2020a; Dong et al., 2019), two in pregnant women (Gardener et al., 2021; Starling et al., 2014a). This included statistical significance in three studies (Cakmak et al., 2022; Dunder et al., 2022; Gardener et al., 2021), and an exposure-response gradient in both studies that examined categorical exposure (Gardener et al., 2021; Liu et al., 2020a). Results in children were less consistent. Four studies reported statistically significant positive associations in at least one analysis (Averina et al., 2021; Blomberg et al., 2021; Jensen et al., 2020a; Mora et al., 2018), but other studies reported inverse (Tian et al., 2020; Kang et al., 2018; Zeng et al., 2015) or null associations. In addition, to the continuous serum lipids measurements, one study (Averina et al., 2021) examined dyslipidemia as a dichotomous outcome (defined as total cholesterol ≥5.17 mmol/L). They reported increased odds of lipidemia with higher exposure (OR [95% CI] vs quartile 1: Q2: 2.34 [1.08, 5.05], Q3: 2.19 [1.01, 4.74]; Q4: 2.36 [1.08, 5.16]). Results for triglycerides were not available for all studies, but a positive association was observed in two studies in adults (Cakmak et al., 2022; Dunder et al., 2022) and one study in pregnant women (Gardener et al., 2021), while the other one study in adults and two studies in pregnant women showed no association. An inverse association was observed in Mora et al. (2018) in children; the direction of this association was not coherent with the reported positive associations for total and LDL cholesterol in the same cohort, which increases uncertainty. Other studies in children indicated no association with triglycerides.

Looking at the *low* confidence studies in adults (Varshavsky et al., 2021; Khalil et al., 2020; Lin et al., 2020b; Christensen et al., 2016; Fu et al., 2014) and adolescents (Koshy et al., 2017), four reported increases in total cholesterol (Lin et al., 2020b; Koshy et al., 2017; Fu et al., 2014) or unspecified high cholesterol (Christensen et al., 2016) with increased exposure, with one being statistically significant (Koshy et al., 2017). Two studies (Varshavsky et al., 2021; Khalil et al., 2020) reported inverse results. The results of all the *low* confidence studies were interpreted with caution due to serious limitations.

Overall, evidence for the association between PFDA exposure and serum lipids is inconsistent, and this inconsistency cannot be easily explained by study confidence level or the participant-demographics. This may be partly explained by narrow exposure contrasts which may have reduced sensitivity and impaired the ability of some studies to observe an effect. However, the strongest associations were observed in studies (Dong et al., 2019; Mora et al., 2018; Starling et al., 2014a) with low PFDA exposure levels (median <0.5 ng/mL). This could be an indication that sensitivity in this body of evidence is adequate, or could be due to residual confounding, such as by other PFAS or the demographics of the study population. There is some support for the PFAS scenario, as PFDA was highly correlated with PFNA (0.7) and moderately correlated with PFOS and

PFOA (0.4) in both Starling et al. (2014a) and Dong et al. (2019), and positive associations were stronger for PFOA in Starling et al. (2014a) and for PFNA, PFOS, and PFOA in Dong et al. (2019). Conversely, in Mora et al. (2018), PFDA was highly correlated with PFOA (0.7) and moderately correlated with PFOS (0.6) and PFNA (0.5), but the observed positive associations were strongest in PFDA, and thus are unlikely to be completely explained by confounding. Given available data, there is not enough evidence to state conclusively whether confounding contributed to these results.

Table 3-30. Associations between PFDA and blood lipids in medium confidence epidemiology studies

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
General population, adults						
Dong et al. (2019)	Cross-sectional study, U.S. (NHANES 2003–2014); 8,950 adults (20–80 yrs)	0.2	β (95% CI) for 1-unit increase	6.6 (−8.5, 21.7)	10.7 (−8.5, 29.9)	NR
Cakmak et al. (2022)	Cross-sectional study, Canada (CHMS 2007–2017); 6,045 participants	0.2 (GM)	% change for increase equivalent to GM	**2.8 (0.2, 5.3)***	**10.7 (5.5, 16.1)***	**7.0 (1.0, 13.2)***
Dunder et al. (2022)	Cohort (2001–2004), Sweden; 864 older adults (70–80 yrs)	0.3 (0.2–0.4)	β (95% CI) for change in exposure and outcome over 10 yrs	**0.23 (0.14, 0.32)***	**0.12 (0.03, 0.20)***	**0.08 (0.04, 0.12)***
Liu et al. (2020a)	Cross-sectional analysis from randomized clinical trial of weight loss; 326 overweight adults	0.4 (0.2–0.5)	Means ± SE for tertiles	T1: 183.1 ± 7.9 T2: 186.6 ± 7.5 T3: 192.1 ± 7.6 *p* = 0.2	NR	T1:138.9 ± 11.3 T2: 119.7 ± 10.7 T3: 129.3 ± 10.8 *p* = 0.3

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
Pregnant women						
Starling et al. (2014a)	Cross-sectional analysis from birth cohort (2003–2004), Norway; 891 women	0.09 (<LOQ–0.2)[a]	β (95% CI) for ln unit increase	1.8 (−2.1, 5.8)	0.2 (−3.3, 3.7) ^	−0.03 (−0.07, 0.01) ^
Gardener et al. (2021)	Pregnancy cohort (2009), U.S., 433 women	0.2 (0.1-0.3)	Means ± CI for quartiles	Positive association with exposure-response gradient*	NR	Positive association with exposure-response gradient*
Yang et al. (2020)	Pregnancy cohort (2013-2014), China, 436 women	1.0 (0.6–1.7)	β (95% CI) for ln unit increase	-0.03 (-0.09, 0.04)	**-0.05 (-0.10, -0.01)***	0.06 (-0.02, 0.14)
Adolescents and children						
Kang et al. (2018)	Cross-sectional study (2012–2014), Korea, 150 children (3–18 yrs)	0.06 (0.04, 0.1)	β (95% CI) for ln unit increase	−3.3 (−7.8, 0.8)	−1.9 (−5.7, 2.0)	−0.04 (−0.1, 0.03)
Blomberg et al. (2021) (additional results with different timing of exposure and outcome measurement are available in the publication)	Birth cohort (2007–2009), Faroe Islands, 459 children (followed to 9 yrs)	0.09 (0.07, 0.1)	β (95% CI) for doubling PFAS and lipids at birth	Overall -0.03 (-0.11, 0.05) Girls 0.05 (-0.07, 0.16) Boys -0.1 (-0.21, 0.00)	Overall -0.02 (-0.07, 0.03) Girls -0.02 (-0.05, 0.09) Boys -0.06 (-0.12, 0.01)	Overall 2.2 (-4.1, 8.8) Girls 7.3 (-2.3, 18) Boys -1.9 (-9.9, 6.8)
			PFAS at birth and lipids at 18 mo	Overall -0.1 (-0.26, 0.06) Girls -0.02 (-0.26, 0.22) Boys -0.17 (-0.39, 0.05)	Overall -0.09 (-0.22, 0.03) Girls -0.03 (-0.22, 0.15) Boys -0.15 (-0.32, 0.02)	Overall 0.33 (-7.9, 9.3) Girls -2.8 (-15, 11) Boys 2.8 (-8.2, 15)
			PFAS and lipids at 9 yrs	**Overall** **0.19 (0.07, 0.32)*** **Girls** **0.2 (0.01, 0.39)*** **Boys** **0.19 (0.02, 0.36)***	**Overall** **0.12 (0.02, 0.22)*** **Girls** **0.19 (0.04, 0.33)*** Boys 0.07 (-0.06, 0.2)	Overall -0.16 (-7.6, 7.9) Girls 2.9 (-8.4, 16) Boys -2.5 (-12, 8.2)
Averina et al. (2021)	Cross-sectional study (2010-2011), Norway; 940 children ~16 yrs)	Girls 0.3 Boys 0.2 (GMs)	β (95% CI) for log increase	**0.35 (0.12, 0.57)***	**0.34 (0.14, 0.54)***	0.01 (-0.15, 0.17)
Jensen et al. (2020a)	Birth cohort (2010–2012), Denmark; 612 children	0.3 (5^{th}-95^{th}: 0.2-0.5)	β (95% CI) for 1 unit increase	3 mo -0.23 (-0.90, 0.43) 18 mo **1.06 (0.08, 2.03)***	3 mo -0.05 (-0.73, 0.62) 18 mo 0.64 (-0.43, 1.71)	3 mo -0.21 (-0.88, 0.47) 18 mo 0.92 (-0.11, 1.95)

Reference	Population	Median exposure in ng/mL (IQR)	Effect estimate	Total cholesterol	LDL	Triglycerides
	(followed to 18 mo)					
Mora et al. (2018)	Birth cohort (1999–2002), U.S.; 682 children (7–8 yrs)	0.3 (0.2–0.5)	β (95% CI) for IQR increase	**6.8 (3.6, 10.1) *** similar for boys and girls	**3.2 (0.6, 5.8) *** similar for boys and girls	−3.6 (−8.2, 1.0) similar for boys and girls
Zeng et al. (2015)	Cross-sectional analysis (2009–2010), Taiwan; 225 adolescents (12–15 yrs)	1.0 (range <LOQ[b]–5.0) (boys)	β (95% CI) for 1-unit increase	−1.3 (−9.0, 6.4)	−0.6 (−6.5, 5.4) ^	0.6 (−10.0, 11.1) ^
Tian et al. (2020)	Birth cohort (2012), China; 306 newborns	2.2 (1.4-3.3) in cord blood	β (95% CI) for ln-unit increase	**-0.12 (-0.19, -0.05)***	-0.09 (-0.18, 0.01)	**-0.09 (-0.18, -0.01)***

***p* < 0.05.**
^*Low* confidence endpoint within *medium* confidence study.
[a]30% below the LOQ.
[b]Less than 6% below the LOQ.
U: uninformative; NR: not reported.
Not all results (e.g., sub-group analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation. Only *medium* confidence studies underwent data extraction.

Other risk factors for cardiovascular disease

Ten studies report on the association between PFDA exposure and other risk factors for cardiovascular disease, including blood pressure in the general population (six studies), hypertensive disorders and blood pressure during pregnancy (four studies), atherosclerosis (two studies), and ventricular geometry (one study). The study evaluations for these outcomes are summarized in Figure 3-70.

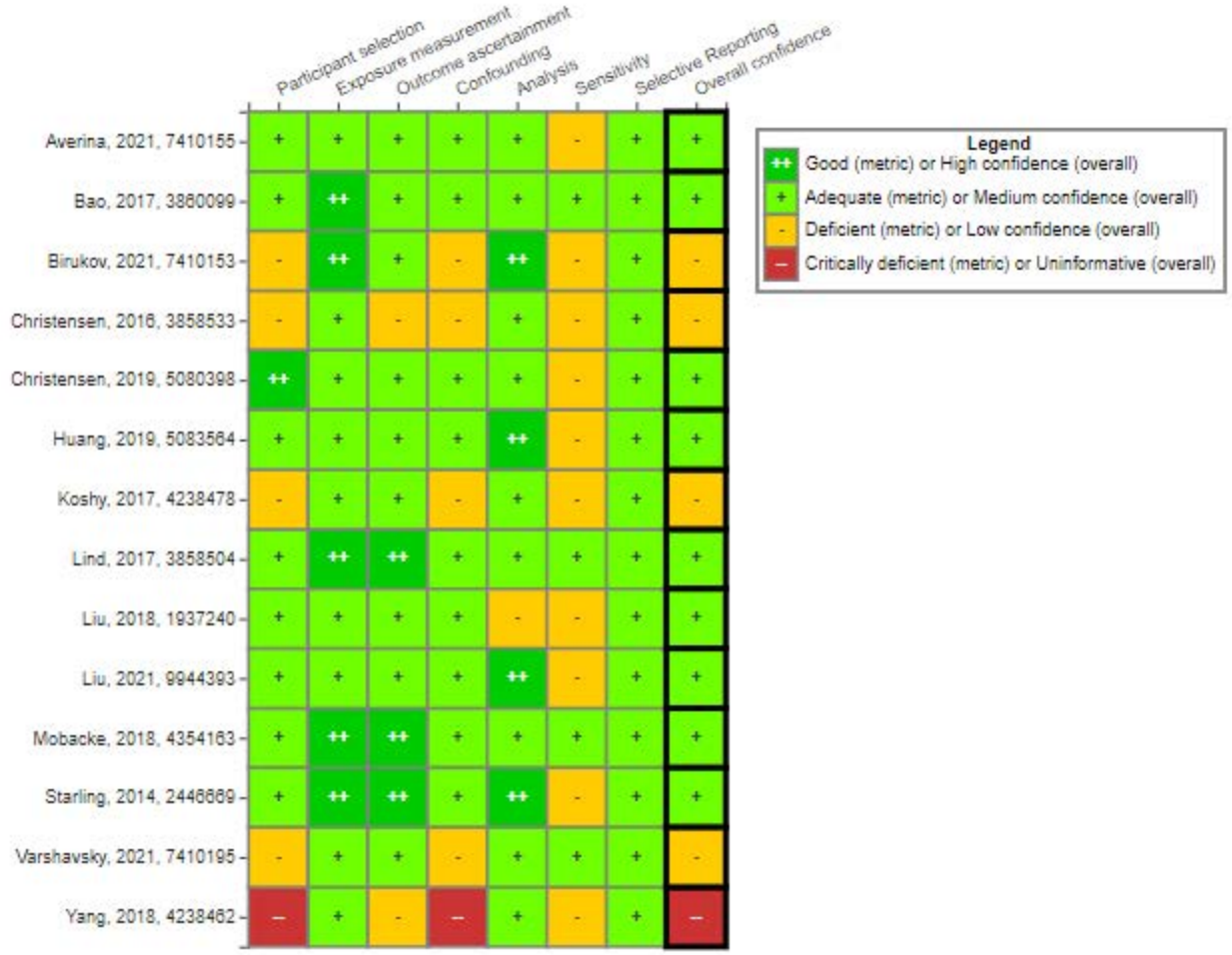


Figure 3-70. Study evaluation results for epidemiology studies of PFDA and cardiovascular risk factors other than serum lipids. Refer to HAWC for details on the study evaluation review: HAWC Human Other Cardiovascular Risk Factors.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2020b) and Jain (2020a).

For blood pressure, one study of blood pressure (Yang et al., 2018) was excluded from further analysis due to critical deficiencies in participant selection in confounding. One medium confidence cross-sectional study (NHANES) reported higher blood pressure with higher PFDA exposure in two publications (Jain, 2020a; Christensen et al., 2019), but the association was nonsignificant and not monotonic across quartiles (OR [95% CI] for Q2 vs. Q1: 1.1 (0.7,1.6), Q3: 1.3 (0.7,2.2), Q4: 1.1 (0.6,1.9) in (Christensen et al., 2019)) and the other four studies, including three medium confidence studies, reported no increase in adults (Liu et al., 2018; Bao et al., 2017; Christensen et al., 2016) or adolescents (Averina et al., 2021).

Out of four studies of hypertensive disorders of pregnancy (see Table 3-31), one *medium* and one *low* confidence study reported positive associations with gestational hypertension Birukov et al. (2021); (Liu et al., 2021a), though neither was statistically significant and Birukov et al. (2021)

did not report a positive association with preeclampsia. The other two *medium* confidence studies reported no increase in the odds of preeclampsia (Huang et al., 2019b; Starling et al., 2014a) or gestational hypertension (Huang et al., 2019b). Associations were in the inverse direction in both studies, but neither was statistically significant. In addition, one low confidence study (Varshavsky et al., 2021) reported positive associations with continuous blood pressure (both systolic and diastolic) during mid-gestation.

Table 3-31. Associations between PFDA and hypertensive disorders of pregnancy in epidemiology studies

Reference, study confidence	Population	Median exposure in ng/mL (IQR)	Effect estimate	Gestational hypertension	Preeclampsia
Starling et al. (2014a), medium	Nested case-control study within cohort in Norway; 1,046 women	0.1	HR (95 CI) for above vs. below median	NR	0.81 (0.63, 1.05)
Huang et al. (2019b), medium	Cross-sectional study in China; 674 women at delivery	0.4 (0.2-0.5)	OR (95% CI) for tertiles vs T1	T2: 1.26 (0.48, 3.31) T3: 0.63 (0.20, 2.00)	T2: 1.16 (0.38, 3.53) T3: 1.00 (0.31, 3.19)
Liu et al. (2021a), medium	Nested case-control study within cohort in China; 544 women	0.4 (0.3-0.7)	OR (95% CI) for tertiles vs T1	T2: 1.24 (0.74, 2.06) T3: 1.48 (0.89, 2.45)	NR
Birukov et al. (2021), low	Cohort in Denmark; 1,436 women	0.6 (0.5-0.9)	HR (95% CI) for doubling of exposure	1.35 (0.86, 2.11)	0.93 (0.71, 1.22)

For atherosclerosis, there was a non-significant increase in the echogenicity of the intima-media complex (a measure of the structural composition of the arterial wall that is an indicator of early change in the carotid artery) and in the number of carotid arteries with atherosclerotic plaques only in women in one *medium* confidence study (Lind et al., 2017b), but no association with atherosclerosis in the *low* confidence study, which did not stratify by sex (Koshy et al., 2017). In the single *medium* confidence study of ventricular geometry (Mobacke et al., 2018), there was a small but statistically significant decrease in relative wall thickness (RWT) ($\beta = -0.02$, 95% CI: −0.04, −0.01) and increase in left ventricular end-diastolic volume ($\beta = 0.95$, 95% CI: 0.11, 1.79). There is some inconsistency in the literature about the adversity of decreased RWT, with some studies indicating increased RWT is associated with hypertension (Li et al., 2001) and concentric left ventricular geometry (de Simone et al., 2005), and others indicating decreased RWT is associated with abnormal left ventricular geometry (Hashem et al., 2015) and ventricular tachyarrhythmia (Biton et al., 2016). In either case, it is difficult to interpret these results without additional studies.

Overall, there is limited evidence of an association between PFDA exposure and cardiovascular risk factors. One low confidence study reported a positive association with blood pressure, and *medium* confidence studies reported associations with atherosclerosis and

ventricular geometry, but no association was observed in medium confidence studies of blood pressure.

Cardiovascular disease

Three studies examined cardiovascular disease and its association with PFDA exposure in adults. All reported on coronary heart disease (Huang et al., 2018; Christensen et al., 2016; Mattsson et al., 2015), while one additionally examined total cardiovascular disease, congestive heart failure, angina pectoris, myocardial infarction (heart attack), and stroke (Huang et al., 2018). Two studies were *medium* confidence (see Figure 3-71), including a case-control study nested within a prospective cohort of farmers and other rural residents in Sweden (Mattsson et al., 2015), while the other (Huang et al., 2018) was based on NHANES, a nationally representative cross-sectional survey in the U.S. The third study was *low* confidence and based on a survey of male anglers in Wisconsin (Christensen et al., 2016). The timing of exposure measurement in all three studies was considered adequate, though the prospective measurement in Mattsson et al. (2015) may be more likely to capture the relevant etiologic period of these chronic outcomes. Exposure levels in the *medium* confidence studies were similar (median = 0.2 ng/mL), and slightly higher in the *low* confidence study (median = 0.5 ng/mL).

For coronary heart disease, Huang et al. (2018) reported significantly higher odds with higher exposure (see Table 3-33). Christensen et al. (2016) also reported higher odds, though not statistically significant, while Mattsson et al. (2015) reported no increase. For other outcomes, Huang et al. (2018) reported higher odds of total cardiovascular disease, angina pectoris, and myocardial infarction, and stroke, though these were not statistically significant and only myocardial infarction and angina pectoris had monotonic gradients across the quartiles (angina pectoris Q2 vs. Q1: 1.16 (0.67,1.99), Q3: 1.21 (0.75,1.95), Q4: 1.23 (0.68,2.24); myocardial infarction Q2: 0.99 (0.65,1.49), Q3: 1.32 (0.90,1.92), Q4: 1.38 (0.83,2.28)). There is not a clear explanation for the differing results in the *medium* confidence studies; both had similar exposure levels (median 0.2 ng/mL). The populations in Mattsson et al. (2015) and Christensen et al. (2016) are fairly homogeneous (farmers/rural residents in Sweden and male anglers in Wisconsin, respectively), in contrast to the nationally representative sample in Huang et al. (2018). It is possible that the prospective exposure measurement in Mattsson et al. (2015) played a role (vs. cross-sectional measurement in Huang et al. (2018) and Christensen et al. (2016)), and the lack of additional prospective studies makes this difficult to interpret. Given that the timing of exposure measurement in Mattsson et al. (2015) is more likely to be during the relevant etiologic window, the lack of association in that study contributes to considerable uncertainty in this body of evidence.

Figure 3-71. Study evaluation results for epidemiology studies of PFDA and cardiovascular disease. Refer to HAWC for details on the study evaluation review: HAWC Human Cardiovascular Disease.

Table 3-32. Associations between PFDA and coronary heart disease in epidemiology studies

Reference, study confidence	Population	Median exposure in ng/mL (IQR)	Coronary heart disease OR (95% CI)
Mattsson et al. (2015), *medium*	Nested case-control study of farmers and rural residents in Sweden, exposure measured 1990–1991 and 2002–2003, cases identified through 2009, N = 462	0.2 (0.1)	Q2: 0.87 (0.49, 1.60) Q3: 1.13 (0.66, 1.94) Q4: 0.92 (0.53, 1.60)
Huang et al. (2018), *medium*	Cross-sectional study of general population in U.S. (NHANES), N = 10,859	0.2 (0.2–0.4)	Q2: 1.50 (0.97, 2.32) Q3: 1.17 (0.77, 1.79) **Q4: 1.84 (1.26, 2.69) ***
Christensen et al. (2016), *low*	Cross-sectional study of male anglers in U.S., N = 154	0.5 (0.3–0.9)	1.12 (0.49, 2.18)

***$p < 0.05$**, NR: not reported.

Diabetes and insulin resistance

Twenty-one studies (23 publications) reported on the relationship between PFDA exposure and diabetes, insulin resistance, fasting blood glucose, or gestational diabetes. A summary of the study evaluations is presented in Figure 3-72, and additional details can be obtained from HAWC.

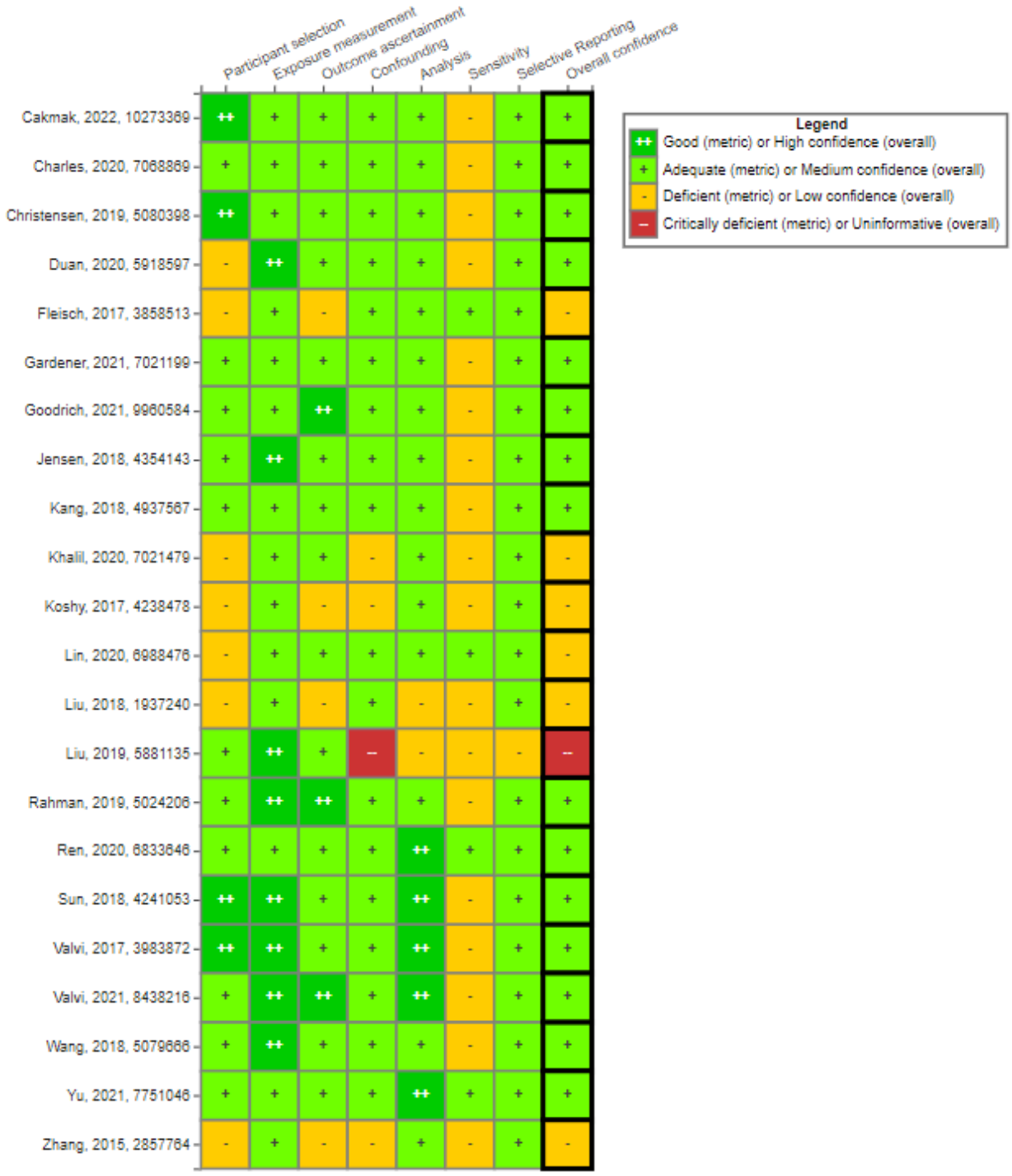


Figure 3-72. Study evaluation results for epidemiology studies of PFDA and diabetes and insulin resistance. Refer to HAWC for details on the study evaluation review: HAWC Human Diabetes and Insulin Resistance.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2021) and Jain (2020a).

For diabetes, due to concerns for reverse causality resulting from metabolic and behavioral changes following a diabetes diagnosis, the optimal epidemiological studies would be longitudinal cohort studies with repeated measurements before onset. Two medium confidence studies evaluated PFDA exposure and incident diabetes (Charles et al., 2020; Sun et al., 2018). Sun et al. (2018), a nested case-control study, found that at the highest tertile of PFDA exposure (range: 0.2–1.95 ng/mL), there was a non-statistically significant inverse (i.e., "protective") association seen with diabetes (OR = 0.7, 95% CI: 0.5, 1.1). Charles et al. (2020), also a nested case-control study, reported results that differed based on the selected control group; an inverse association was observed with controls matched for birth year and year of blood collection, controlling for BMI (OR = 0.89, 95% CI: 0.55, 1.44), while a positive association was observed with controls additionally matched for BMI (OR = 1.52, 95% CI = 0.76, 3.07), though neither was statistically significant.

For insulin resistance and blood glucose, there were several outcome-specific considerations for study evaluation that were influential on the ratings. Homeostatic model assessment (HOMA) is a method for assessing insulin resistance and β-cell function from fasting glucose and insulin measured in the plasma (Matthews et al., 1985). The HOMA of insulin resistance (HOMA-IR) is often used in studies evaluating future risk for diabetes and was considered a primary outcome for this review along with fasting blood glucose. Measures of insulin resistance and blood glucose, including HOMA-IR, are not interpretable in the presence of diabetes, particularly if diabetes is treated with hypoglycemic medication since the treatment will affect insulin production and secretion. Studies that did not consider diabetes status and use of diabetes medications by exclusion, stratification, or adjustment were thus considered deficient for participant selection. For the timing of the exposure measurement, unlike the criteria described for diabetes, exposure and outcome can be assessed concurrently as insulin resistance and blood glucose can represent short-term responses, and establishing temporality was not deemed a major concern.

Sixteen studies examined associations between PFDA exposure and insulin resistance or fasting blood glucose. Nine studies examined associations in adolescents and adults, five studies in pregnant women, and two studies in children. Six studies did not consider diabetes status of participants and were thus considered low confidence (Khalil et al., 2020; Lin et al., 2020b; Kang et al., 2018; Liu et al., 2018; Fleisch et al., 2017; Koshy et al., 2017). The remaining ten studies were medium confidence (Cakmak et al., 2022; Gardener et al., 2021; Goodrich et al., 2021; Valvi et al., 2021; Yu et al., 2021; Duan et al., 2020; Ren et al., 2020; Christensen et al., 2019; Jensen et al., 2018; Wang et al., 2018)six were low confidence.

Results of the insulin resistance and fasting blood glucose are presented in Table 3-34. In all studies of insulin resistance, the results were generally null, and in the low confidence study by Fleisch et al. (2017), an inverse association was observed. In the studies of fasting blood glucose, there was again no clear positive association observed. It is possible that the null associations could be due to poor sensitivity from narrow exposure contrasts in most of the studies, but a

minority of studies had higher exposure levels with corresponding greater contrast and also found no association. Additionally, null, and even inverse associations could be due to outcome misclassification resulting from inclusion of participants with diabetes in some studies. However, based on the current evidence, there is no indication that PFDA exposure is associated with greater insulin resistance or higher fasting blood glucose levels.

Table 3-33. Associations between PFDA and insulin resistance in epidemiology studies

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
General population, adolescents, and adults							
Goodrich et al. (2021)	*Medium*	Cohort and cross-sectional study of adolescents in U.S.; 310 in cohort and 137 in cross-sectional	NR (due to high proportion below the LOD)	NR	NR	"Not associated"	"Not associated"
Koshy et al. (2017)	*Low*	World Trade Center Health Registry (WTCHR) who resided in NYC and were born between Sept. 11, 1993 and Sept. 10, 2001; U.S.; 402 adolescents	Control 0.1 (0.2) WTCHR 0.1 (0.1)	ln-unit change	Beta coefficient (95% CI)	NR	−0.04 (−0.11, 0.03) [#]
Christensen et al. (2019)	*Medium*	Cross-sectional study in U.S. (NHANES 2007–2014); 2975 individuals aged 20 years and older	0.2 (0.1–0.4)	Quartiles	Odds ratio (95% CI)	Q2: 0.9 (0.7, 1.3) Q3: 1.1 (0.7, 1.7) Q4: 0.9 (0.6, 1.5)	NR
Cakmak et al. (2022)	*Medium*	Cross-sectional study in Canada (CHMS 2007-2017); 3,356–6,024 individuals 12 years and older	GM 0.2	Change equivalent to GM	Percent change	-0.3 (-1.4, 0.8)	5.3 (-3.5, 15.0)
Valvi et al. (2017)	*Medum*	Birth cohort in Faroe Islands; 699 young adults	0.2 (0.2-0.3)	Log_2 change	β (95% CI)	Glucose AUC Exposure at 7 yr 0.0 (-0.01, 0.02) Similar with exposure at 14, 22, 28 yr and in men and women	Exposure at 7 yr 0.03 (-0.03, 0.10) Similar with exposure at 14, 22, 28 yr and in men and women
Khalil et al. (2020)	*Low*	Cross-sectional study of firefighters in U.S.; 38 men	0.3 (0.2-0.3)	Log unit change	β (95% CI)	No association (estimates reported on figure)	NR
Liu et al. (2018)	*Low*	Cross-sectional analysis in weight loss clinical trial in U.S.; 621 adults (30–70 yrs)	Male 0.4 (0.3–0.5) Female 0.4 (0.3–0.6)	n/a	Spearman correlation	0.08	0.05

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
Lin et al. (2020b)	*Low*	Cross-sectional study of older adults living near a high contamination area in Taiwan; 397 adults (55–75 yrs)	Median (range) 1.7 (0.6-27)	Quartiles	β (95% CI)	Women Q2: -4.83 (-13.34, 3.68) Q3: -4.33 (-12.91, 4.26) Q4: -5.72 (14.37, 2.94) Men Q2: -5.38 (-19.68, 8.92) Q3: 2.67 (-11.7, 17.05) Q4: 3.9 (-11.1, 18.9)	NR
Duan et al. (2020)	*Medium*	Cross-sectional study in China; 294 adults	2.1 (1.0–4.1)	1% increase	Percent change	0.009 (−0.002, 0.020)	NR
Pregnant women							
Gardener et al. (2021)	*Medium*	Pregnancy cohort in U.S.; 433 pregnant women	0.2 (0.1-0.3)	Quartiles	Means (95% CI)	NR	Insulin: No association (estimates reported on figure)
Jensen et al. (2018)	*Medium*	Birth cohort in Denmark; 649 pregnant women (15–49 yrs)	0.3 (0.2–0.5)	Two-fold change	% Change (95% CI)	−1.3 (−3.6, 1.0)	−1.5 (−13.5, 12.1)
Wang et al. (2018)	*Medium*	1:2 matched case control of pregnant women in China; 84 cases and 168 noncases	Controls 0.3 (0.2–0.4) Cases 0.3 (0.2–0.4)	Dichotomous exposure (tertiles of outcome)	Odds ratio (95% CI)	Medium vs. Lowest FBG 1.3 (0.7–2.4) Highest vs. Lowest FBG 1.0 (0.5–1.8)	NR
Yu, 2021,	*Medium*	Pregnancy cohort in China; 2,747 pregnant women	1.7 (1.4)	Log-unit change	β (95% CI)	0.01 (-0.02, 0.04) 1 hr post glucose tolerance test 0.12 (0.01, 0.22) 2 hr post 0.08 (-0.002, 0.17)	NR

Reference	Confidence	Population	Median exposure (IQR) in ng/mL or as specified	Exposure change	Effect estimate	Fasting blood glucose	Insulin resistance (HOMA-IR)
Ren et al. (2020)	*Medium*	Pregnancy cohort in China; 856 pregnant women	2.0 (1.3–3.2)	ln-unit change	OR (95% CI) for high glucose 1 hr post glucose tolerance test 1.61 (1.10, 2.44)	1.24 (0.87, 1.76)	NR
Children							
Fleisch et al. (2017)	*Low*	Birth cohort in U.S.; 665 mother-children's pairs	GM (IQR) Mid-childhood 0.3 (0.2, 0.5)	Quartiles	Beta coefficient (95% CI)	NR	Mid-childhood Q2: −7.1 (−22.1, 10.6) **Q3: −31.3 (−42.8, −17.5) *** **Q4: −21.5 (−34.0, −6.7)***
Kang et al. (2018)	*Low*	Cross-sectional study in South Korea; 150 children (3–18 yrs)	0.06 (0.04–0.1)	ln-unit change	Beta coefficient (95% CI)	−0.2 (−1.3, 0.9)	NR

***p*-value or *p*-trend < 0.05**.

HOMA-IR was log-transformed.

Note: Not all results (e.g., sub-group analyses, different exposure classification) were extracted from each study if additional results did not change the interpretation.

NR = not reported.

Six studies reported on the association between PFDA exposure and gestational diabetes (Liu et al., 2019b; Rahman et al., 2019; Wang et al., 2018; Valvi et al., 2017; Zhang et al., 2015). Four studies were *medium* confidence, one was *low* confidence, and one (Liu et al., 2019b) was *uninformative* due to lack of control for confounding in single-pollutant models. The three *medium* confidence studies were inconsistent, with one (Valvi et al., 2017) reporting higher odds of gestational diabetes with higher exposure (OR for doubling of exposure: 1.2 (0.7,2.0)), but the association was not statistically significant and non-monotonic (OR for tertile 2: 2.0 (0.9,4.1), tertile 3: 1.0 (0.5,2.3)). Two *medium* confidence studies reported close to null association with gestational diabetes and PFDA exposure (OR: 1.02 (0.86, 1.20) in the overall cohort in Rahman et al. (2019), OR 0.95 (0.78, 1.16) in Yu et al. (2021)), and the other *medium* confidence study Wang et al. (2018) reported a non-statistically significant inverse association (OR: 0.85 (0.30–2.92)). The *low* confidence study (Zhang et al., 2015) reported no association (OR: 1.0 (0.7–1.5)).

Overall, for diabetes and insulin resistance, there were no clear associations with higher PFDA exposures. Results were generally null or in the inverse direction. While it is possible that a positive association with these outcomes exists but was obscured by poor sensitivity and/or bias, there is no clear explanation for the inconsistency based on study confidence, design, or population.

Adiposity

Thirteen studies reported on the association between PFDA exposure and obesity or related outcomes. Two studies were excluded due to critical deficiencies in participant selection (Yang et al., 2018) and confounding (Zhao et al., 2022; Yang et al., 2018). Of the 11 remaining studies, four were cohorts that examined early life exposure to PFDA and adiposity at 18 months (Karlsen et al., 2017), at 4–8 years of age (Bloom et al., 2022), at 5 years of age (Chen et al., 2019; Karlsen et al., 2017), and at 13 years of age (Janis et al., 2021); one was a clinical trial of weight loss diets in adults that examined weight change (Liu et al., 2018); and one was a cohort of adults living near a uranium processing site (Blake et al., 2018). All of these were classified as *medium* confidence. Five studies (three in adults and two in children) were cross-sectional (Lind et al., 2022; Wise et al., 2022; Thomsen et al., 2021; Domazet et al., 2020; Christensen et al., 2019) and were low confidence due to the potential for reverse causation resulting from metabolic changes in obese individuals. The evaluations are summarized in Figure 3-73.

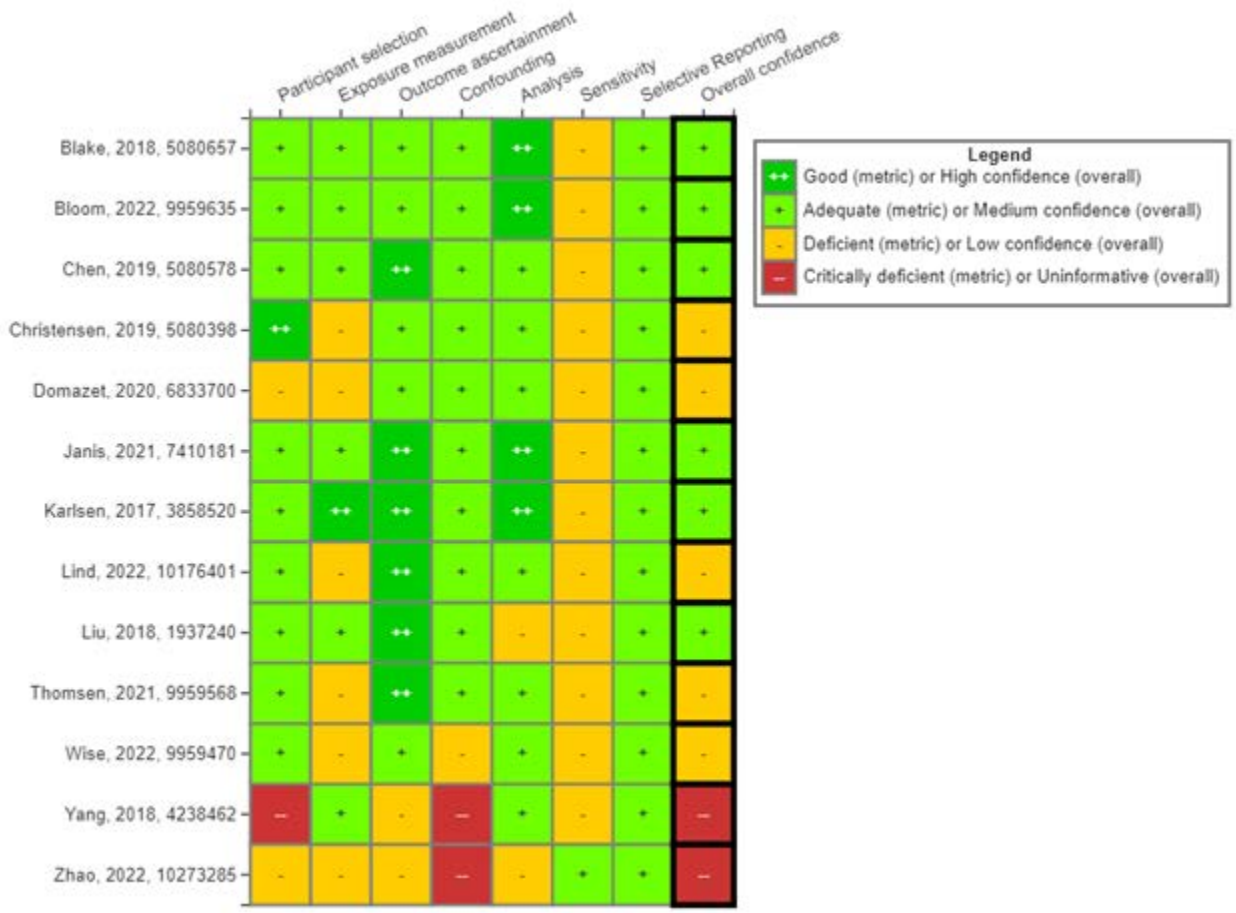


Figure 3-73. Study evaluation results for epidemiology studies of PFDA and adiposity. Refer to HAWC for details on the study evaluation review: HAWC Human Adiposity.

Multiple publications of the same study: Christensen et al. (2019) includes Jain (2020a).

The available studies look at several different outcomes and populations, so are generally not directly comparable (see Table 3-35). In the five studies in adults, one medium confidence study reported higher BMI with higher exposure (Blake et al., 2018) and the other *medium* confidence study reported greater weight gain following a weight loss trial (Liu et al., 2018), with only the latter being statistically significant. Of the three *low* confidence cross-sectional studies, two reported statistically significant inverse associations with BMI in women (Lind et al., 2022; Wise et al., 2022), while the third also reported an inverse, though not statistically significant, association with waist circumference. In children, one *medium* confidence birth cohort (Karlsen et al., 2017) reported a slightly higher proportion of overweight participants with higher exposure at 18 months when maternal exposure was modeled as a continuous variable (RR = 1.14, 95% CI 0.91,1.43), but this was not statistically significant and not monotonic when modeled in

tertiles (RR T2 vs. T1 = 0.90 (95% CI: 0.71, 1.15), T3 vs. T1 = 1.03 (95% CI: 0.82, 1.31))or in follow-up of the children at 5 years. However, a cross-sectional analysis by Karlsen et al. (2017) in this population at 5 years indicated lower BMI and incidence of children who were overweight with higher exposure. A second *medium* confidence birth cohort study reported non-significant inverse associations in girls and non-significant positive associations in boys at 5 years (Chen et al., 2019). The other two medium confidence cohort studies, including a birth cohort with exposure measurement in gestation and follow-up to 4–8 yrs (Bloom et al., 2022) and a cohort with exposure measurement in mid-childhood (age 8) and follow-up to age 13 (Janis et al., 2021) were null overall with regard to BMI and fat mass. The two low confidence cross-sectional studies reported inverse associations with fat mass (Domazet et al., 2020) and measures of fat obtained with MRI and Dual X-ray absorptiometry (Thomsen et al., 2021). Overall, there is some limited evidence of an association between PFDA exposure and adiposity in adults in two *medium* confidence studies, but there is considerable *uncertainty*, and this association was not observed in studies of children.

Table 3-34. Associations between PFDA and adiposity in epidemiology studies

Reference, study confidence	Population	Median exposure (IQR) in ng/mL	Effect estimate	BMI	Waist circumference	Other
Adults						
Blake et al. (2018), *medium*	Prospective cohort near a uranium processing site in U.S.; 210 adults	0.1 (0.1, 0.2)	% change (95% CI) for IQR increase in exposure	0.7 (−1.3, 2.7)	NR	NR
Christensen et al. (2019), *low*	NHANES, cross-sectional in U.S.; 2,975 adults (20+ yr)	0.2 (0.1, 0.4)	OR (95% CI) for increased WC by quartiles (ref Q1)	NR	Q2: 0.9 (0.6, 1.2) Q3: 0.9 (0.5, 1.5) Q4: 0.8 (0.5, 1.3)	NR
Liu et al. (2018), *medium*	Clinical trial of weight loss diet in U.S.; 621 adults	Male 0.4 (0.3–0.5) Female 0.4 (0.3–0.6)	Mean difference	NR	NR	Weight gain following trial T1: 2.5 ± 0.9 T2: 3.1 ± 0.9 T3: 4.2 ± 0.8, p-trend: 0.03
Children						
Karlsen et al. (2017), *medium*	Prospective birth cohort in Faroe Islands; 444 children at 18 mo and 371 at 5 yr		β (95% CI) for BMI; Relative risk for overweight	18 mo 0.1 (−0.1, 0.3) 5 yr (−0.04 (−0.2, 0.1)	NR	Overweight 18 mo 1.1 (0.9, 1.4) 5 yr 1.0 (0.6, 1.7)
Chen et al. (2019), *medium*	Prospective birth cohort in China; 404 children at 5 yr	0.4 (range 0.2–2.0)	β (95% CI) for log-unit change	Girls: −0.2 (−0.4, 0.1) Boys: 0.1 (−0.3, 0.5)	Girls: −0.7 (−1.5, 0.1) Boys: 0.2 (−0.8,1.0)	Body fat percentage (%) Girls: −1.1 (−2.3, 0.2) Boys: 1.1 (−0.2, 2.3)
			β (95% CI) for tertiles (ref T1)	Girls T2: −0.1 (−0.7, 0.4) T3: 0.0 (−0.6, 0.5) Boys T2: −0.2 (−0.8, 0.4) T3: 0.2 (−0.5, 0.8)	Girls T2: −0.6 (−2.2, 1.0) T3: −0.5 (−2.2, 1.1) Boys T2: −0.9 (−2.5, 0.7) T3: 0.5 (−1.1, 2.1)	Girls T2: −0.6 (−3.2, 1.9) T3: −1.5 (−4.1, 1.0) Boys T2: 0.5 (−1.5, 2.6) T3: 2.0 (−0.1, 4.1)

NR = not reported.

Metabolic syndrome

The current criteria for clinical diagnosis of metabolic syndrome include the following: larger waist circumference; elevated triglycerides ≥150 mg/dL (1.7 mmol/L); reduced HDL-C <40 mg/dL (1.0 mmol/L) in males and <50 mg/dL (1.3 mmol/L) in females; elevated blood pressure: systolic ≥130 and/or diastolic ≥85 mm Hg; and elevated fasting glucose ≥100 mg/dL (Alberti et al., 2009). Main considerations are that three abnormal findings out of five in the criteria would qualify a person for the metabolic syndrome and that country- or population-specific cut points for waist circumference should be used (Alberti et al., 2009).

Three studies reported on the association between PFDA exposure and metabolic syndrome. One study was *uninformative* due to critical deficiencies in participant selection (Yang et al., 2018). The remaining two studies were cross-sectional, with one (Christensen et al., 2019) being *medium* confidence and one being *low* confidence (Lin et al., 2020b). Christensen et al. (2019) found an exposure-dependent, significant inverse association between PFDA exposure and metabolic syndrome (OR: 0.72; 95%CI: 0.54, 0.97 with ln (PFDA); quartile 2: 0.93; 95%CI: 0.64, 1.35, quartile 3: 0.71; 95%CI: 0.43, 1.18, and quartile 4: 0.56; 95%CI: 0.31, 1.01). Lin et al. (2020b) also reported an inverse association (not statistically significant) in women (OR (95% CI) for quartiles vs. Q1, Q2: 0.68 (0.33, 1.4); Q3: 0.78 (0.38, 1.61); Q4: 0.51 (0.24, 1.08) but reported a positive association (also not statistically significant) in men (Q2: 0.94 (0.31, 2.85); Q3: 1.43 (0.48, 4.22); Q4: 1.9 (0.63, 5.77).

Animal studies

There is a single study available in experimental animals that evaluated endpoints related to cardiometabolic effects following short-term exposure to PFDA (NTP, 2018). The study exposed female and male SD rats to PFDA doses of 0, 0.156, 0.312, 0.625, 1.25 and 2.5 mg/kg-day for 28 days via gavage and included endpoints such as serum lipids, histopathology, and organ weights. Confidence in the study was rated as high during study evaluation for these endpoints with no outstanding issues regarding risk of bias or sensitivity (see Figure 3-74).

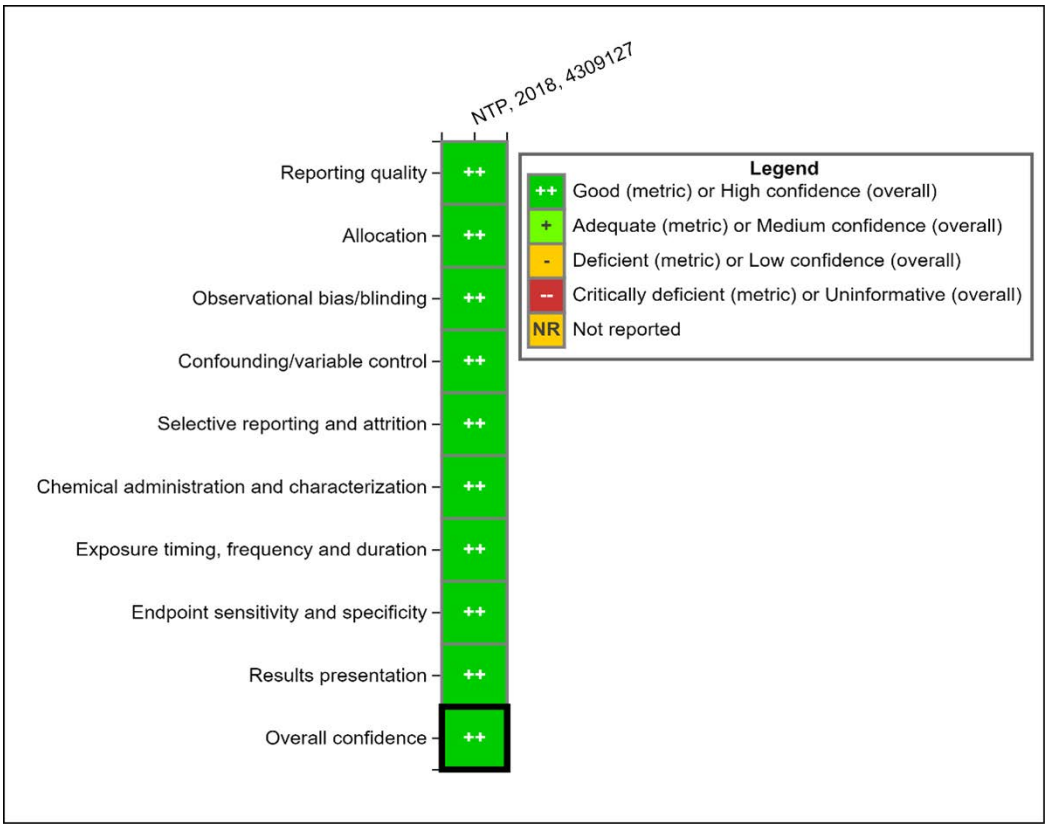


Figure 3-74. Evaluation results for animal study assessing effects of PFDA exposure on cardiometabolic effects. Refer to HAWC for details on the study evaluation review.

Histopathology

The heart and blood vessel were examined histologically in rats in the control and high-dose groups (2.5 mg/kg-day) at study termination (see Figure 3-75). An increase in the incidence of granulomatous inflammation of the epicardium (2/10 rats; moderate severity) was reported in high-dose females after PFDA exposure. Granulomas are focal, inflammatory tissue responses that arise from a broad range of etiologies, including infectious and non-infectious processes (Boros and Revankar, 2017). This lesion was not observed in exposed males or in the controls. Results for blood vessel histopathology were null. The biological significance of the histopathological observations in females is unknown given the sparse information available.

Serum lipids

Cholesterol is important for maintaining cell membrane integrity and transport and is also used as a precursor for the synthesis of steroid hormones, bile acids and other substances in the body. Triglycerides are an essential source of energy storage and production. Both cholesterol and triglycerides are routinely evaluated in blood lipid panels as cardiovascular risk measures. Cholesterol and triglyceride levels were measured in rat serum after 28-day exposure (see

Table 3-36 and Figure 3-75. Dose-related decreases in triglyceride levels were reported in male and female rats exposed to PFDA, with the largest changes occurring in males at the highest doses (35% and 52% compared to controls at 1.25 and 2.5 mg/kg-day, respectively). A downward trend ($p < 0.01$) was reported for cholesterol levels in females, reaching 35% compared to controls at 2.5 mg/kg-day. In males, cholesterol decreased 14–38% compared to controls across 0.156–2.5 mg/kg-day, but the effects did not display a significant trend. The findings should be interpreted with caution given the known species differences in lipid metabolism and blood cholesterol levels between rodents and humans that may impact the evaluation of the human relevance of the observed responses (Getz and Reardon, 2012; Davidson, 2010).

Table 3-35. Percent change relative to controls in serum lipids in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Triglycerides					
Male S-D rats	14	−2	−21	**−35**	**−52**
Female S-D rats	27	18	−7	−23	−27
Cholesterol					
Male S-D rats	**−27**	**−38**	**−27**	−12	−14
Female S-D rats	1	−8	0	−9	**−35**

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

Organ weight

Terminal absolute and relative heart weights were measured in all exposed animals (see Table 3-37 and Figure 3-75). It is unclear which metric (i.e., absolute, or relative) would be more appropriate to evaluate effects on heart weight in the presence of significant body weight changes (Bailey et al., 2004). As such, both absolute and relative measures were considered herein. Absolute heart weight showed a decreasing trend ($p < 0.01$) in males and females, with 15–37% decreases compared to controls at doses of 1.25 and 2.5 mg/kg-day. In contrast, changes in relative heart weights did not show a significant trend. The reductions in absolute heart weight coincide with reductions in body weight observed in these animals at the high-dose groups (≥1.25 mg/kg-day) (see Section 3.2.10 on General toxicity effects for additional details).

Table 3-36. Percent change relative to controls in heart weights in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Absolute heart weight					
Male S-D rats	5	−2	2	**−18**	**−37**
Female S-D rats	1	1	−2	**−15**	**−36**
Relative heart weight					
Male S-D rats	2	−1	6	4	1
Female S-D rats	−3	−3	−2	−3	1

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

Endpoint Name	Organ	Study Name	Outcome Confidence	Exposure Design	Species, Strain (Sex)	Trend Test Result
Triglyceride (TRIG)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
					Rat, Sprague-Dawley (Harlan) (♀)	significant
Cholesterol (CHOL)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
					Rat, Sprague-Dawley (Harlan) (♀)	significant
Histopathology	Heart	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
Epicardium, Granulomatous Inflammation	Heart	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
Histopathology	Blood Vessel	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not applicable
					Rat, Sprague-Dawley (Harlan) (♀)	not applicable
Heart Weight, Absolute	Heart	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
					Rat, Sprague-Dawley (Harlan) (♀)	significant
Heart Weight, Relative	Heart	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
					Rat, Sprague-Dawley (Harlan) (♀)	not significant

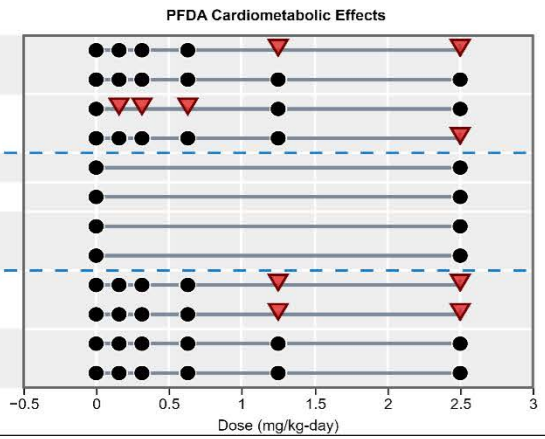


Figure 3-75. Cardiometabolic effects following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/pfda-cardiometabolic-effects/).

Evidence integration

The evidence of an association between PFDA exposure and cardiometabolic effects in humans is *sligh*t, with an indication of higher serum lipids, adiposity, cardiovascular disease, and possible markers of atherosclerosis with higher PFDA exposure. While most results were imprecise and not statistically significant, exposure contrasts for PFDA in the study populations were relatively narrow, which is interpreted to result in low sensitivity to detect an effect. However, there is inconsistency across studies for similar outcomes, so there is considerable uncertainty in the evidence. There is no evidence of an association with diabetes, insulin resistance, and metabolic syndrome, but the null results are difficult to interpret due to concerns for sensitivity.

Overall, the animal evidence is *indeterminate* given that the observed changes fail to establish a coherent pattern of adverse cardiometabolic effects in animals following short-term PFDA exposure. The evidence in animals is limited to a *high* confidence study in rats exposed via gavage for 28 days that examined cardiovascular histopathology, serum lipids and heart weights (NTP, 2018). Dose-related decreases in triglyceride levels occurred in males and females and cholesterol also decreased dose-dependently in females. However, the biological significance of these responses is unclear. Absolute heart weights decreased dose-dependently in rats at the highest doses (≥1.25 mg/kg-day) but confidence in the results is reduced by potential confounding with decreased body weights and a lack of corroborative findings from histopathological evaluations or other organ weight measures (relative heart weight was unchanged). A major limitation in the animal toxicity database of this chemical is the lack of studies examining prolonged or chronic oral exposures. In addition, for some cardiometabolic endpoints (i.e., serum lipids), it would be preferred if studies were available in models that are more physiologically relevant to humans given species differences in lipid metabolism between humans and rodents (Getz and Reardon, 2012; Davidson, 2010). In the absence of such studies or mechanistic information on these responses, the human relevance of effects on rodent lipid profiles cannot be determined.

Overall, ***evidence suggests*** that PFDA exposure has the potential to cause cardiometabolic effects in humans under sufficient exposure conditions (see Table 3-38). This conclusion is based on evidence of an association between PFDA exposure and certain cardiometabolic outcomes (serum lipids, adiposity, cardiovascular disease, and atherosclerosis) in a small number of epidemiological studies with median exposure levels from 0.1–0.4 ng/mL; however, issues with inconsistency across studies raise considerable uncertainty. Moreover, evidence in animals is sparse and largely uninterpretable regarding its relevance to humans.

Table 3-37. Evidence profile table for PFDA exposure and cardiometabolic effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.7: Human studies)					⊕⊙⊙ ***Evidence suggests*** *Primary basis:* Some coherent effects in a small number of *medium* confidence epidemiological studies, but data is largely inconsistent. Evidence from a *high* confidence rat study was *indeterminate*. *Human relevance:* The utility of the observed serum lipid effects in rats for informing human health hazard is uncertain given the species differences in lipid metabolism between humans and rodents. *Cross-stream coherence, susceptibility, and other inferences:* No specific factors are noted.
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
<u>Serum lipids</u> 14 *medium* and 6 *low* confidence studies	• Five of six *medium* confidence studies in adults (including two in pregnant women) reported higher serum total cholesterol with higher PFDA exposure (p <0.05 in three studies). • In children, results were inconsistent.	• *Consistency* of direction of association across studies in adults for total cholesterol. • Exposure-response gradient in the only two studies that examined categorical exposure.	• *Imprecision* in most positive associations • *Lack of coherence* across measures (total cholesterol and triglycerides) in some studies	⊕⊙⊙ *Slight* Positive associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in some studies, but with the exception of total cholesterol in adults, findings were inconsistent or incoherent across studies. Exposure levels were low, which may explain the lack of association in some studies.	
<u>Other cardiovascular risk factors</u> 9 *medium* and 4 *low* confidence studies	• Studies of blood pressure in the general population were largely null. Three of five studies reported hypertension or a positive association with blood pressure among pregnant women, but there was inconsistency among *medium* confidence studies. • There was a non-significant increase	• No factors noted	• *Unexplained* inconsistency across studies for blood pressure • *Imprecision* in positive associations observed for blood pressure and atherosclerosis		

Evidence stream summary and interpretation					Evidence integration summary judgment
	in the number of carotid arteries with atherosclerotic plaques in women in one study. • One study reported statistically significant changes in ventricular geometry.				
<u>Cardiovascular disease</u> 2 *medium* and 1 *low* confidence studies	• One *medium* and one *low* confidence studies reported higher odds of coronary heart disease (the former being statistically significant), but another *medium* confidence study was null. • Higher odds of angina pectoris, myocardial infarction, and stroke were reported in the single study that examined them.	• No factors noted	• *Unexplained inconsistency* across *medium* confidence studies, possibly related to timing of exposure measurement • *Imprecision* in results of specific cardiovascular conditions		
<u>Diabetes and insulin resistance</u> 15 *medium* and 6 *low* confidence studies	• One study reported higher odds of gestational diabetes with higher PFDA exposure, but the	• No factors noted	• Unexplained inconsistency across studies		

Evidence stream summary and interpretation					Evidence integration summary judgment
	association was non-monotonic and not statistically significant. Other studies reported either null or inverse associations with gestational diabetes. • Two studies of incident diabetes and 16 studies of insulin resistance indicated primarily null associations with PFDA exposure. • Low sensitivity across majority of studies				
<u>Adiposity</u> 6 *medium* and 5 *low* confidence studies	• One study in adults reported an increase in weight gain (significant trend) and one reported higher BMI with higher PFDA exposure, but other studies reported null or inverse associations • Low sensitivity across studies	• No factors noted	• Unexplained inconsistency across studies		
<u>Metabolic syndrome</u> 2 *medium* confidence studies	• Inverse association between metabolic syndrome and PFDA	• No factors noted	• No factors noted		

Evidence stream summary and interpretation					Evidence integration summary judgment
	exposure in two studies (one reported a positive association in men).				
Evidence from in vivo animal studies (see Section 3.2.7: Animal studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
Histopathology 1 *high* confidence study in rats for 28 d	• No significant effects in heart and blood vessel histopathology in rats up to 2.5 mg/kg-d	• *High* confidence study	• No factors noted	⊙⊙⊙ *Indeterminate* Lack of coherent, adverse effects indicative of cardiometabolic toxicity.	
Serum lipids 1 *high* confidence study in rats for 28 d	• Decreases in triglyceride (males and females) and cholesterol levels (females only) in rats at ≥1.25 mg/kg-d for 28 d	• *Dose-response* gradient for most effects • *High* confidence study	• Unclear biological significance of decreases in lipids		
Organ weight 1 *high* confidence study in rats for 28 d	• Decreases in absolute (but not relative) heart weight in rats at doses ≥1.25 mg/kg-d	• Dose-response gradient for absolute heart weights • *High* confidence study	• *Unexplained inconsistency* across heart weight measures • *Potential confounding* by body weight decrease (particularly since only absolute weights affected)		

C = cohort study; CS = cross-sectional study; CC = case-control study.

3.2.7. NEURODEVELOPMENTAL EFFECTS

Human studies

Neurodevelopment

There are 13 studies (19 publications) of PFDA and neurodevelopmental outcomes in humans. The study evaluations are summarized for Figure 3-76. In the case of multiple publications for the same study population, they were evaluated under one record if the selection procedures for the analysis population were similar but evaluated under different records if selection procedures were significantly different (see figure footnote for details). All but one study (Gump et al., 2011) was *medium* confidence, however all but (Niu et al., 2019) were *deficient* for study sensitivity due to limited exposure contrast. With the exception of Gump et al. (2011), all studies were birth cohorts or case-controls studies nested in cohorts that evaluated maternal exposure to PFDA during pregnancy and/or during childhood. Functionally there is considerable overlap between different domains of neurodevelopment, but for the purposes of this review, the outcomes were categorized: eight studies (9 publications) examined Attention Deficient Hyperactivity Disorder (ADHD), attention , or related behaviors (Dalsager et al., 2021b; Harris et al., 2021; Skogheim et al., 2021; Luo et al., 2020; Vuong et al., 2018; Høyer et al., 2017; Oulhote et al., 2016; Liew et al., 2015; Gump et al., 2011), eight studies (ten publications) examined cognition and summary measures of neurodevelopment (Yao et al., 2022; Harris et al., 2021; Skogheim et al., 2020; Niu et al., 2019; Harris et al., 2018; Liew et al., 2018; Lyall et al., 2018; Vuong et al., 2018; Vuong et al., 2016; Wang et al., 2015), five studies examined autism spectrum disorder (ASD) or social behaviors (Skogheim et al., 2021; Shin et al., 2020; Niu et al., 2019; Lyall et al., 2018; Liew et al., 2015), three examined motor effects (Yao et al., 2022; Niu et al., 2019; Harris et al., 2018), and one examined congenital cerebral palsy (Liew et al., 2014).

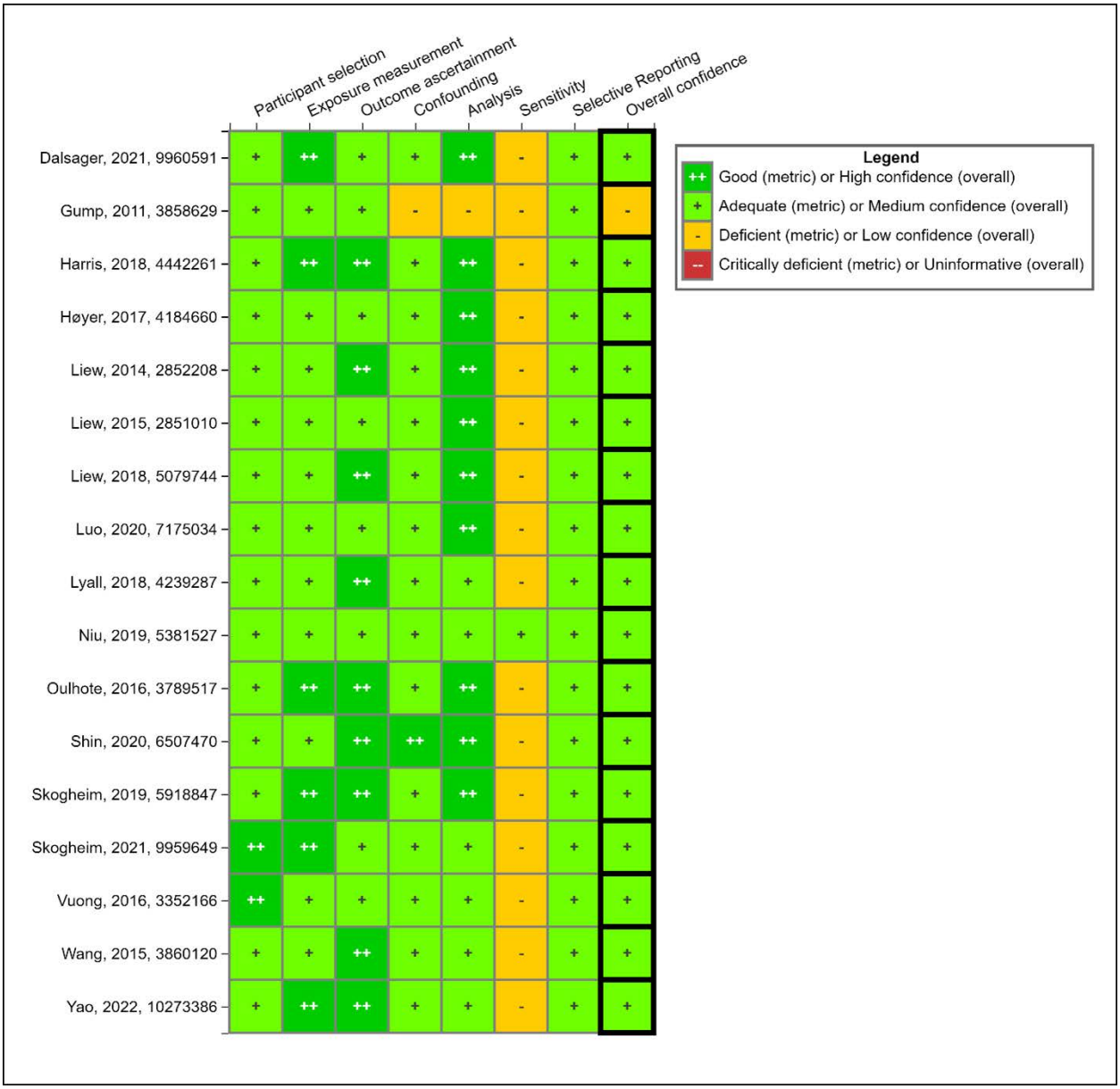


Figure 3-76. Study evaluation results for epidemiology studies of PFDA and neurodevelopmental effects. Refer to HAWC for details on the study evaluation review: https://hawc.epa.gov/summary/visual/assessment/100500072/pfda-and-neurodevelopmental-outcomes/.[a-c]

[a]Multiple publications of the same study population:
Project Viva - Harris et al. (2018) also includes (Harris et al., 2021)
HOME study - Vuong et al. (2016) also includes (Vuong et al., 2018)

[b]Four publications with data from the Danish National Birth Cohort were evaluated separately due to significantly different selection procedures but should not be considered independent: (Liew et al., 2014); (Liew et al., 2015); (Liew et al., 2018); (Luo et al., 2020).

[c]Two publications with data from the Norwegian Mother Father and Child Cohort were evaluated separately due to significantly different selection procedures but should not be considered independent: (Skogheim et al., 2020) and (Skogheim et al., 2021).

Most of the eight studies (reported in nine publications) examining ADHD or related behaviors reported associations with greater difficulties in attention or behavior problems, but there is some inconsistency within and across studies and imprecision in the results. Results for the *medium* confidence studies are displayed in Table 3-39. Notably, the two studies with the most clinically relevant outcome measure (Skogheim et al., 2021; Liew et al., 2015) examined diagnosed ADHD and found no increase in the odds of diagnosis (effect estimates were in the inverse direction). The remaining *medium* confidence studies (including another publication using the same population as Liew et al. (2015), resulting in six studies) examined scores on neurobehavioral assessments including the Strengths and Difficulties Questionnaire (SDQ), the Child Behavior Checklist (CBC), and the Behavior Rating Inventory of Executive Function (BRIEF). With the exception of Luo et al. (2020), which reported inconsistent results across child ages, all of these studies reported associations consistent with greater difficulties in attention or behavior problems with higher PFDA exposure, though effect estimates were small in most studies. This included statistically significant associations in Harris et al. (2021) and Oulhote et al. (2016) with SDQ scores and an exposure-response gradient across categories in Harris et al. (2021) and Høyer et al. (2017). However, in most studies, the confidence intervals were wide. It is possible that the limited study sensitivity could explain the non-significant findings, but this would likely not explain the inconsistency with studies of the more apical outcome of ADHD diagnosis, and thus there is uncertainty in the findings overall. Finally, a *low* confidence cross-sectional study examined inter-response time (IRT) at age 9–11 and found statistically significant decreases in IRT, which indicates poor response inhibition (a primary deficit in children with ADHD) as the test is designed to reward longer response times (Gump et al., 2011).

For the other neurodevelopmental outcomes, results were less consistent. In the eight studies of cognition and summary neurodevelopmental scores, Vuong et al. (2018), reported higher odds of "at risk" scores for metacognition and global executive indices at ages 3 and 8 (statistically significant for the global executive composite, OR 2.95, 95% CI: 1.20, 7.23). Nonstatistically significant decreases in IQ or similar scores were reported in two studies (Harris et al., 2018; Wang et al., 2015), but the remaining studies did not report associations with IQ (Liew et al., 2018), executive function (Harris et al., 2021), communication and problem solving (Niu et al., 2019), working memory (Skogheim et al., 2020), adaptive or language developmental quotient (Yao et al., 2022), or intellectual disability (Lyall et al., 2018). Among the five studies of ASD and social behavior, four examined diagnosed ASD; three of these reported inverse associations (statistically significant in one) (Skogheim et al., 2021; Shin et al., 2020; Liew et al., 2015) and one reported a null finding (Lyall et al., 2018). One study examined personal-social skills and found a positive association with problems which was statistically significant in girls (Niu et al., 2019). Two of three studies of motor effects reported *non-statistically significant* associations with reduced motor performance (Niu et al., 2019; Harris et al., 2018). Lastly, one study of congenital cerebral palsy

found no association with PFDA exposure (Liew et al., 2014). Due to the poor sensitivity of the available studies, it is difficult to interpret the primarily null results for these outcomes.

Table 3-38. Results for *medium* confidence epidemiology studies of PFDA exposure and behavioral and attention effects

Study name, reference(s)	Measured Outcome	Exposure measurement timing	Estimate type (adverse direction[a])	Sub-population / N	Group or unit change	Exposure Median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
Norwegian Mother, Father, and Child cohort **Skogheim et al. (2021)**	Diagnosed ADHD	Maternal (2nd trimester)	OR (↑)	1,801	Q1	0.02–0.13	Ref		
					Q2	0.13–0.17	0.86	0.65	1.13
					Q3	0.17–0.23	0.77	0.59	1.02
					Q4	0.23–1.5	0.61	0.46	0.81
Danish National Birth Cohort **Liew et al. (2015)** **Luo et al. (2020)**	Diagnosed ADHD	Maternal (1st trimester)	RR (↑)	760	Ln-unit increase in exposure	0.2 (0.1–0.2)	**0.76***	0.64	0.91
	Externalizing problems at 7 yrs		OR (↑) (odds of elevated score)	2,421	Per doubling of exposure		1.09	0.78	1.53
	Internalizing problems at 7 yrs						1.03	0.72	1.47
	Total SDQ score at 7 yrs						1.11	0.87	1.43
	Externalizing problems at 11 yrs			2,070			0.95	0.70	1.28
	Internalizing problems at 11 yrs						0.95	0.72	1.26
	Total SDQ score at 11 yrs						0.86	0.68	1.08
Odense child cohort **Dalsager et al. (2021b)**	ADHD symptom score on CBC	18 mo	IRR (↑) (relative difference in score)	775	Per doubling of exposure	0.2	0.98	0.88	1.09
		Maternal (1st trimester)		1,113		0.3	1.02	0.95	1.09
		18 mo	OR (↑) (odds of elevated score)	775	Per doubling of exposure	0.2	1.06	0.78	1.44
		Maternal (1st trimester)		1,113		0.3	1.08	0.85	1.37
HOME study **Vuong et al. (2018)**	Behavioral regulation index on BRIEF	3 yrs	OR (↑) (odds of elevated score)	208	Ln-unit increase in exposure	0.2	1.95	0.83	4.62
		8 yrs				0.2	1.70	0.59	4.88
Project Viva	Externalizing problems	7–11 yrs	Mean Difference (↑)	628	Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.2	-0.5	0.9

Study name, reference(s)	Measured Outcome	Exposure measurement timing	Estimate type (adverse direction[a])	Sub-population / N	Group or unit change	Exposure Median (IQR) or range (quartiles)	Effect Estimate	CI LCL	CI UCL
Harris et al. (2021)					Q3	0.4–0.4	0.3	-0.4	1.0
					Q4	0.5–1.9	0.5	-0.2	1.2
	Internalizing problems				Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.2	-0.4	0.7
					Q3	0.4–0.4	0.4	-0.2	0.9
					Q4	0.5–1.9	0.6	0.0	1.1
	Total SDQ score				Q1	<0.1–0.2	Ref		
					Q2	0.3–0.3	0.4	-0.6	1.3
					Q3	0.4–0.4	0.7	-0.4	1.7
					Q4	0.5–1.9	**1.1***	0.1	2.1
Faroe Island cohort (Oulhote et al., 2016)	Externalizing problems	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	**0.45***	0.02	0.87
		Maternal (32 wks gestation)		539		0.3 (0.2–0.4)	0.26	-0.29	0.81
	Internalizing problems	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	0.27	-0.11	0.65
		Maternal (32 wks gestation)		539		0.3 (0.2–0.4)	0.26	-0.29	0.81
	Total SDQ score	5 yrs	Mean Difference (↑)	508	Per doubling of exposure	0.3 (0.2–0.4)	**0.72***	0.07	1.38
		Maternal (32 wks gestation)		539		0.3 (0.2–0.4)	-0.01	-0.98	0.96
INUENDO (Biopersistent organochlorines in diet and human fertility) (Høyer et al., 2017)	SDQ hyperactivity score at 5–9 yrs	Maternal (second trimester median)	Regression Coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.13	-0.10	0.36
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.11	-0.22	0.44
					High exposure	2.0–18.8	0.13	-0.27	0.53
	Total SDQ score at 5–9 yrs	Maternal (second trimester median)	Regression Coefficient (↑)	1,023	ln-unit increase in exposure	1.5 (10th–90th 0.7–3.4)	0.40	-0.15	0.95
					Low exposure	0.2–1.2	Ref		
					Medium exposure	1.2–2.0	0.07	-0.71	0.85
					High exposure	2.0–18.8	0.65	-0.30	1.61

***p* <0.05**

SDQ: Strengths and Difficulties Questionnaire. Externalizing problems calculated from conduct and hyperactivity subscales; Internalizing problems calculate from emotional and peer subscales.
BRIEF: Behavior Rating Inventory of Executive Function
[a] The arrows indicate the direction the effect estimate will be if there is an association between PFHxS and reduced behavior. For all the tests included here, higher scores indicate more difficulties/behavior problems/ADHD diagnosis. For ratio measures such as odds ratios (OR), an effect estimates greater than 1 indicates more difficulties/behavior problems, while for regression coefficients and mean differences, an effect estimates greater than 0 indicates more difficulties/behavior problems

Animal studies

There are no available animal toxicity studies informing of potential neurodevelopmental effects of PFDA via any relevant exposure route and duration.

Evidence Integration

The evidence for potential neurodevelopmental effects in humans is considered *slight.* Associations between PFDA exposure and outcomes related to attention and behavior were reported in multiple epidemiological studies, though there was inconsistency between these findings and the more clinically relevant measure of ADHD diagnosis. Results for other neurodevelopmental effects were largely inconsistent, though poor sensitivity due to limited exposure contrast may explain the lack of association in some studies. No animal toxicity studies are available. Altogether, based on the available human studies, the ***evidence suggests*** that PFDA exposure might cause neurodevelopmental effects in humans under sufficient exposure conditions[4] (see Table 3-40).

Table 3-39. Evidence profile table for PFDA neurodevelopmental effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.7: Human studies)					⊕⊙⊙ ***Evidence suggests***
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	*Primary basis:* *Slight* evidence of attention and behavior effects in humans. *Human relevance, cross-stream coherence, susceptibility, and other inferences:* Evidence comes from studies in humans at a susceptible lifestage (*in utero* or childhood exposure).
<u>ADHD and related behaviors</u> 7 *medium* and 1 *low* confidence studies	• 5/6 studies examining behavioral issues and/or attention problems reported positive associations but the two studies examining ADHD diagnosis (the most clinically relevant outcome) reported inverse findings.	• *Consistency* in direction of association for studies of behavior and attention	• *Unexplained inconsistency* with studies of ADHD diagnosis • *Imprecision* in most study results	⊕⊙⊙ *Slight* There is some evidence of greater problem behaviors and decreased attention with increasing PFDA exposure but there is remaining uncertainty due to inconsistency and imprecision.	
<u>Other neurodevelopmental effects</u> 14 *medium* confidence studies	• Some studies reported decreases in cognition or motor scores, but findings were inconsistent across studies. No association was observed with ASD/social behavior or cerebral palsy.	• No factors noted	• Unexplained inconsistency		

3.2.8. ENDOCRINE EFFECTS

Human studies

Thyroid effects

Twenty-three studies examined thyroid hormones and PFDA exposure. A summary of the study evaluations is presented in Figure 3-77, and additional details can be obtained from HAWC. Two studies were considered *uninformative* and excluded from further analysis due to critical deficiencies in confounding and analysis (Seo et al., 2018) or serious deficiencies in several domains (Kim et al., 2011). Sixteen studies were classified as *medium* confidence and five studies were classified as *low* confidence (Liu et al., 2021b; Itoh et al., 2019; Zhang et al., 2018a; Ji et al., 2012; Bloom et al., 2010). Of the *medium* confidence studies, five were cross-sectional, nine were prospective cohorts, one was a retrospective cohort, and one was participants from a randomized clinical trial of energy-reduced diets (functionally equivalent to a prospective cohort).

In addition to the general considerations described in Section 1.2.2, there were several outcome-specific considerations for study evaluation that were influential on the ratings. First, for outcome ascertainment, collection of blood during a fasting state and at the same time of day for all participants (or adjustment for time of collection) is preferred for measurement of thyroid hormones to avoid misclassification due to diurnal variation (van Kerkhof et al., 2015). Studies that did not consider these factors (e.g., by study design or adjustment) were not excluded but were considered *deficient* for the outcome ascertainment domain. For participant selection, it was considered important to account for current thyroid disease and/or use of thyroid medications; studies that did not consider these factors by exclusion or another method were considered *deficient* for the participant selection domain. Concurrent measurement of exposure with the outcome was considered appropriate for this outcome since circulating hormone levels can change quickly in response to a change in exposure and the half-life of PFDA in humans is long. All the available studies analyzed PFDA in serum or plasma using appropriate methods (as described in the protocol). Thyroid hormones were analyzed using standard and well-accepted methods in all studies. Overall, while most studies were considered *medium* confidence, nearly all of them had limitations in outcome ascertainment and/or study sensitivity (primarily due to low PFDA exposure levels in the study populations). These ascertainment issues and other (non-differential) sources of measurement error are likely to bias the results towards the null, and thus null associations are difficult to interpret.

Figure 3-77. Study evaluation results for epidemiology studies of PFDA and thyroid effects. Refer to HAWC for details on the study evaluation review: HAWC Human Thyroid Effects

Multiple publications of single study: Berg et al. (2017) includes Berg et al. (2015). Aimuzi et al. (2019) and Aimuzi et al. (2020) examine the same birth cohort but are considered separately because the populations are different (neonates/cord blood in Aimuzi et al. (2019) and pregnant women in Aimuzi et al. (2020). These studies should not be considered fully independent.

Twelve studies examined associations with thyroid hormones in adults (11 for T4, 8 for T3, and 11 for TSH), including those focused on pregnant women. Results were mixed across studies of the same hormone, with no clear pattern to explain the inconsistency (e.g., study confidence or population characteristics). For T4 (both free and total), two *medium* confidence studies (Inoue et al., 2019; Blake et al., 2018) and one low confidence study (Liu et al., 2021b) in general population adults and one *medium* confidence study in pregnant women during early gestation (Aimuzi et al., 2020) reported small positive associations (higher levels with higher exposure), but these differences were imprecise (wide confidence intervals and p >0.05) and in Inoue et al. (2019), nonmonotonic across quartiles of exposure (Inoue et al. (2019): % difference (95% CI): Q2: 1.0 (−2.3, 4.3), Q3: 1.5 (−2.3, 5.4), Q4: −0.5 (−3.6, 2.7); Blake et al. (2018): 2.5% change, 95% CI:= −2.9, 8.3); Aimuzi et al. (2020): β (95% CI): 0.05 (-0.03, 0.13)). One *low* confidence study (Zhang et al., 2018a) in women with premature ovarian insufficiency (POI) reported non-statistically significant lower levels of free T4 with higher exposure (β (95% CI) = −1.19 (−2.66, 0.28)). The other seven available studies (six *medium* confidence) did not report a positive or negative association (Cakmak et al., 2022; Itoh et al., 2019; Reardon et al., 2019; Liu et al., 2018; Yang et al., 2016a; Berg et al., 2015; Wang et al., 2014a). For T3, two *medium* confidence studies in pregnant women reported positive associations (Aimuzi et al., 2020; Wang et al., 2014a), with statistical significance in one (Wang et al., 2014a), β (95% CI) = 0.002 (0, 0.003); Aimuzi et al. (2020): β (95% CI): 0.05 (-0.03, 0.13)). In contrast, three studies, one *medium* and two *low* confidence reported inverse associations (lower T3 with higher PFDA exposure) (Berg et al. (2015), mean differences vs. the Q1 (95% CI) referent: Q2: −0.04 (−0.08, 0.04), Q3: −0.05 (−0.08, 0), Q4: −0.10 (−0.14, −0.06); Liu et al. (2021c): % change (95% CI) per ln-unit increase in PFDA: -3.79 (-7.69, 0.27); and Zhang et al. (2018a) in women with POI, β (95% CI) = −0.56 (−1.27, 0.16)). Effect sizes in both directions were close to null. The remaining studies reported no association (Itoh et al., 2019; Reardon et al., 2019; Liu et al., 2018; Yang et al., 2016a). Of the 11 studies reporting on TSH, two *medium* and two *low* confidence studies reported higher TSH with higher exposure (Inoue et al. (2019): % difference (95% CI): Q2: −2.7 (−21.4, 20.6), Q3: 0.4 (−21.5, 28.5), Q4: 3.6 (−16.7, 28.8);Blake et al. (2018): 11% change, 95% CI: −4.5, 28.8 and Zhang et al. (2018a): β (95% CI) = 0.85 (−0.03, 1.72); Liu et al. (2021b): % change (95% CI) per ln-unit increase in PFDA: 9.53 (-6.15, 27.92)), but these estimates were imprecise (wide confidence intervals) and, in Inoue et al. (2019), were non-monotonic across quartiles of exposure. The results in Blake et al. (2018), which was the only study with repeated measures of TSH, were not robust as it changed to an inverse association when only the first exposure measurements were included in the model, rather than repeated measures. In addition, one *medium* confidence study reported a non-significant inverse association (Cakmak et al. (2022): % change (95% CI) for a one mean increase in PFDA: -7.0 (-17.2, 4.4)). The remaining studies reported no association between TSH and PFDA exposure (Aimuzi et al., 2020; Itoh et al., 2019; Reardon et al., 2019; Yang et al., 2016a; Berg et al., 2015; Wang et al., 2014a; Wang et al., 2013). In addition, two *medium* confidence studies (Kim et al.,

2020a; Kang et al., 2018) examined associations in children and adolescents and reported no association with free T4 or TSH (both studies) or T3 (Kim et al., 2020a).

Seven *medium* confidence studies and one *low* confidence study examined associations with thyroid hormones in neonates. For T4 (total or free), there were seven studies available, and only one reported an association; Liang et al. (2020) reported an inverse association with T4 but not total T4 (β (9% CI for ln-unit increase in PFDA: -5.07 (-9,78, -0.37)). The remaining studies reported no (Guo et al., 2021; Aimuzi et al., 2019; Itoh et al., 2019; Shah-Kulkarni et al., 2016; Yang et al., 2016a; Wang et al., 2014a). For total T3, two out of six *medium* confidence studies reported higher T3 with higher PFDA Shah-Kulkarni et al. (2016): β (95% CI) for ln-unit increase in PFDA: 2.4 (-0.27, 5.09), stronger association in girls (β = 3.93 vs 1.02); Liang et al. (2020): 0.06 (0.03, 0.09)). In contrast, one study reported lower T3 ($p < 0.05$) in boys with maternal thyroid antibody negative but higher T3 in boys with maternal thyroid antibody positive ($p > 0.05$) and girls (Itoh et al., 2019). The remaining three studies reported no association (Guo et al., 2021; Aimuzi et al., 2019; Yang et al., 2016a; Wang et al., 2014a). For TSH, eight studies were available. Three reported inverse associations between TSH and PFDA exposure, but in Itoh et al. (2019), this was observed only in boys with maternal thyroid antibody positive, while in Shah-Kulkarni et al. (2016), the association was observed only in girls and not statistically significant. The association was observed in the overall population in Wang et al. (2014a), but this was also not statistically significant. In addition, one study reported a positive association with TSH Liu et al. (2021b). The remaining studies reported no association (Guo et al., 2021; Aimuzi et al., 2019; Yang et al., 2016a; Berg et al., 2015). It is possible that the lack of consistency was due to differences in the timing of exposure measurement (maternal sampling at median 18 weeks in Berg et al. (2017), second trimester in Itoh et al. (2019), third trimester in Wang et al. (2014a), and 1–2 days before delivery in Yang et al. (2016a), and cord blood sampling in Shah-Kulkarni et al. (2016), Aimuzi et al. (2019), Guo et al. (2021); Liu et al. (2021b), but this is not possible to evaluate further due to the lack of multiple studies per sampling period other than cord blood.

Overall, the evidence for the association between PFDA exposure and thyroid effects in human studies is inconsistent. A few studies do suggest an association between thyroid hormones and PFDA exposure, but other studies are null, and the direction of association is not consistent across studies. Even in the studies that observed associations, there is not clear coherence across outcomes, where one would expect a decrease in T4 and T3 to correspond with an increase in TSH, or vice versa, though this could be explained by secondary hypothyroidism as discussed below. For most studies, the exposure levels were low (median exposure was less than 0.5 ng/mL) and there were narrow exposure contrasts, which along with potential for outcome misclassification in most studies, reduced the study sensitivity and could have impaired the ability of these studies to observe a true effect. However, this poor sensitivity would not explain the observed differences in the direction of association, and thus considerable uncertainty remains.

Animal studies

Two studies in the database of toxicity studies for PFDA evaluated endocrine effects. One study exposed female Sprague-Dawley rats for 28 days (0, 0.125, 0.25, and 0.5 mg/kg-day) and examined the adrenal glands (weight and histopathology) (Frawley et al., 2018). The second study examined the following endpoints in both male and female Sprague-Dawley rats after a 28-day gavage exposure (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day): thyroid hormone levels, histopathology, and organ weights (NTP, 2018).

Thyroid hormones levels

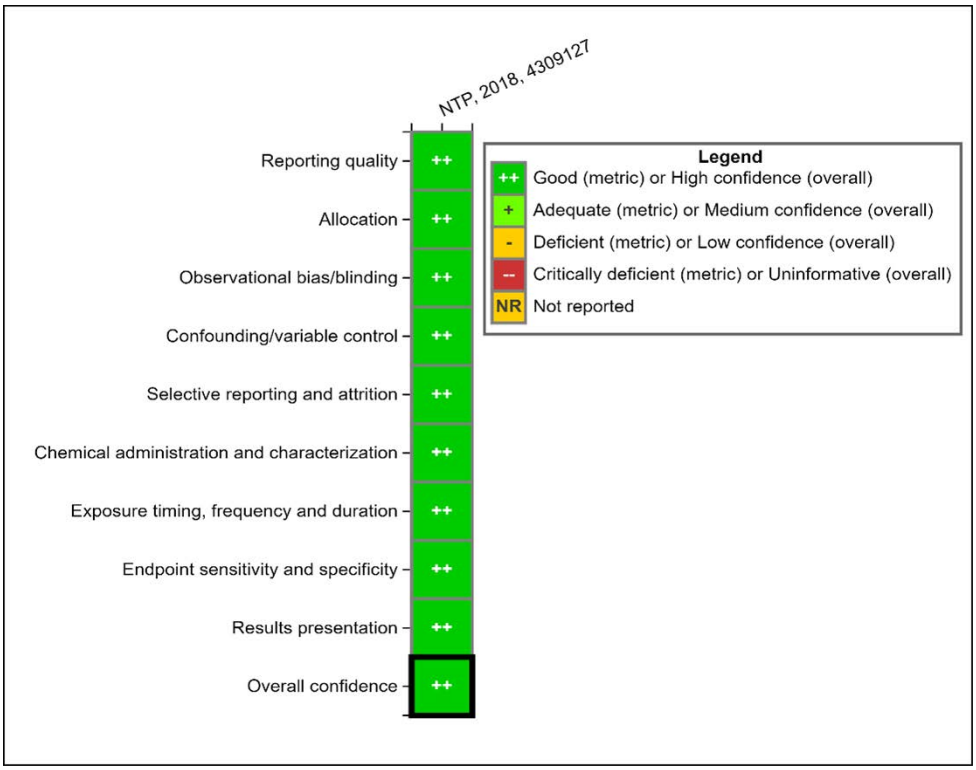


Figure 3-78. Thyroid hormone levels animal study evaluation heatmap. Refer to HAWC for details on the study evaluation review.

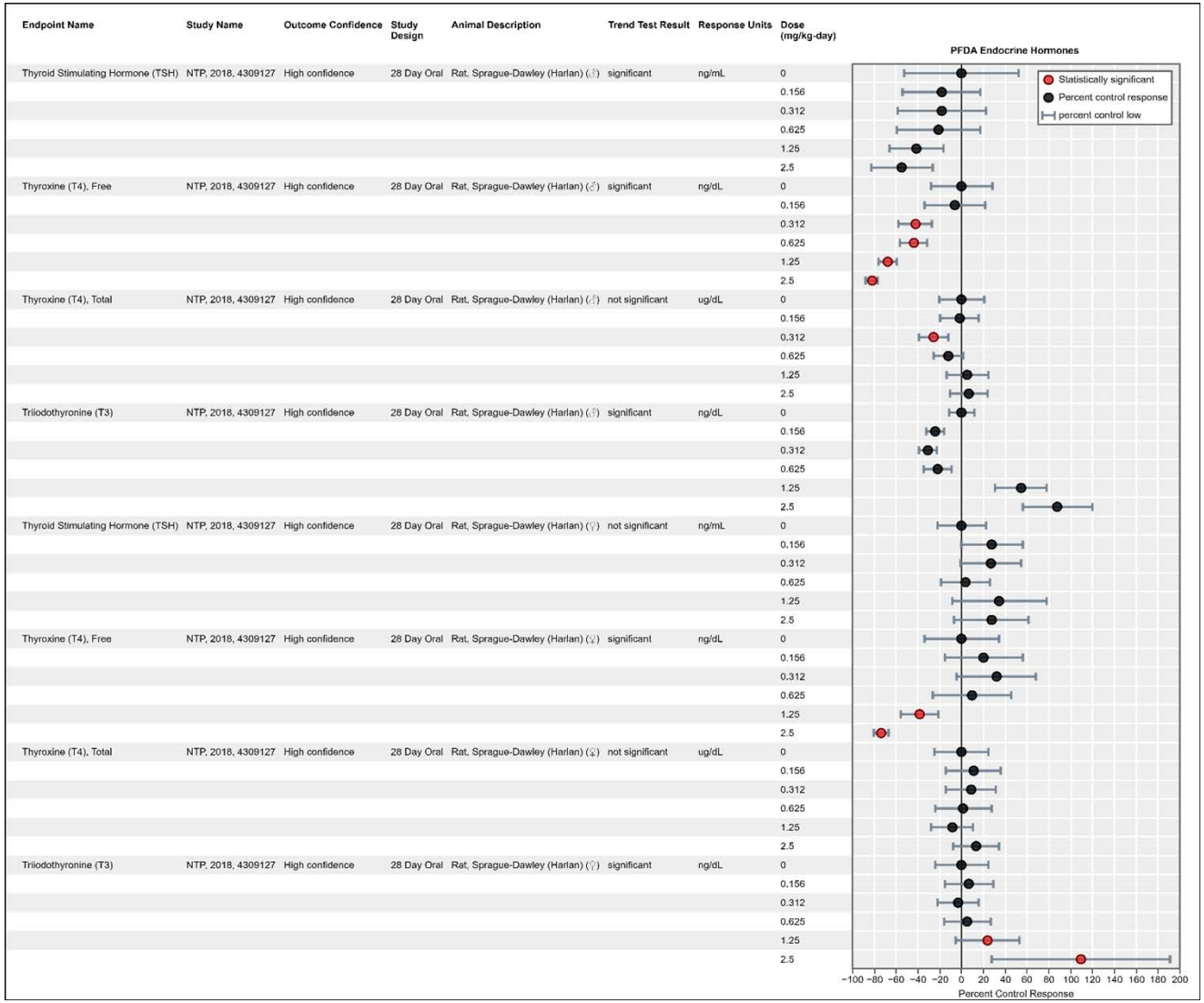
In the NTP (2018) study which was considered *high confidence* (see Figure 3-79), thyroid hormones were measured in male and female rats exposed to 0–2.5 mg/kg-day for 28 days (see Figure 3-79 and Table 3-41). For thyroid-stimulating hormone (TSH), a statistically significant decreasing trend (18 to 55%) was observed in male rats, but a significant decrease compared to controls was not reported at any dose. No statistically significant change for TSH was observed in the female rats but increases ranged from 3 to 35% with the lowest effect occurring at 0.625 mg/kg-day. A statistically significant increasing trend was reported for T3 in male (22 to 88%) and female rats with significant increases (24–109%) reported at ≥1.25 mg/kg-day for females only. A statistically significant decreasing trend in free thyroxine (fT4) was reported in

male and female rats with significant decreases at ≥0.312 mg/kg-day in males (42–82%) and at ≥1.25 mg/kg-day in females (39–74%). A statistically significant decrease in total thyroxine (tT4) was observed in males only at 0.312 mg/kg-day and was unchanged in females at all doses. fT4 is the preferred measurement over tT4 in adult animals given that the level of tT4 can be dependent on the amount of serum binding proteins while fT4 is available to be utilized by the body. The effects of PFDA on fT4 and TSH in male and female rats are consistent with secondary hypothyroidism, which is characterized by decreased T4 and decreased or normal levels of TSH (Lewiński and Stasiak, 2017). However, there is uncertainty in this conclusion given that changes in fT4 and T3 are often expected to occur in the same direction, with T3 being the more active hormone form and formation of T3 contingent upon the deiodination of fT4. The potential mechanism and interpretation for an observation of decreasing fT4 with increasing T3 is unknown and unexamined in the PFDA evidence base.

Table 3-40. Percent changes relative to controls in thyroid hormone levels in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	**0.312**	**0.625**	**1.25**	**2.5**
Thyroid-stimulating hormone (TSH)					
Female S-D rats	28	27	3	35	27
Male S-D rats	−18	−18	−22	−41	−55
Triiodothyronine (T3)					
Female S-D rats	7	−4	5	**24**	**109**
Male S-D rats	−24	−31	−22	54	88
Free thyroxine (fT4)					
Female S-D rats	20	32	10	**−39**	**−74**
Male S-D rats	−6	**−42**	**−44**	**−68**	**−82**
Total thyroxine (tT4)					
Female S-D rats	11	9	1	−9	13
Male S-D rats	−2	**−26**	−12	5	7

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



Endpoint Name	Study Name	Outcome Confidence	Study Design	Animal Description	Trend Test Result	Response Units	Dose (mg/kg-day)
Thyroid Stimulating Hormone (TSH)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/mL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Free	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Total	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant	ug/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Triiodothyronine (T3)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroid Stimulating Hormone (TSH)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant	ng/mL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Free	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Thyroxine (T4), Total	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant	ug/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5
Triiodothyronine (T3)	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant	ng/dL	0
							0.156
							0.312
							0.625
							1.25
							2.5

Figure 3-79. PFDA thyroid hormone levels after short-term oral exposure (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100000026/pfda-endocrine-hormones/).

Histopathology

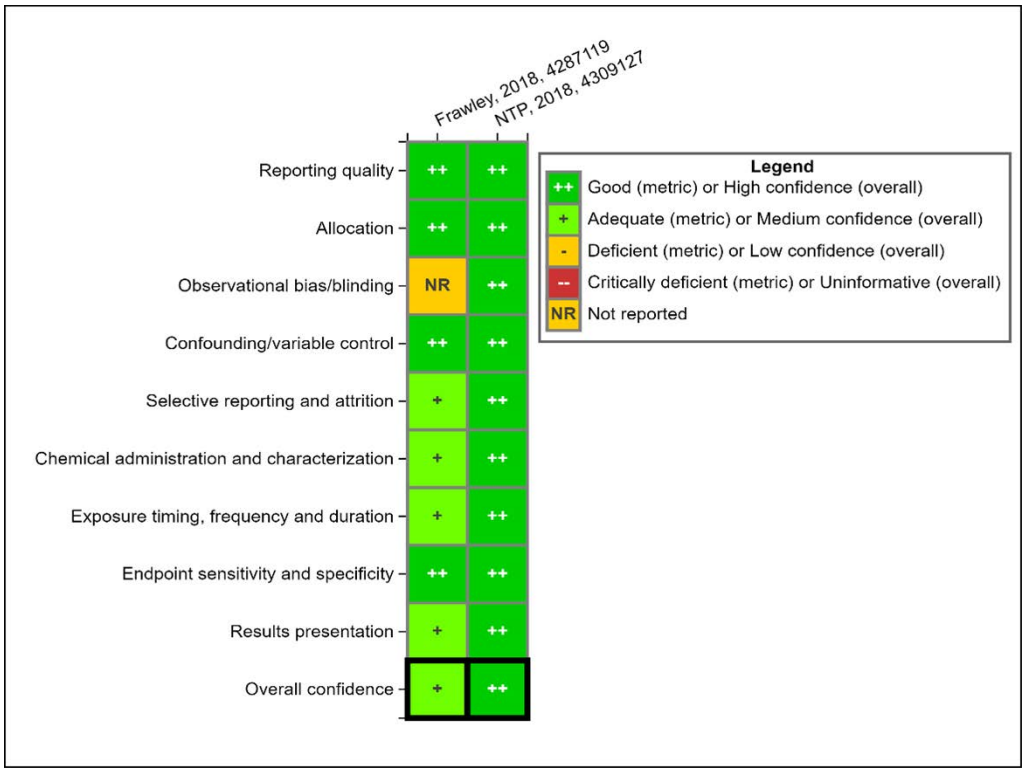


Figure 3-80. Endocrine histopathology animal study evaluation heatmap.
Refer to HAWC for details on the study evaluation review.

Both the NTP (2018) and Frawley et al. (2018) studies performed histopathological examinations to examine PFDA-related effects. The NTP (2018) study was considered *high confidence* while the Frawley et al. (2018) study was evaluated as *medium confidence* due to incomplete reporting of the null data (see Figure 3-80). NTP (2018) performed histopathological examination of the thyroid gland, adrenal cortex and medulla, parathyroid gland, and pituitary gland in both male and female rats (NTP, 2018). Histopathology was examined for the thyroid gland at all doses; all other endocrine tissues were examined only in the control and high-dose (2.5 mg/kg-day) groups. NTP (2018) reported that there were no tissue changes observed in any of the examined organs in either sex (see Figure 3-81). Results from the histopathological examination of the adrenal glands in female rats from the Frawley et al. (2018) were qualitatively reported as being unchanged by PFDA exposure (Frawley et al., 2018).

Endpoint Name	Study Name	Outcome Confidence	Exposure Design	Species, Strain (Sex)	Trend Test Result
Adrenal Gland Histopathology	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Parathyroid Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Pituitary Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant
Thyroid Gland Histopathology	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				Rat, Sprague-Dawley (Harlan) (♀)	not significant

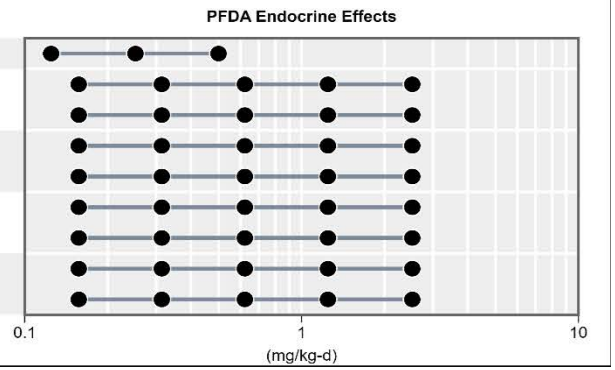


Figure 3-81. PFDA endocrine histopathology (results can be viewed by clicking the HAWC link: details: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/PFDA-Endocrine-Histopath-Animal/).

Organ weight

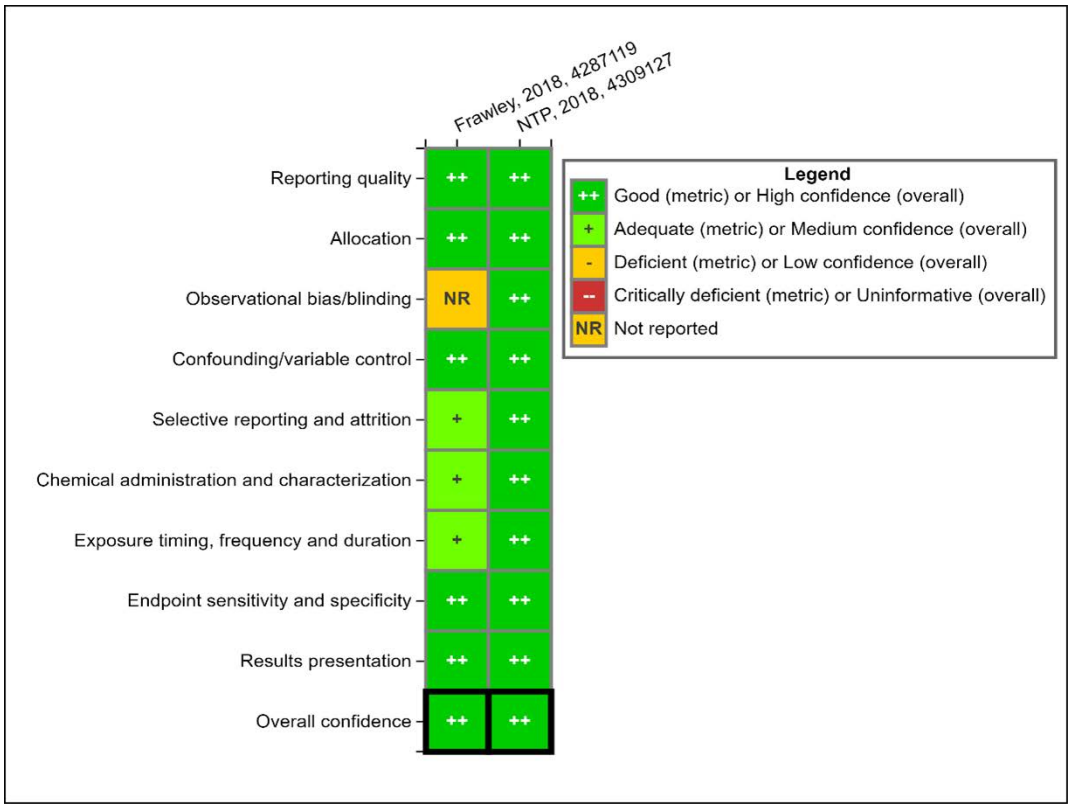


Figure 3-82. PFDA endocrine organ weights animal study evaluation heatmap. Refer to HAWC for details on the study evaluation review.

Both the NTP (2018) and Frawley et al. (2018) studies evaluated PFDA effects on endocrine organ weights and were considered *high* confidence for this outcome (see Figure 3-82). As indicated above, both studies measured adrenal weights. Only the NTP (2018) study measured thyroid weight; both sexes in rats demonstrated a statistically significant trend in relative thyroid weight with statistically significant increases reported at ≥1.25 mg/kg-day in male rats (43% at both 1.25 and 2.5 mg/kg-day) and at ≥0.312 mg/kg-day in female rats (27–45%). For absolute thyroid weight in male rats, there was no significant trend, and no significant change was observed at any dose tested. In female rats, there was no significant trend but significant increases (33–34%) were observed at doses ranging from 0.312 to 1.25 mg/kg-day but not at the highest dose tested (2.5 mg/kg-day). Relative (to body weight) thyroid weight is the preferred measure for this organ particularly in the presence of body weight changes (Bailey et al., 2004). Significant reductions in body weight were observed in the NTP (2018) study at the two highest doses tested (≥1.25 mg/kg-day; refer to the Section 3.2.10 on General toxicity effects for additional details). For adrenal weight in female rats, no significant changes were observed in rats at doses up to 1.25 mg/kg-day in both studies. A statistically significant decrease (36%) for absolute adrenal gland weight was observed at the highest dose group (2.5 mg/kg-day) in female rats from the NTP

(2018) study; no change was reported for relative adrenal weight in females in this study. A statistically significant decrease (15–21%) was reported in absolute adrenal gland weight in male rats at all dose groups. Conversely, relative adrenal weight in males was significantly increased (50%) at the highest dose tested (NTP, 2018). The toxicological significance of the adrenal organ-weight changes is unclear; the opposing direction of absolute and relative organ-weight changes suggests a confounding effect of body-weight changes (refer to the General toxicity section for more detail on body-weight effects) at the same doses. Furthermore, No PFDA-induced histopathological changes on the adrenal gland were observed (see discussion above).

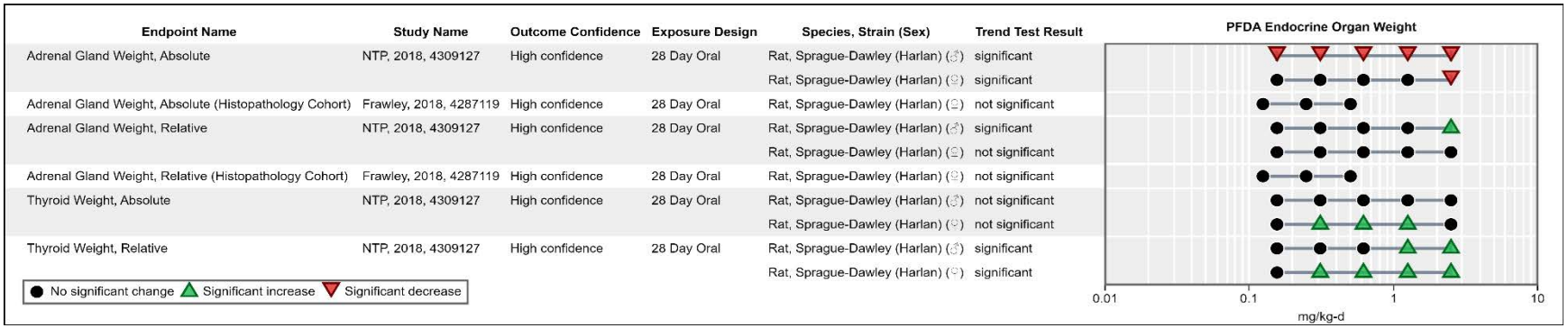


Figure 3-83. PFDA endocrine organ weight (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/PFDA-Endocrine-Organ-Weight/).

Mechanistic studies and supplemental information

In support for PFDA-induced changes on thyroid hormones observed in rats from the 28-day NTP (2018), structurally related PFAS (e.g., PFNA; PFOA) have been shown to effect thyroid hormone levels in rodents. Specifically, PFNA induced hypothyroxinemia in rodents. Hypothyroxinemia has been defined in humans as a low percentile value of serum free T4 (ranging from the 2.5th percentile to the 10th percentile of free T4), with a TSH level within the normal reference range (Alexander et al., 2017).

Additionally, multiple high-dose, intraperitoneal (i.p.) injection studies have demonstrated that PFDA affects T3 and T4 serum levels. Specifically, decreases in serum T4 have been repeatedly observed in rats exposed to PFDA via i.p. injection at doses ranging from 20 to 80 mg/kg (Gutshall et al., 1989, 1988; Van Rafelghem et al., 1987a; Langley and Pilcher, 1985). Evaluations of PFDA effects on T3 varied among i.p. studies in rats. Langley and Pilcher (1985) observed an initial significant decrease in T3 levels starting at 12 hours post PFDA exposure (75 mg/kg i.p.) as compared to pair-fed controls, which remained significantly decreased until day 4 of the study. Following day four of the study, there were no significant differences in T3 serum levels between pair-fed and PFDA-exposed animals, while serum T4 levels remained significantly diminished through day eight of the study as compared to the pair-fed controls. Gutshall et al. (1989) also reported significant decreases in T3 at 75 mg/kg i.p. in rats at 12 and 24 hours after PFDA exposure. Conversely, no changes in T3 were observed in rats exposed via i.p. to PFDA at doses up to 80 mg/kg-day (Gutshall et al., 1988; Van Rafelghem et al., 1987a). However, the inconsistencies in PFDA effects on T3 levels could be due to differences in experimental design and the time at which thyroid hormones were measured. In the studies that showed no effect on T3 levels in rats (Gutshall et al., 1988; Van Rafelghem et al., 1987a), thyroid hormones were measured at 7 and 14 days after PFDA treatment compared to the positive studies that showed effects on hormone levels at 12 to 48 hours after exposure.

Under normal physiologic conditions, neurons in the hypothalamus release thyroid releasing hormone (TRH) to stimulate thyrotrophs of the anterior pituitary gland to release thyroid stimulating hormone (TSH). TSH plays a number of important metabolic functions including stimulation of the thyroid gland to release triiodothyronine (T3) and thyroxine (T4). When increased T3 and T4 serum levels reach above a certain blood concentration threshold, secretion of TRH from the hypothalamus is inhibited via a negative feedback loop.

To evaluate whether PFDA altered the ability of the pituitary and thyroid glands of the PFDA exposed animals to respond to a physiological stimulation, Gutshall et al. (1989) challenged male Wistar rats with 500 µg/kg TRH at 15 or 22 hours post a single, high-dose 75 mg/kg (i.p.) PFDA exposure and found that although the percent response changes in T4 and T3 compared to baseline (i.e., pre-TRH challenge) were similar between the control and PFDA exposed animals, the absolute values for T4 and T3 in the sera from PFDA exposed animals was significantly less than that of their control counterparts following TRH stimulation (Gutshall et al., 1989). These data

indicate that PFDA may alter the ability of the glands in the hypothalamic-pituitary-thyroid axis (HPT) to respond to physiological stimulation (Gutshall et al., 1989). Additional studies would help clarify whether this observation is relevant in other species and at lower, more physiologically relevant levels of PFDA exposure. Impaired responsiveness of the hypothalamic-pituitary-thyroid axis to hormonal stimulation could explain the results from the 28-day study in rats (NTP, 2018) in which TSH and T4 were both decreased by PFDA exposure in male rats; this mechanistic information does not however provide insight on why T3 was increased in the presence of decreased TSH and T4.

Additionally, the high dose, i.p. study by Gutshall et al. (1989) showed that PFDA is able to displace T4 from plasma proteins (Gutshall et al., 1989). The fate of the displaced (i.e., free) T4 is unknown, but the authors postulated increased biliary excretion may be a potential route of T4 loss. Using a fluorescence displacement assay, Ren et al. (2016) reported that PFDA binds to transthyretin, a major transport protein for thyroid hormone, with the potential to displace T4 from the transport protein in occupational exposure settings. It is unclear how these mechanistic data which indicate that PFDA decreases protein binding of T4, support the PFDA-induced effects on thyroid hormone homeostasis observed in rats from the NTP (2018) study. A decrease in protein binding of T4 could result in increased fT4 (unbound form) and a decrease in tT4 (bound form). Conversely, decreased fT4 was observed in rats while tT4 was decreased only at the mid-dose in males and unchanged in female rats exposed to PFDA (NTP, 2018). Interestingly, evaluation of unsaturated binding capacity of thyroid-binding proteins, measured by T3 uptake analysis showed that T3 uptake was significantly reduced in the 80 mg/kg PFDA exposed animals as compared to the pair-fed controls (Van Rafelghem et al., 1987a). Under in vitro conditions, Ren et al. (2015) reported binding of PFDA to the human thyroid receptor but that PFDA did not exhibit antagonistic or agonistic effects on the thyroid receptor pathway (Ren et al., 2015).

Kelling et al. (1987) sought to determine the effects of PFDA on the thyroid by evaluating the hepatic activities of L-glycerol-3-phosphate dehydrogenase, malic enzyme, and glucose-6-phosphate dehydrogenase, which are enzymes that are sensitive to thyroid status. The activity of these enzymes is increased during hyperthyroidism and decreased during hypothyroidism (Mariash et al., 1980). Similar to the study performed by Langley and Pilcher, SD male rats received a single, high dose i.p. injection of either 20, 40, or 80 mg/kg PFDA and then hepatic subcellular fractions were prepared following euthanasia. These hepatic fractions were then used to assay the activity of L-glycerol-3-phosphate dehydrogenase, lactate dehydrogenase, malic enzyme, and glucose-6-phosphate dehydrogenase. PFDA significantly increased the activity of L-glycerol-3-phosphate dehydrogenase, cytosolic lactate dehydrogenase and cytosolic malic enzyme as compared to their pair-fed and ad libitum controls indicating that the increase of enzyme activity is a direct result of PFDA exposure and not a secondary effect caused by decreased food intake and subsequent loss in body weight (Kelling et al., 1987). Similar effects of PFDA on L-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme in rats were also reported by

Gutshall et al. (1989). There was no significant difference in glucose-6-phosphate dehydrogenase activity, hepatic DNA content or protein content. These data indicate that while evidence such as decreased serum T4 in rats exposed to PFDA is suggestive of a lessened thyroid state, the activation of thyroid sensitive enzymes is increased in rats exposed to PFDA.

Overall, there is uncertainty in the relevance of the mechanistic studies and supplemental information to the thyroid effects observed in rats from the NTP (2018) study. Specifically, the doses from these studies (20–80 mg/kg) are much higher than those used in the 28-day study (0.156–2.5 mg/kg-day) (NTP, 2018) and have been shown to cause overt systemic toxicity including a "wasting syndrome" (refer to the General toxicity section), which could confound the interpretation of the mechanistic data. Additionally, the mechanistic studies and supplemental information are shorter duration in which rats were exposed to PFDA via i.p. injection rather than gavage as was done in the NTP (2018) study. Furthermore, a data gap exists because there are no mechanistic studies available that determined the effect of PFDA on the activities of deiodinases, which convert free T4 to T3. Data on how PFDA might affect deiodinase activity could inform the mechanism by which PFDA was observed to decrease fT4 while increasing T3 in rats from the NTP (2018) study.

Evidence Integration

There is *indeterminate* evidence of an association between PFDA exposure and endocrine related effects in studies of exposed humans. The evidence is largely null, but there are concerns for study sensitivity. The observed associations are inconsistent across studies and not coherent across thyroid hormones.

There is *indeterminate* animal evidence of endocrine toxicity- specifically, thyroid effects, with PFDA based on incoherent evidence from a single *high* confidence short term study in rats (a second short term study examined adrenal effects). PFDA was shown to cause changes in thyroid hormone levels, some of which may be interpreted as suggestive of secondary hypothyroidism, a phenotype characterized by decreased T4 and decreased or normal levels of TSH (Lewiński and Stasiak, 2017); however, the PFDA data are not entirely coherent with such a hypothesis. Specifically, in the NTP (2018) study, significant trends were reported for decreased TSH and fT4 (but not tT4) in male rats at ≥0.312 mg/kg-day, while significant trends were also reported for increased T3 (the latter findings are not coherent with hypothyroidism). Likewise, in females, increased T3 and decreased fT4 was observed at ≥1.25 mg/kg-day. High dose PFDA exposure-induced decreases in total T4 were consistently observed in multiple, high dose i.p. studies in rats. The cause of secondary hypothyroidism is thought to be due to impaired responsiveness of the hypothalamus-pituitary-thyroid axis (Lewiński and Stasiak, 2017). Consistent with this, PFDA was shown to impair the response of the hypothalamic-pituitary-thyroid axis to TRH stimulation in rats from a high dose i.p. study (Gutshall et al., 1989). These data provide mechanistic insight and biological plausibility for how PFDA could be decreasing serum levels of T4. Furthermore, there was coherence with increased relative thyroid weight and decreased fT4 serum levels at

≥1.25 mg/kg-day in male and female rats. A previous study observed increased relative thyroid weight in a rat model of methimazole-induced hypothyroidism (Soukup et al., 2001). Also, an enlarged thyroid is a symptom of hypothyroidism in humans (IQEHC, 2014). In support for PFDA-induced changes on thyroid hormone homeostasis, structurally related PFAS compounds (e.g., PFNA; PFOA) have been shown to effect thyroid hormone levels in rodents. However, several aspects of the available animal data decrease the strength or certainty of the evidence informing thyroid effects, which was only available from a single oral exposure study. Whereas the NTP (2018) study reported changes in fT4 and TSH in rats that may indicate secondary hypothyroidism, there was an increase in T3 that cannot be explained. Furthermore, there are no mechanistic studies that determined the effect of PFDA on deiodinase activity that could offer insight on how PFDA decreased fT4 and TSH while increasing T3. Additionally, while fT4 was decreased in male and female rats from the NTP (2018) study, a consistent decrease in tT4 was not observed. However as noted above, fT4 not tT4 is the preferred measure in adult animals. Whereas there was potential coherence between decreased fT4 and increased thyroid weight in rats, it is unclear how thyroid weight and T3 were increased in the absence of increased TSH or histopathological changes.

Uncertainty is also associated with the mechanistic studies and supplemental information. Specifically, inconsistent results were observed for effects on T3 in rats exposed to PFDA via i.p. injection and results from the protein binding studies (Gutshall et al., 1989) suggest that PFDA decreased protein binding of T4, which could result in increased fT4 and decreased tT4, which is not consistent with the results from the NTP (2018) study. The mechanistic database is also limited in that there are no studies that investigated the effects of PFDA on deiodinase activity. Furthermore, the activities of thyroid-sensitive hepatic enzymes (e.g., L-glycerol-3-phosphate dehydrogenase) were increased in rats exposed to PFDA via the i.p. route suggesting that thyroid activity may not be decreased due to PFDA treatment. In general, the interpretation and relevance of the mechanistic studies and supplemental information to thyroid effects observed in the NTP (2018) study is unclear given that these studies used doses that were much higher (i.e., 20–80 mg/kg-day, as compared to ≤2.5 mg/kg-day) and associated with overt systemic toxicity. Additionally, the mechanistic studies and supplemental information are of shorter duration and rats were exposed to PFDA via i.p. injection rather than gavage as was done in the NTP (2018) study.

In addition to the uncertainty in the available evidence in adults, due to the sparse evidence base available, concern remains for potential susceptible populations to PFDA-induced endocrine effects in susceptible populations including young individuals exposed during gestation, early childhood, and puberty. Importantly, T3 and T4 levels play critical roles in bone growth and brain development (O'Shaughnessy et al., 2019) at these various life stages. However, at the present time few epidemiological studies and no animal toxicological studies have addressed the potential for PFDA-induced effects in these populations. A primary delineating feature between adult animals

and developing offspring is that adults have a considerable reserve thyroid hormone capacity whereas developing offspring do not. Thus, there is an elevated concern regarding the potential for decreases in thyroid hormones during developmental life stages due to the critical endocrine dependency of in utero and neonatal development.

Taken together, there is ***inadequate evidence*** across human, animal, and mechanistic data to determine whether PFDA exposure would cause endocrine effects in humans. This conclusion is based on inconsistent evidence from human studies and from a single high confidence rat study investigating PFDA doses ≤2.5 mg/kg-day that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and T4; increased thyroid weight; no histopathology) that cannot be interpreted based on the currently available evidence base.

Table 3-41. Evidence profile table for PFDA exposure and endocrine effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.6: Human studies)					⊙⊙⊙ *Inadequate Evidence* *Primary basis:* Single *high* confidence study in rats showing mixed effects on thyroid hormone levels that cannot be reliably interpreted. *Human relevance:* Given the general conservation of thyroid function across rodents and humans, evidence in animals is presumed relevant to humans in the absence of evidence to the contrary. *Cross-stream coherence:* No factors noted. *Susceptible populations and lifestages:* None identified, as a hazard is not supported by the current evidence. *Other inferences:* None
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgement**	
Thyroid hormones 16 *medium* and 5 *low* confidence studies	• Results from studies of thyroid hormones were inconsistent. Most results were null, but study sensitivity was limited which hinders interpretation. Positive and inverse associations were observed in a few studies, but there was a lack of *consistency* of direction of association across studies.	• No factors noted	• Unexplained inconsistency • *Incoherence* in direction of association across hormones • Lack of association in studies with limited sensitivity	⊙⊙⊙ *Indeterminate* While a subset of studies suggests changes in thyroid hormone levels with higher levels of PFDA, there is considerable uncertainty due to inconsistency across studies and endpoints.	
Evidence from in vivo animal studies (see Section 3.2.6: Animal studies)					
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream summary**	
Thyroid hormones 1 *high* confidence study	• Significantly decreased trend for TSH in males. • Significant increased trend for T3 in males. • Increased T3 in females at ≥1.25 mg/kg-d. • Decreased fT4 was reported at ≥0.312 mg/kg-d in males	• *Consistency* for decreased fT4 in male and female rats in a *high* confidence study. • *Dose-response gradient* for decreased TSH (males only), decreased fT4 (males and females), and	• *Lack of expected coherence* across thyroid measures (the pattern of changes is inconsistent with any currently available understanding of adverse thyroid-related changes)	⊙⊙⊙ *Indeterminate* There is mixed evidence from a single *high* confidence rat study that reported largely incoherent effects on thyroid hormone homeostasis and thyroid structure (i.e., increased T3, decreased TSH and fT4, but not tT4;	

Evidence stream summary and interpretation					Evidence integration summary judgment
	and at ≥1.25 mg/kg-d in females. • No change in tT4	increased T3 (males and females). • Supportive evidence for decreased fT4 from supplemental (mechanistic and i.p.) studies. •	• Unexplained inconsistency across T4 (free and total) measurements	increased thyroid weight; no histopathology) that cannot be reliably interpreted based on the currently available evidence base.	
<u>Histopathology</u> 1 *high* confidence study and 1 *medium* confidence study	• No PFDA-induced histopathological changes were observed for the thyroid gland, adrenal cortex and medulla, parathyroid gland, and pituitary gland.	• No factors noted.	• No factors noted		
<u>Organ weights</u> 2 *high* confidence studies	• Decreased absolute adrenal gland weight in males at ≥0.156 mg/kg-d and females at 2.5 mg/kg-d (NTP, 2018). • Increased relative adrenal gland weight in males at 2.5 mg/kg-d (NTP, 2018). • Increased absolute thyroid in females at 0.312 to 1.25 mg/kg-d but not at the highest	• *Consistency* for increased relative thyroid weight in male and female rats across two *high* confidence studies. • *Dose-response gradient* for decreased absolute adrenal gland weight (males and females), increased relative adrenal gland weight	• No factors noted		

Evidence stream summary and interpretation					Evidence integration summary judgment
	dose tested (2.5 mg/kg-d) (NTP, 2018). • Increased relative thyroid weight in males at ≥1.25 mg/kg-d and females at ≥0.312 mg/kg-d (NTP, 2018).	(males only), and increased relative thyroid weight (males and females). • *Coherence* of increased thyroid weight and decreased fT4.			
Mechanistic evidence and supplemental information (see subsection above)					
Biological events or pathways (or other information)	**Primary evidence evaluated Key findings, interpretation, and limitations**			**Evidence stream summary**	
Hypothalamic-pituitary-thyroid axis	*Interpretation:* The results suggest that PFDA may impair the ability of the hypothalamic-pituitary-thyroid axis to respond to physiological stimulation. *Key findings:* • Decreased T3 and T4 levels after TRH stimulation in vivo. *Limitations:* one-time i.p. exposure; single study.			The mechanistic and supplementary data provide limited, inconsistent information on how PFDA may be affecting thyroid hormone homeostasis, and the results may be confounded by overt systemic toxicity due to the high doses used in the i.p. studies.	
Plasma protein binding	*Interpretation:* The results suggest that PFDA may impair the binding of thyroid hormones to plasma transport proteins. *Key findings:* • PFDA decreased the plasma protein uptake of T3 and T4. *Limitations:* one-time i.p. exposure; few studies.				
Activity of thyroid sensitive hormones	*Interpretation:* The data indicate activation of thyroid sensitive enzymes in a manner that suggests PFDA increases thyroid activity in rats. *Key findings:* • PFDA increased the activities of L-glycerol-3-phosphate dehydrogenase, cytosolic lactate dehydrogenase and cytosolic malic enzyme, which are thyroid-sensitive hormones. *Limitations:* one-time i.p. exposure; few studies.				

Evidence stream summary and interpretation			Evidence integration summary judgment
Binding to thyroid receptor	*Interpretation:* PFDA is capable of binding to the thyroid hormone receptor. *Key findings:* Under in vitro conditions, PFDA was shown to bind to the human thyroid hormone receptor. PFDA did not exhibit antagonistic or agonistic effects on the thyroid receptor pathway. Limitations: Single study available.		
Other evidence	*Interpretation:* Effects after i.p. injection is consistent with results in orally exposed rats. *Key findings:* Altered T3 and T4 levels. *Limitations:* Effects on T3 levels were inconsistent among the i.p. studies, which could be due to differences in experimental design and the time at which thyroid hormones were measured		

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3.2.9. URINARY EFFECTS

Human Studies

Nine epidemiology studies (14 publications) investigated the relationship between PFDA exposure and urinary effects, including glomerular filtration rate (GFR) and uric acid (see Figure 3-84). Two studies were considered uninformative due to lack of consideration of potential confounding (Zhang et al., 2019; Seo et al., 2018). The remaining studies were classified as low confidence primarily due to concerns for reverse causality (with potential for bias away from the null). In essence, as described in Watkins et al. (2013), decreased renal function could plausibly lead to higher levels of PFAS (including PFDA) in the blood due to reduced excretion. This hypothesis is supported by data presented by Watkins et al. (2013), though there is some uncertainty in their conclusions due to the use of modeled exposure data as a negative control and the potential for the causal effect to occur in addition to reverse causality. The results least likely to be affected by reverse causality were analyses in two studies stratified by glomerular filtration stage, Jain (2019); (Zeng et al., 2019c) and one study with a prospective design Blake et al. (2018).

Three studies (Lin et al., 2020b; Blake et al., 2018; Qin et al., 2016) reported associations between PFDA exposure and impaired renal function (i.e., lower GFR, higher serum uric acid), though only Blake et al. (2018) was statistically significant and the associations in Qin et al. (2016) and Lin et al. (2020b) were limited to one sex (girls in Qin et al. (2016) and men in Lin et al. (2020b)) (see Table 3-43). Conversely, Wang et al. (2019) reported higher GFR and lower odds of chronic kidney disease with higher exposure. The remaining studies report null associations with renal function, including the studies that stratified by glomerular function stage. Overall, there is unexplained inconsistency in the direction of the association. More importantly, because of the potential for reverse causation for this outcome, there is considerable uncertainty in interpreting the available evidence.

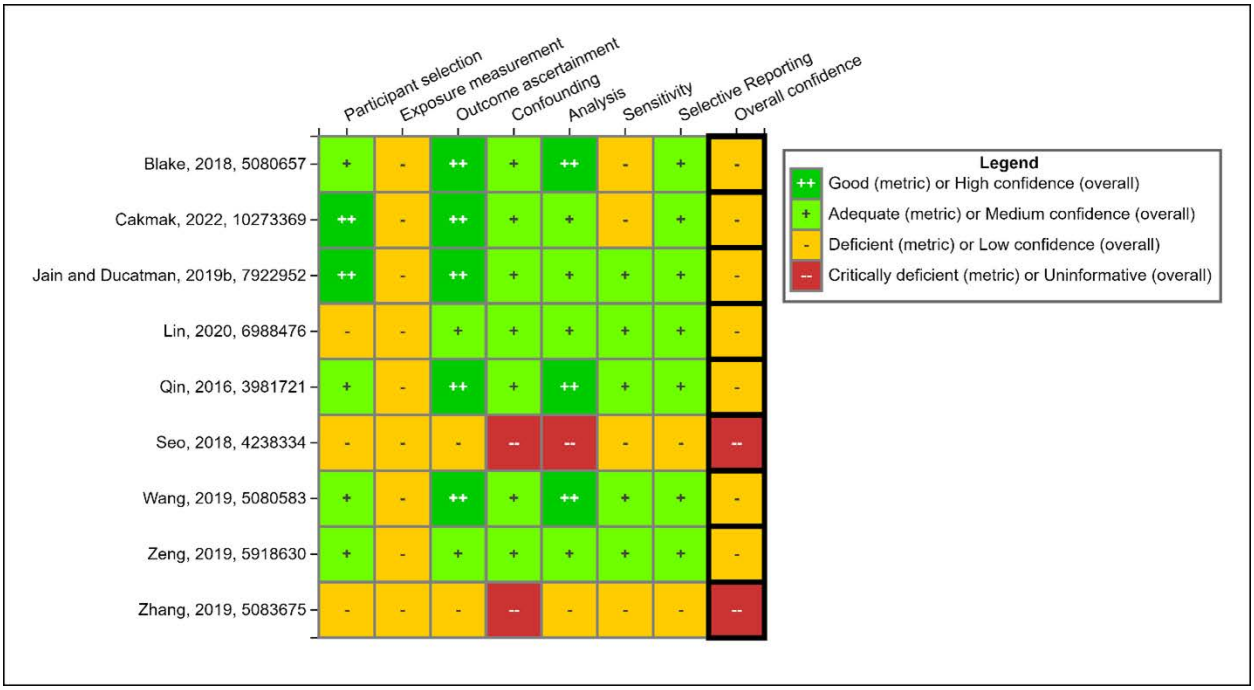

Figure 3-84. Urinary effects human study evaluation heatmap. Refer to HAWC for details on the study evaluation review: HAWC Human Urinary Effects.

Table 3-42. Associations between serum PFDA and urinary effects in *low* confidence epidemiology studies

Reference	Population	Median exposure (IQR) (ng/mL)	Result			
Glomerular filtration rate						
Blake et al. (2018)	Prospective cohort of residents near a uranium processing site (1990–2008); U.S.; 210 adults	0.1 (0.1–0.2)	Percent change (95% CI) in eGFR per IQR change in PFDA **−2.2 (−4.3, −0.1) ***			
Jain (2019)	Cross-sectional study (NHANES) (2007–2014); U.S.; 4,057 adults	0.2 in GF-1 group	Adjusted geometric mean (95% CI) by glomerular function stage			
			GF stage	All participants	Men	Women
			GF-1	0.25 (0.24, 0.26)	0.26 (0.25, 0.28)	0.23 (0.22, 0.24)
			GF-2	0.27 (0.25, 0.29)	0.28 (0.26, 0.31)	0.26 (0.24, 0.28)
			GF3-A	0.33 (0.26, 0.43)	0.31 (0.25, 0.38)	0.37 (0.35, 0.39)
			GF-3B/4	0.23 (0.19, 0.28)	0.21 (0.21, 0.22)	0.24 (0.19, 0.31)
Wang et al. (2019)	Cross-sectional study (2015–2016); China; 1,612 adults	0.9 (0.5, 1.5)	Mean change (95% CI) in eGFR per ln-unit change in PFDA **1.04 (0.27, 1.81) ***			
Uric acid						
		Mean (SD)	β (95% CI) in serum uric acid for quartiles vs Q1			

Reference	Population	Median exposure (IQR) (ng/mL)	Result		
Scinicariello et al. (2020)	Cross-sectional study (NHANES) (2009–2014); U.S.; 4,917 adults	0.2 (0.01)	Without chronic kidney disease Q2: 0.00 (-0.09, 0.10) Q3: -0.05 (-0.17, 0.07) Q4: 0.12 (0.00, 0.24) With chronic kidney disease Q2: 0.34 (-0.03, 0.72) Q3: 0.19 (-0.13, 0.52) Q4: 0.26 (-0.09, 0.61)		
			OR (95% CI) in hyperuricemia for quartiles vs Q1 Without chronic kidney disease Q2: 0.94 (0.66, 1.34) Q3: 0.86 (0.57, 1.25) Q4: 1.30 (0.94, 1.80) With chronic kidney disease Q2: 1.32 (0.66, 2.65) Q3: 0.98 (0.60, 1.61) Q4: 1.26 (0.64, 2.46)		
Zeng et al. (2019c)	Cross-sectional study (2015–2016); China; 384 adults	0.9 (0.5-1.5)	Mean difference per log-unit change in PFDA 0.01 (-0.06, 0.08)		
Qin et al. (2016)	Cross-sectional study (2009–2010); Taiwan; 225 children and adolescents (mean age: 13.6 yr)	0.9 (0.8–1.2)	Mean change (95% CI) in serum uric acid per ln-unit change in PFDA		
			All participants 0.08 (−0.11, 0.28)	Boys 0.05 (−0.23, 0.34)	Girls 0.18 (−0.09, 0.46)
			OR (95% CI) for high uric acid per quartile change in PFDA		
			1.3 (0.8, 1.9)	1.0 (0.6, 1.7)	1.8 (0.9, 3.7)
Lin et al. (2020b)	Cross-sectional study (2016-2017); Taiwan; 397 older adults (55–75 yrs)	1.6 (1.2–2.4)	β (95% CI) in serum uric acid for quartiles vs Q1		
			All participants NR	Men Q2: 0.31 (-0.38, 0.99) Q3: 0.68 (-0.02, 1.37) Q4: 0.68 (-0.04, 1.4)	Women Q2: -0.09 (-0.45, 0.27) Q3: -0.1 (-0.02, 1.37) Q4: -0.18 (-0.54, 0.19)
Creatinine					
Cakmak et al. (2022)	Cross-sectional study (2007-2017); Canada; 6,045 adults	Mean 0.2	% change per 1 mean increase in PFDA -1.5 (-3.7, 0.7)		
Chronic kidney disease					
Wang et al. (2019)	Cross-sectional study (2015–2016); China; 1,612 adults	0.9 (0.5, 1.5)	OR (95% CI) for chronic kidney disease per ln-unit change in PFDA **0.7 (0.6, 0.9) ***		

******p* **< 0.05.**

Animal Studies

A 28-day study in female B6C3F1/N mice and two, 28-day studies in male and female S-D rats are available to examine effects relevant to the evaluation of urinary system toxicity after PFDA exposure (Frawley et al., 2018; NTP, 2018). The studies report on histopathology, serum

biomarkers of effect and organ weights. Overall study confidence was *high* for most endpoints evaluated in these studies with the exception of histopathology in Frawley et al. (2018), which had incomplete reporting of null data (results were only discussed qualitatively) resulting in a *medium* confidence rating (see Figure 3-85).

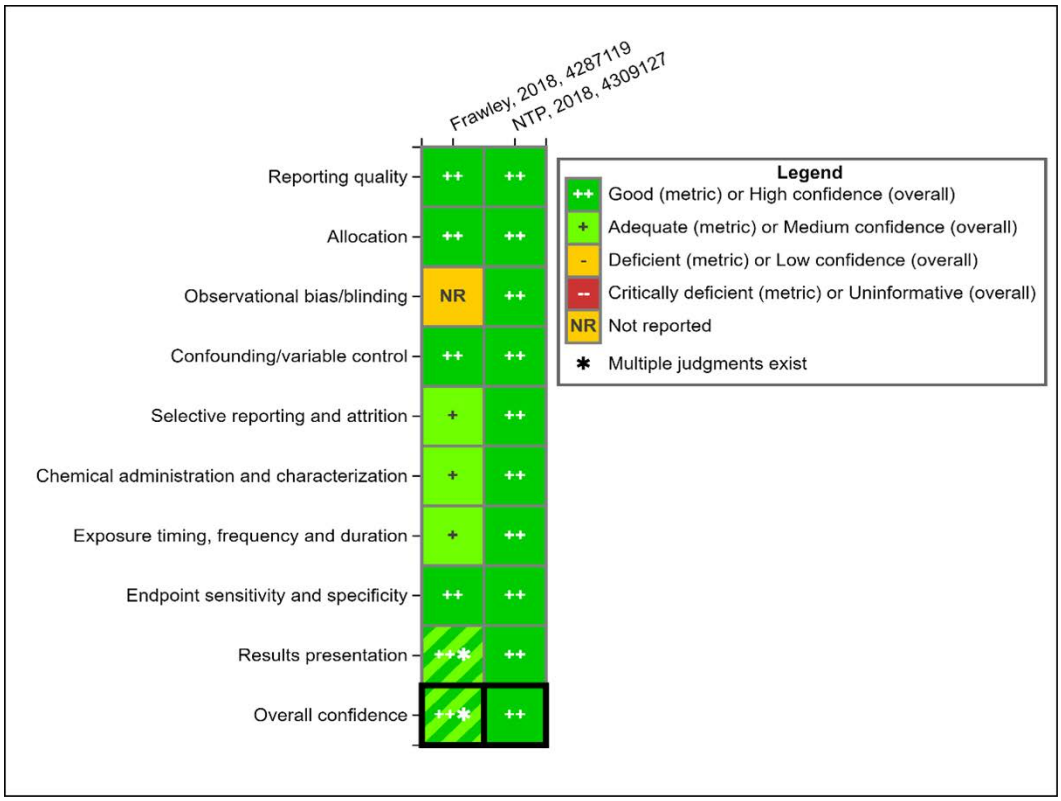

Figure 3-85. Evaluation results for animal studies assessing effects of PFDA exposure on urinary effects. Refer to HAWC for details on the study evaluation review.

Histopathology

The kidney and urinary bladder were evaluated for histopathology across a *high* confidence (NTP, 2018) and a *medium* confidence study (Frawley et al., 2018) in rats exposed for 28 days (see Figure 3-86). NTP (2018) found no evidence of histopathological lesions in the urinary bladder of males and females at the only dose examined (2.5 mg/kg-day). Chronic progressive nephropathy (CPN) graded as minimal occurred in the kidneys of nearly all dose groups, including controls, in this study (NTP, 2018) (see Figure 3-86). A reduction in the incidence of CPN was noted in males and females at the highest dose tested (0% and 30% incidence at 2.5 mg/kg-day in females and males respectively compared to 60% in controls) (NTP, 2018); but there was no clear dose-response effect and incidences were in some instances increased at doses lower than 2.5 mg/kg-day (i.e., 0.156–1.25 mg/kg-day) in both sexes, as compared to controls. The other 28-day gavage study reported no effects in kidney histopathology in female rats up to doses of

0.5 mg/kg-day (Frawley et al., 2018). Taken together, the high dose decrease in CPN incidence in rats in one study is not interpreted as biologically significant, and overall, the histopathology data were considered null.

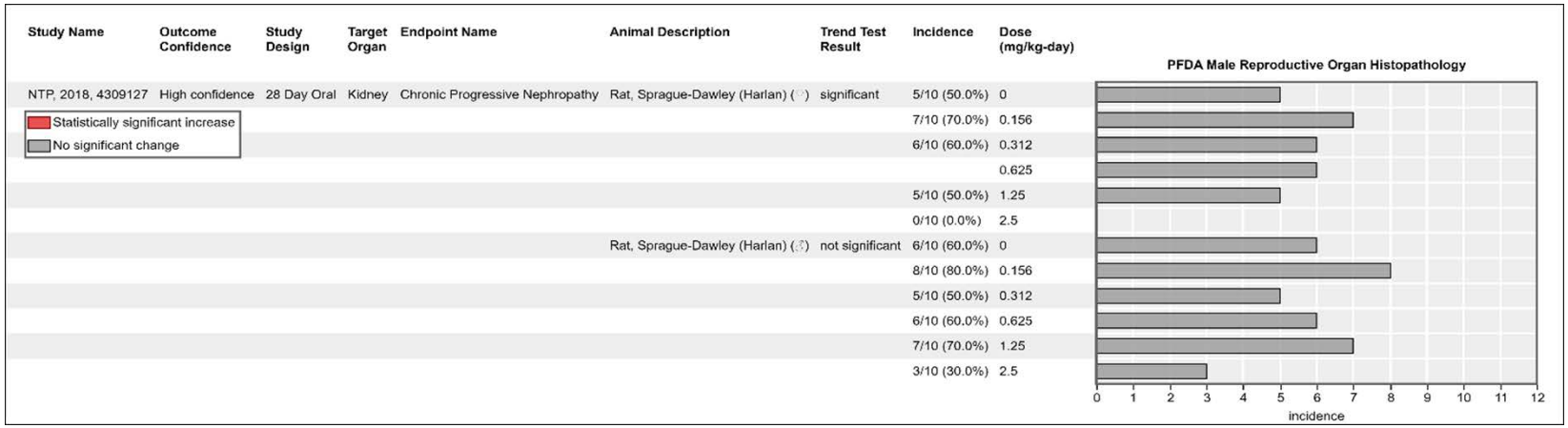

Figure 3-86. Kidney histopathology effects following exposure to PFDA in 28-day rat study (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/PFDA-Kidney-Histopathology-effect-size-animal/).

Serum biomarkers

Serum biomarkers of kidney injury and/or function, namely blood urea nitrogen (BUN) and creatinine were measured in rats in one *high* confidence study (NTP, 2018) (see Table 3-44 and Figure 3-87). Creatinine is a waste product of creatine metabolism produced in muscle tissue and BUN is a waste product of protein metabolism in the liver. Both creatinine and BUN are removed from the blood by the kidneys and often used as indicators of kidney function. Dose-related increases in circulating BUN levels occurred in males and females, most notably at 1.25 and 2.5 mg/kg-day (25–50% compared to controls). In contrast, a significant downward trend was reported for creatinine levels, reaching 4–11% decrease compared to controls at ≥1.25 mg/kg-day. The decreases in creatinine levels were accompanied by significant decreases in glucose levels at similar doses (31–51% compared to controls; data not shown in Table 3-44 or Figure 3-87) and likely reflect the marked systemic toxicity associated with high-dose PFDA exposure (see Section 3.2.10 on General toxicity effects for more details) (NTP, 2018).

Table 3-43. Percent change relative to controls in serum biomarkers of kidney function in a 28-day rat study after PFDA exposure (NTP, 2018)

Animal group	Dose (mg/kg-d)				
	0.156	0.312	0.625	1.25	2.5
Blood urea nitrogen (BUN)					
Male S-D rats	−9	−13	5	**25**	**25**
Female S-D rats	4	−2	11	**38**	**50**
Creatinine					
Male S-D rats	0	4	−8	**−11**	**−11**
Female S-D rats	−4	−5	−3	−4	**−10**

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

Organ weight

Absolute and relative kidney weights were measured in the two 28-day gavage studies using mice and/or rats (Frawley et al., 2018; NTP, 2018). There are some uncertainties surrounding the most toxicologically relevant organ weight metric so both absolute and relative kidney weights were evaluated herein (Craig et al., 2015; Bailey et al., 2004) (see Table 3-45 and Figure 3-87). Absolute and relative kidney weights of female rats displayed an upward trend, reporting increases of up to 11% and 13%, respectively, at a dose of 0.5 mg/kg-day in 1 out of 2 study cohorts exposed to similar experimental conditions (Frawley et al., 2018). Kidney weights (absolute and relative) increased in response to PFDA exposure in the second study cohort, but the changes were relatively small (0–5%) and a dose-related trend was not established. No appreciable body weight changes were reported in this study up to the highest dose tested (0.5 mg/kg-day) (Frawley et al., 2018). A separate study observed significant increases in relative kidney weight of 12–45% compared to controls in male and female rats at doses ≥0.625 mg/kg-day (NTP, 2018).

Conversely, absolute kidney weight increased significantly in females by 9 and 15% at 0.312 and 0.625 mg/kg-day, respectively, but decreases were observed in both males and females at 2.5 mg/kg-day (10 and 15% from controls, respectively) (NTP, 2018). The apparent decreases in absolute kidney weight at higher doses may be associated with concurrent reductions in body weight occurring in the exposed animals (up 38% compared to controls at 2.5mg/kg-day) (see Section 3.2.10 on General toxicity effects for more details) (NTP, 2018). In mice, kidney weights were mostly unchanged by PFDA treatment (0.045–0.71 mg/kg-day) (Frawley et al., 2018). In addition to the uncertainties due to confounding effects with decreased body weight at the highest PFDA doses (≥1.25 mg/kg-day), the observed kidney weight changes in rats are not supported by significant histopathological findings in these animals (Frawley et al., 2018; NTP, 2018).

Table 3-44. Percent change relative to controls in kidney weights (absolute and relative to body weight) due to PFDA exposure in short-term oral toxicity studies

Animal group	Dose (mg/kg-d)						
	0.045	0.089	0.125–0.179	0.25–0.36	0.5–0.71	1.25	2.5
Absolute kidney weight							
28 d; female S-D rats –Histopathology cohort Frawley et al. (2018)			6	6	**11**		
28 d; female S-D rats – MPS cohort Frawley et al. (2018)			2	2	5		
28 d; female S-D rats NTP (2018)			6	**9**	**15**	6	**–15**
28 d; male S-D rats NTP (2018)			5	–1	8	–2	**–10**
28 d; female B6C3F1/N mice Frawley et al. (2018)	1	9	1	–1	–3		
Relative kidney weight							
28 d; female S-D rats –Histopathology cohort Frawley et al. (2018)			7	**9**	**13**		
28 d; female S-D rats – MPS cohort Frawley et al. (2018)			3	0	4		
28 d; female S-D rats NTP (2018)			2	5	**15**	**20**	**34**
28 d; male S-D rats NTP (2018)			2	0	**12**	**24**	**45**
28 d; female B6C3F1/N mice Frawley et al. (2018)	–2	1	2	–5	–7		

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

Effect	Endpoint Name	Organ	Study Name	Outcome Confidence	Experiment Name	Species, Strain (sex)	Trend Test Result
Clinical Chemistry	Blood Urea Nitrogen (BUN)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Creatinine (CREAT)	Blood	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
Histopathology	Chronic Progressive Nephropathy	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Histopathology	Kidney	Frawley, 2018, 4287119	Medium confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not reported
	Urinary Bladder Histopathology	Bladder	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	not applicable
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not applicable
Organ Weight	Kidney Weight, Absolute (Histophatology Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Absolute (MPS Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Right Kidney Weight, Absolute	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Kidney Weight, Absolute (Hematology Study)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant
	Kidney Weight, Relative (Histopathology Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Relative (MPS Cohort)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	not significant
	Right Kidney Weight, Relative	Kidney	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	significant
				High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	significant
	Kidney Weight, Relative (Hematology Study)	Kidney	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	not significant

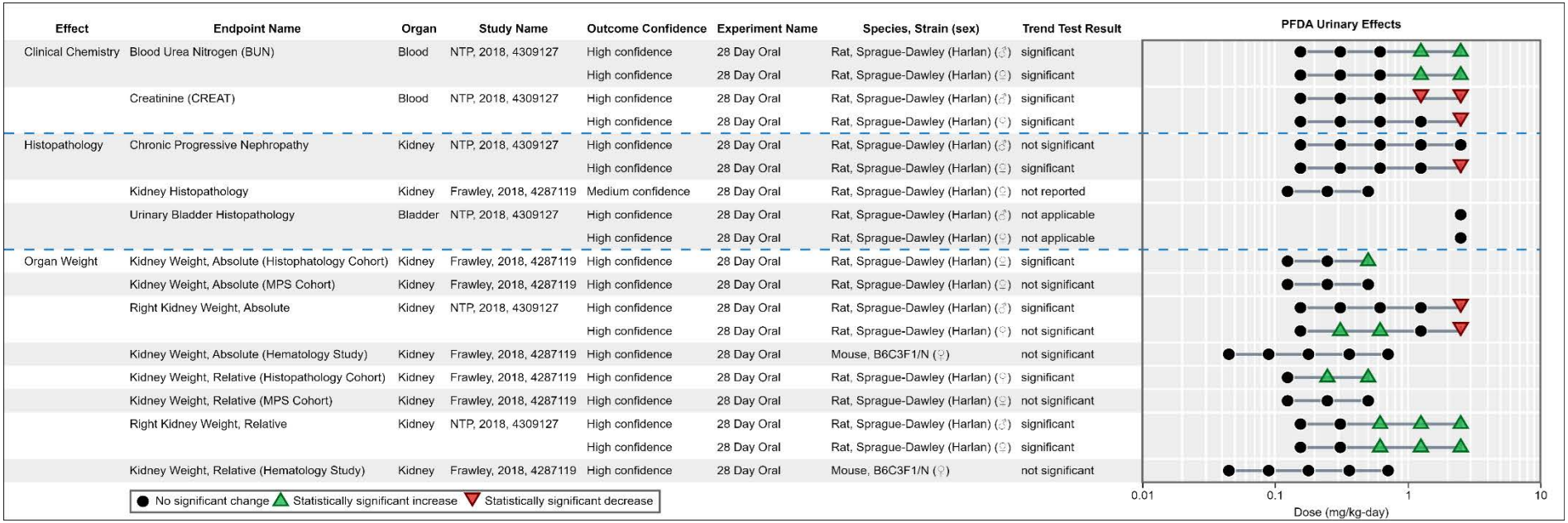

Figure 3-87. Urinary effects following exposure to PFDA in short-term oral studies in animals (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100500072/pfda-urinary-effects/).

Evidence Integration

The evidence for potential urinary system effects in humans is considered *indeterminate.* Associations between PFDA exposure and impaired renal function were reported in two *low* confidence epidemiological studies. However, there is considerable uncertainty in the interpretation of these findings due to the potential for reverse causation and some unexplained inconsistency in the direction of association across studies.

The evidence for potential urinary system effects in experimental animals is limited to three *high/medium* confidence studies in rats (Frawley et al., 2018; NTP, 2018) and one *high* confidence study in mice with exposure for 28 days (Frawley et al., 2018). Although alterations in BUN and creatine levels were observed at ≥1.25 mg/kg-day in rats, there is no coherent pattern of effects (BUN levels increased and creatinine levels decreased) or supportive information (i.e., histopathology) to determine the toxicological relevance of the changes that occurred (NTP, 2018). Histopathological examinations of rat kidney and urinary bladder were mostly unremarkable across two studies (Frawley et al., 2018; NTP, 2018). Finally, the interpretation of the absolute and relative kidney weight changes in rats at doses ≥0.312 mg/kg-day is complicated by the lack of coherent histopathological findings (Frawley et al., 2018; NTP, 2018), inconsistencies in the direction of changes across experiments, and confounding effects from significant body weight reductions at the highest doses tested (≥1.25 mg/kg-day) (NTP, 2018). In summary, the sparse and uncertain evidence from animal studies is considered *indeterminate*. The absence of any long-term studies (subchronic/chronic) via the oral route or other relevant routes of exposure increases uncertainty in the evaluation of potential urinary system toxicity in animals following PFDA exposure.

Altogether, based on the available human and animal studies, there is ***inadequate evidence*** to assess whether PFDA exposure can cause urinary system effects in humans (see Table 3-46).

Table 3-45. Evidence profile table for PFDA urinary effects

Evidence stream summary and interpretation					Evidence integration summary judgment
Evidence from studies of exposed humans (see Section 3.2.8: Human studies)					⊙⊙⊙ ***Inadequate Evidence*** *Primary basis:* Evidence from epidemiological studies and experimental animals is *indeterminate*. *Human relevance, cross-stream coherence, susceptibility, and other inferences:* No specific factors are noted.
Studies, outcomes, and confidence	**Key findings and interpretation**	**Factors that increase strength or certainty**	**Factors that decrease strength or certainty**	**Evidence stream judgment**	
Seven *low* confidence studies	• Three studies reported some associations between PFDA exposure and impaired renal function (i.e., lower GFR or higher serum uric acid). • One study reported associations in the opposite direction and three others were null	• No factors noted	• *Low* confidence studies due to potential for reverse causality • Unexplained inconsistency	⊙⊙⊙ *Indeterminate* There is some evidence of urinary effects with PFDA exposure across two *low* confidence studies but considerable concerns for reverse causality and inconsistency.	
Evidence from in vivo animal studies (see Section 3.2.8: Animal studies)					
<u>Histopathology</u> 1 *high and 1 medium* confidence studies in rats for 28 days	• Mostly null findings for kidney and urinary bladder histopathology in rats up to 2.5 mg/kg-d across two studies. • A high dose (2.5 mg/kg-d) *decrease* in the incidence of CPN in rats reported in one study was not interpreted as biologically significant.	• No factors noted	• No factors noted	⊙⊙⊙ *Indeterminate* Lack of coherent effects in *high* and *medium* confidence studies in rats and mice exposed up to 2.5 mg/kg-d for 28 d.	

Evidence stream summary and interpretation					Evidence integration summary judgment
<u>Serum biomarkers</u> 1 *high* confidence study in rats for 28 d	• Increased BUN levels and decreased creatinine levels in rat serum at ≥1.25 mg/kg-d (alterations in creatinine levels coincide with body weight reductions)	• *High* confidence study	• *Lack of expected coherence* in the directionality of BUN and creatinine changes • Potential confounding by body weight decreases		
<u>Organ weight</u> 2 *high* confidence studies (encompassing 4 experiments) in mice and/or rats for 28 d	• Absolute and relative kidney weight changes in rats at doses ≥0.312 mg/kg-d (directionality of effects varied across experiments and organ weight measures); no effects in mice up to 0.71 mg/kg-d	• *High* confidence studies	• *Unexplained inconsistency* across experiments, species, and organ weight measures		

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3.2.10. GENERAL TOXICITY

The potential for PFDA exposure-induced general toxicity is specifically discussed given that PFDA has been shown to cause a "wasting syndrome" in rodents, which is characterized by decreased food intake and reduced body weight (Goecke-Flora et al., 1995). In animals, decreased body weights can be indicative of non-specific overt toxicity and some effects that occur at doses associated with this and other frank effects should be interpreted cautiously when drawing conclusions about organ-/system-specific hazards. Thus, this section informs judgments drawn for other potential health hazards, but a specific evidence integration judgment is not drawn.

Human Studies

No human studies were available to inform the potential for PFDA exposure to cause general toxicity.

Animal Studies

Animal toxicity studies reporting general toxicity with repeated dose exposure to PFDA include two 28-day gavage studies, four dietary exposure studies (7–14 days) in mice and/or rats, and two drinking water studies (12–49 days) in mice. The endpoints measured in these studies include body weight (Li et al., 2022; Wang et al., 2020; Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Takagi et al., 1992, 1991), clinical observations (NTP, 2018) and survival (Wang et al., 2020; NTP, 2018) (Figure 3-67). Three studies (Li et al., 2022; Frawley et al., 2018; NTP, 2018) were evaluated as *high* confidence for all general toxicity endpoints tested (see Figure 3-88). Four studies (Wang et al., 2020; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992) were evaluated as *medium confidence* for all general toxicity endpoints tested while the Takagi et al. (1991) study was evaluated as *low confidence* for the body weight endpoint (see Figure 3-67). Key issues regarding study quality evaluation in the *medium* and *low confidence* studies were related to exposure sensitivity (no analytical verification methods or quantitative data on food consumption), allocation/randomization of animals into experimental groups, and deficiencies in data reporting (see Figure 3-88).

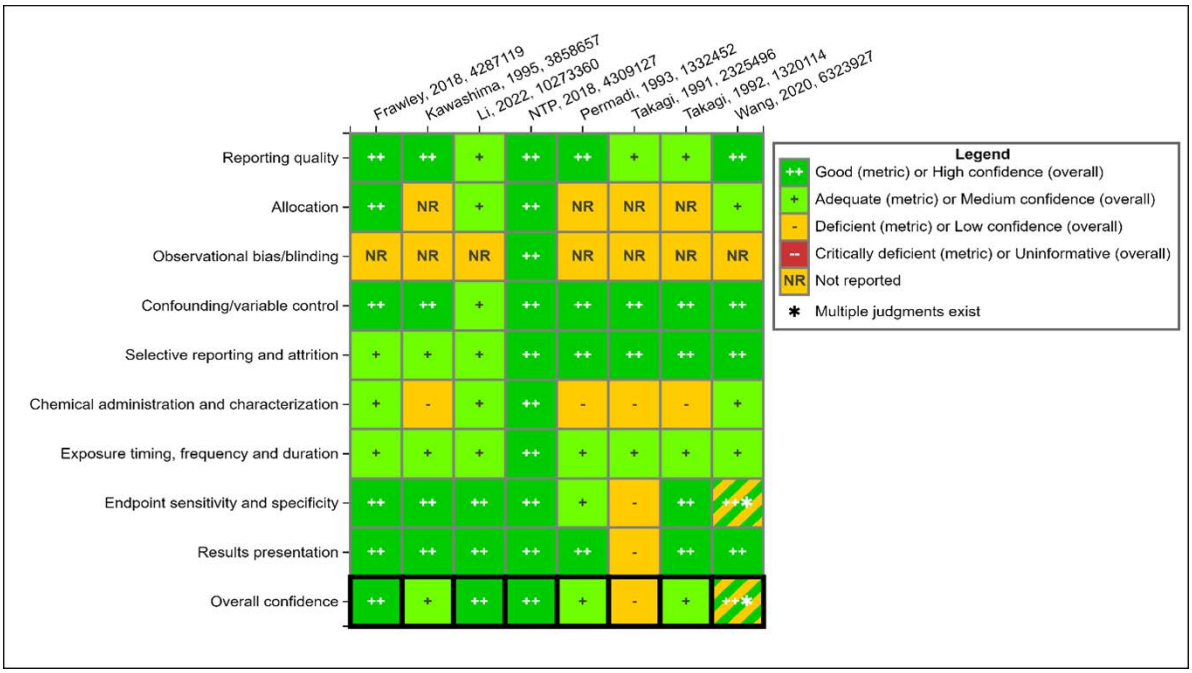


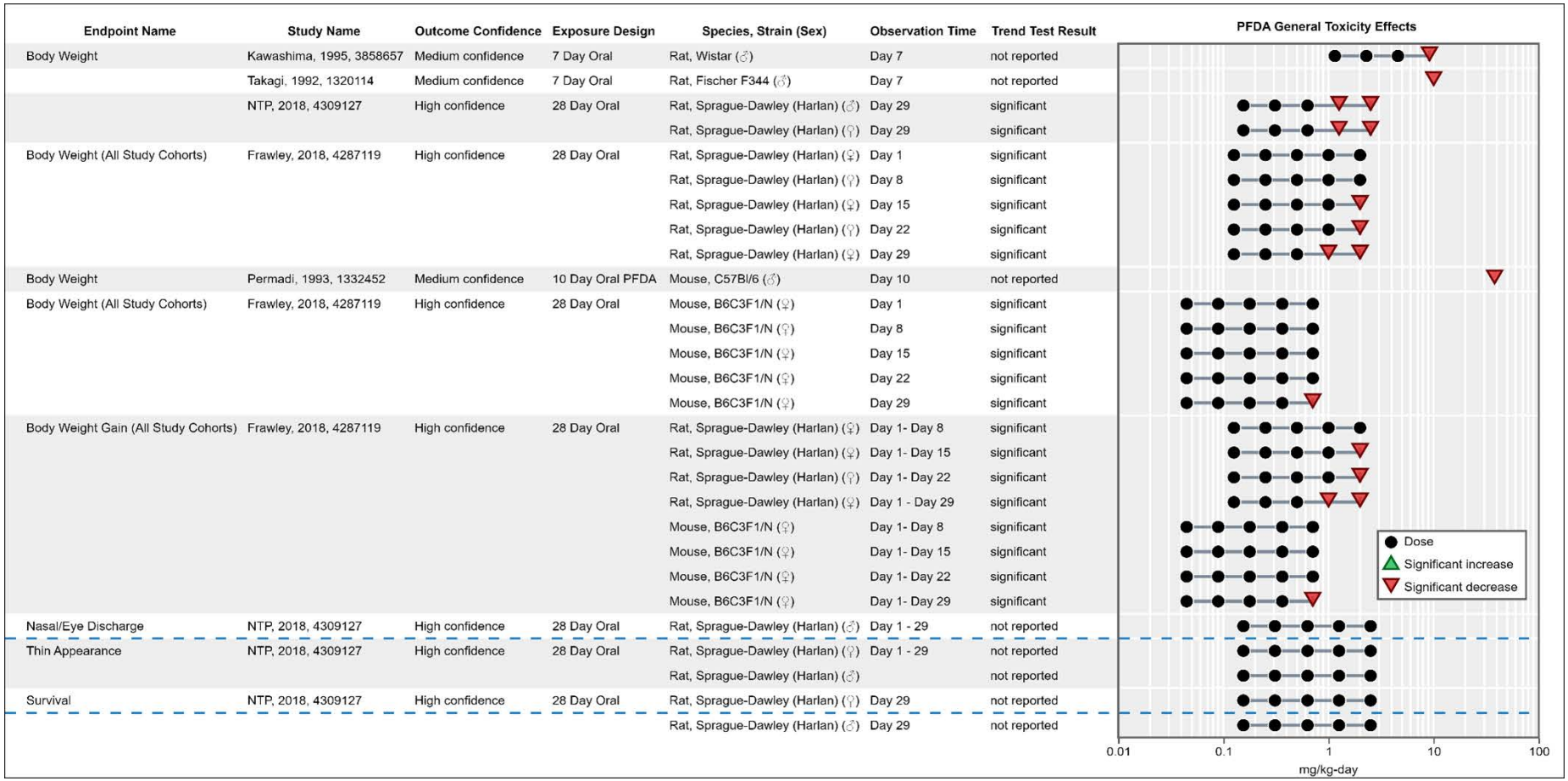
Figure 3-88. PFDA general toxicity animal study evaluation heatmap. Refer to HAWC for details on the study evaluation review.

Body weight

PFDA-induced body weight suppression was observed to be dose-dependent in short-term animal studies in rats (Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Takagi et al., 1992, 1991) and mice (Li et al., 2022; Wang et al., 2020; Frawley et al., 2018; Permadi et al., 1993) (Figure 3-68). In rats treated with doses ranging from 1.0–10 mg/kg-day, reductions in mean body weight and body weight gain ranged from 4–38% and 21–103% respectively, compared to controls. In mice, changes in body weight were less than 5% at doses ≤0.71 mg/kg-day but decreases reached 53% at 6.6 mg/kg-day. In the 28-day high confidence study that included multiple study cohorts (Frawley et al., 2018), the study authors reported that 2 out of 88 rats in the 2.0 mg/kg-day exposure group were euthanized due to marked reductions in body weight (>20%) occurring within the first 5 days of the study initiation (Frawley et al., 2018). This evidence of PFDA-induced acute toxicity was also observed in several single intraperitoneal (i.p.) injection studies as discussed below. Furthermore, PFDA-induced decreased body weight in female rats was more severe with longer treatment durations (Frawley et al., 2018). For example, body weight was decreased by 4% at Day 15, by 13% at Day 22, and 22% at Day 29 at 2.0 mg/kg-day. Also, in this study, reduced body weight was observed to be more sensitive to dose at Day 29 compared to earlier time points (statistically significant at 1.0 mg/kg-day on Day 29 compared to 2.0 mg/kg-day for Days 15 and 22). The NTP (2018) study also showed similar results for multiple timepoint data for body weight. For example, in male rats treated with the highest dose (2.5 mg/kg-day), body weight was decreased by 13, 27, and 38% on Day 15, Day 22, and Day 29, respectively.

Clinical observations and survival

Clinical observations and survival data are available from a *high confidence* gavage study in S-D rats exposed for 28 days (NTP, 2018). Additionally, a *medium confidence* study reported effects on survival in male CD-1 mice exposed to PFDA in the drinking water for 49 days (Wang et al., 2020). PFDA exposure was associated (albeit not statistically significant) with thin appearance in male and female S-D rats at the highest exposure dose tested (2.5 mg/kg-day) (see Figure 3-89). The incidence rate was 30% in males and 10% in females compared to 0% for the corresponding controls. Nasal/eye discharge was observed in 1 out 10 male rats in the control, 0.156, 0.0625, 1.25 and 2.5 mg/kg-day exposure groups. No other clinical observations were reported. All exposed animals survived and were euthanized at study termination. In summary, 28-day gavage exposure to PFDA caused mild clinical symptoms in rats (thin appearance) but had no effect on survival in this study. However as discussed above, Frawley et al. (2018) reported that two (of 88) rats were euthanized due to severe weight loss caused by 5 days of exposure to PFDA at 2.0 mg/kg-day. In mice exposed to PFDA for up to 49 days, the mortality rate was reported to be significantly increased at 6.6 mg/kg-day (Wang et al., 2020).



Endpoint Name	Study Name	Outcome Confidence	Exposure Design	Species, Strain (Sex)	Observation Time	Trend Test Result
Body Weight	Kawashima, 1995, 3858657	Medium confidence	7 Day Oral	Rat, Wistar (♂)	Day 7	not reported
	Takagi, 1992, 1320114	Medium confidence	7 Day Oral	Rat, Fischer F344 (♂)	Day 7	not reported
	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	Day 29	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 29	significant
Body Weight (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 8	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 15	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 22	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 29	significant
Body Weight	Permadi, 1993, 1332452	Medium confidence	10 Day Oral PFDA	Mouse, C57Bl/6 (♂)	Day 10	not reported
Body Weight (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Mouse, B6C3F1/N (♀)	Day 1	significant
				Mouse, B6C3F1/N (♀)	Day 8	significant
				Mouse, B6C3F1/N (♀)	Day 15	significant
				Mouse, B6C3F1/N (♀)	Day 22	significant
				Mouse, B6C3F1/N (♀)	Day 29	significant
Body Weight Gain (All Study Cohorts)	Frawley, 2018, 4287119	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 8	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 15	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1- Day 22	significant
				Rat, Sprague-Dawley (Harlan) (♀)	Day 1 - Day 29	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 8	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 15	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 22	significant
				Mouse, B6C3F1/N (♀)	Day 1- Day 29	significant
Nasal/Eye Discharge	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♂)	Day 1 - 29	not reported
Thin Appearance	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 1 - 29	not reported
				Rat, Sprague-Dawley (Harlan) (♂)		not reported
Survival	NTP, 2018, 4309127	High confidence	28 Day Oral	Rat, Sprague-Dawley (Harlan) (♀)	Day 29	not reported
				Rat, Sprague-Dawley (Harlan) (♂)	Day 29	not reported

Figure 3-89. PFDA general toxicity effects (results can be viewed by clicking the HAWC link: https://hawcprd.epa.gov/summary/data-pivot/assessment/100000026/pfda-general-toxicity-effects/).

Mechanistic studies and supplemental information

Several intraperitoneal (i.p.) studies using a single injection, have demonstrated that PFDA induces a "wasting syndrome" in rodents, which is characterized by decreased food intake and reduced body weight (Goecke-Flora et al., 1995). In these studies, decreased body weight (5 to 72% compared to controls or pretreatment values) was observed in rats at doses ranging from 20 to 100 mg/kg PFDA (Unkila et al., 1992; Bookstaff et al., 1990; Chen et al., 1990; Ylinen and Auriola, 1990; Gutshall et al., 1988; Van Rafelghem and Andersen, 1988; Van Rafelghem et al., 1988a; Kelling et al., 1987; Langley and Pilcher, 1985; Olson and Andersen, 1983). Generally, across rodent species, i.p. injection of PFDA at doses ≥20 mg/kg-day, even acutely, caused generalized acute toxicity. Whereas significant decreases in food intake were also observed in rats at 40 to 80 mg/kg, body weights were reduced compared to both ad-libitum and pair-fed controls suggesting that PFDA-decreased body weight is not only related to reduced food intake but also a direct effect of PFDA on body weight. In guinea pigs, body weight gain (32% decrease) and food intake (11% decrease) were significantly reduced at 20 mg/kg PFDA via the i.p. route (Chinje et al., 1994). In a study that tested multiple species, rats lost a maximum of 45% of their pretreatment body weight at 50 mg/kg PFDA, hamsters lost 26% at 50 mg/kg and 41% at 100 mg/kg, and mice lost 25% at 150 mg/kg (Van Rafelghem et al., 1987b). Multiple other i.p. studies reported effects on body weight and food intake, but the data were presented qualitatively or graphically, and percent changes were not calculated. Doses for these studies ranged from 10 to 100 mg/kg (Kudo and Kawashima, 2003; Wilson et al., 1995; Chen et al., 1994; Glauert et al., 1992; Arand et al., 1991; Powers and Aust, 1986). Most of the studies described here utilized a single injection of PFDA, highlighting the acute toxicity and rapid weight loss caused by PFDA treatment. It is important to note that the doses used in the mechanistic/supplemental studies are much higher than the doses in which body weight was decreased in some of the toxicity studies. For example, decreases in body weight interpreted as biologically significant were observed in rats at ≥1.25 mg/kg-day from the NTP (2018) study.

Summary of animal and mechanistic/supplemental information

The available studies for PFDA-induced general toxicity were mostly *high* and *medium* confidence (see Figure 3-89) and evaluated endpoints related to general toxicity (body weight, clinical observations, and survival) in multiple strains (S-D, Wistar, Fisher F344, C57BL/6N and B6C3F1/N) of male and female rats and mice via gavage and dietary exposure for up to 28 (Frawley et al., 2018; NTP, 2018; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991). Reduced body weight was consistently observed in all available animal studies, with biologically significant effects occurring at doses as low as 1.25 mg/kg-day in rats from the NTP (2018) study. The consistent effect of PFDA on body weight that appears to be time- and dose-related coupled with clinical observations (i.e., thin appearance) in rats provide support for PFDA-induced general toxicity. Furthermore, multiple acute i.p. studies across different species reported

decreased body weight indicative of "wasting syndrome" at doses ranging from 20 to 100 mg/kg, but primarily at ≥40 mg/kg-day.

3.2.11. OTHER HEALTH EFFECTS

Short-term oral exposure studies (*high/medium* confidence) in experimental animals evaluated potential health effects related to the hematological, respiratory, digestive, dermal, musculoskeletal, and adult nervous system (please see Section 3.2.7 for the synthesis of evidence on neurodevelopmental effects). The available evidence from these animal studies is briefly summarized below. Given the limitations of the evidence base and the lack of consistent or coherent effects of PFDA exposure, there is ***inadequate evidence*** to determine whether any of the evaluated outcomes below might represent potential human health hazards of PFDA exposure. Additional studies on these health effects could modify these interpretations.

Animal studies

Other health effects

Hematological parameters were evaluated across two studies in male and/or female S-D rats and one study in female B6C3F1/N mice, all with gavage exposure for 28-days (Frawley et al., 2018; NTP, 2018). No significant effects were found in mice up to 0.71 mg/kg-day (Frawley et al., 2018). In rats, mean corpuscular hemoglobin (amount of hemoglobin per red blood cell [RBC]; MCH) and mean corpuscular hemoglobin concentration (amount of hemoglobin per unit of RBC volume; MCHC) decreased at the two highest doses (0.25 and 0.5 mg/kg-day) in one study (Frawley et al., 2018); however, the changes did not show a dose-response gradient and were relatively small (6–7% compared to controls). In the other rat study, a significant dose-related trend was reported for several hematological parameters (NTP, 2018). Erythrocyte (RBCs) counts increased (9–23%) in males and females and hematocrit (proportion of RBCs in blood; 6–16%) and hemoglobin (7–19%) concentrations increased in females only at doses ≥1.25 mg/kg-day. These changes were accompanied by decreases in reticulocyte counts (immature RBCs) of 54-91%, and slight decreases in mean corpuscular volume (average volume of RBCs; decreases of 3–7%) and MCH (4%) and slight increases in MCHC (2–4%) in males and females at similar doses. In addition, the platelet count in females decreased by up to 30% in females at the highest dose group, 2.5 mg/kg-day. In summary, although there is some potential evidence of hematological effects in rats with PFDA exposure (NTP, 2018), the observed changes occurred mostly in the presence of significant systemic toxicity (i.e., reduced body weights at ≥2.5 mg/kg-day), which limits the interpretation of the findings.

Histopathology of the dermal, musculoskeletal, nervous, and special senses (eye and harderian gland) systems was examined in the control and 2.5 mg/kg-day dose groups in adult S-D rats in one 28-day study that reported null findings (NTP, 2018). The digestive and respiratory systems were examined histologically in S-D rats across two, 28-days studies (Frawley et al., 2018;

NTP, 2018). No lesions were identified in stomach or lungs of rats at doses of 0.125–0.5 mg/kg in one study (Frawley et al., 2018). The second study found lesions in the esophagus, forestomach, lungs and nose of exposed rats (NTP, 2018). Increased incidence of forestomach lesions (epithelium hyperplasia, inflammation, and ulcer) was reported in males and inflammation was reported in the lungs and esophagus of females. The incidence rates for these lesions were low (10–20%) and restricted to the highest dose group (2.5 mg/kg-day). The nose lesions (epithelium degeneration, hyperplasia, and chronic inflammation) were increased in both males and females (10–50% incidence) across 0.158–2.5 mg/kg-day, but there was no clear dose-response relationship, and these morphological changes were also observed in the control group (0–20% incidence). Overall, the limited information available for these organ systems impedes further evaluation of the biological significance of the histopathological results.

3.3. CARCINOGENICITY

3.3.1. CANCER

Human studies

Eight studies evaluated the risks of cancer associated with exposures to PFDA (Velarde et al., 2022; Liu et al., 2021b; Omoike et al., 2021; Lin et al., 2020a; Tsai et al., 2020; Wielsøe et al., 2017; Christensen et al., 2016; Hardell et al., 2014). Five cancer studies by (Velarde et al., 2022; Omoike et al., 2021; Lin et al., 2020a; Wielsøe et al., 2017; Christensen et al., 2016) were evaluated as '*Uninformative*.'

The study of risks of prostate cancer (Hardell et al., 2014) was *low* confidence due to concern about the exposure measurement not representing the etiologically relevant time period, potential for confounding, insufficiencies in the analysis, and concerns about sensitivity (see Figure 3-90). Hardell et al. (2014) reported a non-significantly increased risk of prostate cancer among men with PFDA concentrations in blood that were above the median value. The study of risks of thyroid cancer (Liu et al., 2021b) was low confidence due to concern about the exposure measurement not representing the etiologically relevant time period, deficiencies regarding the outcome definition, and potential for confounding, (see Figure 3-90). Liu et al. (2021b) reported significantly decreased risk of thyroid cancer associated with increasing quartiles of PFDA. The study of risks of breast cancer (Tsai et al., 2020) was low confidence due to concern about the exposure measurement not representing the etiologically relevant time period, potential for confounding, and concerns about low sensitivity (see Figure 3-90). Tsai et al. (2020) reported non-significantly increased risk of breast cancer per ln-transformed unit increase in PFDA concentration in blood among women <= 50 years of age; and non-significantly decreased risk of breast cancer per ln-transformed unit increase in PFDA concentration in blood among women > 50 years of age. In summary, the available epidemiologic evidence on PFDA and the risks of cancer is limited and generally *uninformative*.

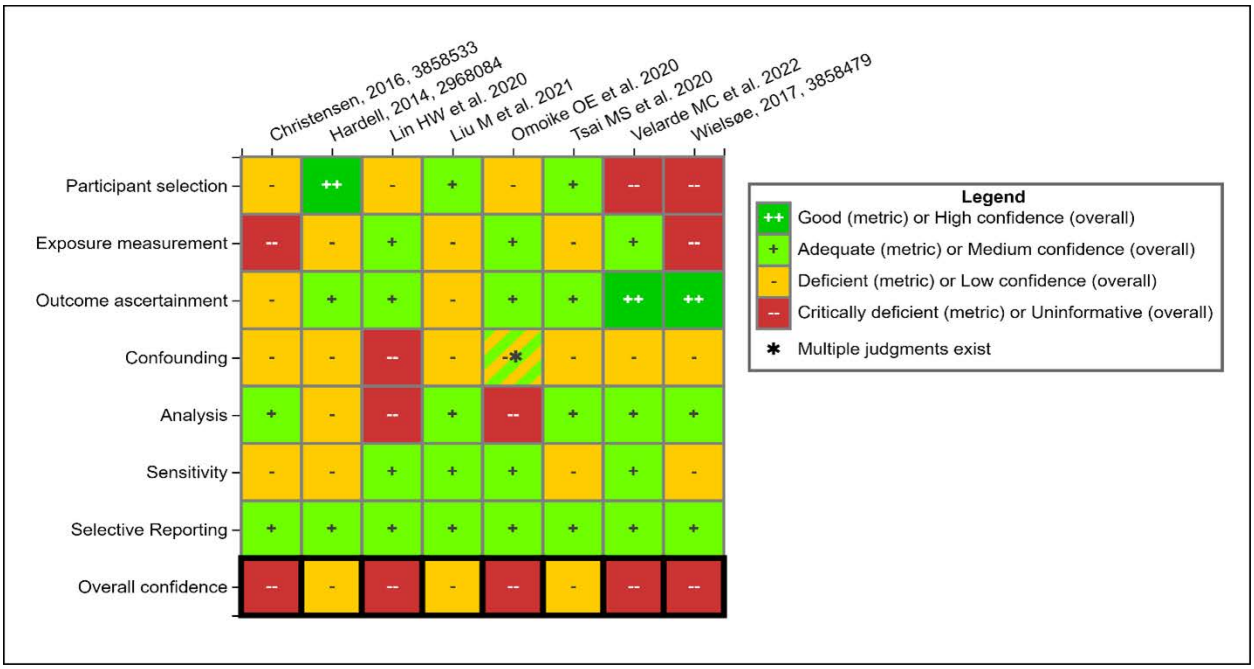


Figure 3-90. Study evaluation results for epidemiology studies of PFDA and cancer. Refer to HAWC for details on the study evaluation review: HAWC Human Cancer.

Animal studies

There are no long-term animal bioassay studies available for PFDA. One short-term study reported null findings for neoplastic histopathology in male and female rats gavaged with doses of 0–2.5 mg/kg-day for 28 days (NTP, 2018). The study performed a complete necropsy of control and PFDA-exposed groups, examining various tissues (i.e., esophagus, intestine, liver, pancreas, salivary glands, stomach, blood vessel, heart, adrenal cortex, adrenal medulla, parathyroid gland, pituitary gland, thyroid gland, epididymis, preputial gland, prostate seminal vesicle, testes, clitoral gland, ovary, uterus, bone marrow, lymph node, spleen, thymus, mammary gland, skin, bone, brain, lung, nose, eye, harderian gland, kidney and urinary bladder). However, the study was considered *low* confidence for the assessment of carcinogenicity due to the inadequacy of the short-term exposure duration for evaluating the long-term development of potential cancers. Although 28-day studies may be able to provide some information on preneoplastic lesions, the study duration does not cover the entire spectrum of tumor development and promotion for nearly all cancer types and thus they are insensitive.

Mechanistic studies and supplemental information

The scope of the analysis for evaluating putative mechanisms of carcinogenicity for PFDA focused on the synthesis of genotoxicity studies based on data availability. A more comprehensive

and rigorous mode of action (MOA) investigation was not attempted due to the sparse and *low* confidence human and animal studies available, as well insufficient information for the evaluation of alternative carcinogenic mechanisms (e.g., mitogenesis, inhibition of cell death, cytotoxicity with reparative cell proliferation and immune suppression) or considerations for human relevance of tumor responses in animals, susceptible populations and lifestages and anticipated shape of dose-response relationships. This is in agreement with the proposed framework for cancer MOA analysis in the EPA Guidelines for Carcinogen Risk Assessment, which states that "the framework supports a full analysis of mode of action information, but it can also be used as a screen to decide whether sufficient information is available to evaluate or whether the data gaps are too substantial to justify further analysis" (U.S. EPA, 2005).

Studies evaluating the genotoxic, mutagenic and clastogenic potential of PFDA from in vitro assays with prokaryotic organisms and mammalian cells and in vivo assays in rats and mice are summarized in Table 3-47. Mutagenicity test results in *S. typhimurium* (TA98, TAl00, TA1535, TA1537, and TA1538) and *E. coli* strains (WP2 *uvrA* pKM101) across several studies were consistently negative for PFDA in the presence or absence of S9 rat liver metabolism system (NTP, 2005; Kim et al., 1998; Godin et al., 1992; Myhr et al., 1990). Similarly, PFDA had no effect on mutation frequency in L5178Y mouse-lymphoma cells and in the HGPRT forward mutation assay in Chinese hamster ovary (CHO) cells with or without S9 metabolic activation (Godin et al., 1992; Myhr et al., 1990; Rogers et al., 1982).

PFDA was inactive for the in vitro transformation of BALB/C-3T3 mouse cells (Godin et al., 1992) and in the sister chromatic exchange (SCE) assays in CHO cells but induced chromosomal aberrations indicative of clastogenic effects under conditions of S9 metabolic activity (Godin et al., 1992; Myhr et al., 1990). PFDA caused DNA double-strand breaks (DSB) in human gastric adenocarcinoma AGS and SGC cell lines, although the details of the study exposure methodology including information on the test article concentrations were not provided (Liu et al., 2019a). The mechanisms of PFDA-induced DSB were attributed to the downregulation of X-ray repair cross complementing 4 (XRCC4) expression and nonhomologous end-joining (NHEJ) inactivation. These events lead to impairment of DNA damage repair and inhibition of p53 expression and apoptosis, contributing to the observed alterations in cell sensitivity to chemotherapy (Liu et al., 2019a). Elevated levels of DSB were also detected in mice with PFDA treatment (dosing regimen was not specified) (Liu et al., 2019a). Xu et al. (2019b) also showed increases in DNA strand breaks, 8OHdG formation and ROS levels, indicative of oxidative DNA damage in primary mouse hepatocytes exposed to PFDA. In vivo experiments in rats showed increase in oxidative DNA damage (8OHdG levels) in liver tissue after dietary PFDA treatment at 10 mg/kg-day for 2 weeks (Takagi et al., 1991) but no effects were reported with a lower dose (1.4 mg/kg-day) administered via i.p. for up to 8 weeks (Kim et al., 1998). There were no effects on frequency of micronucleated polychromatic or normochromatic erythrocytes in blood after repeated dose PFDA treatment (0.156–2.5 mg/kg-day) via gavage (NTP, 2012). PFDA was not associated with induction of unscheduled DNA

synthesis (UDS) in primary hepatocytes isolated from rats after single-dose exposure (≥11 mg/kg); however, increase in S-phase DNA synthesis was observed in the exposed rats (Godin et al., 1992; Myhr et al., 1990).

In summary, PFDA does not appear to elicit a strong genotoxic response as demonstrated by the lack of activity in most assays described above, including mutagenicity tests in prokaryotic organisms and mammalian cells; SCE and cell transformation assays in vitro; and UDS, oxidative DNA damage and micronucleus assays in rats. Nevertheless, there is some evidence of potential clastogenic effects in CHO cells, S-phase induction in rat hepatocytes, double strand DNA breaks in human and mouse gastric cells and oxidative DNA damage in primary mouse hepatocytes.

Table 3-46. Test evaluating genotoxicity and mutagenicity

Test	Materials and methods	Results	Conclusions	References
Genotoxicity studies in prokaryotic organisms				
Ames assay	*S. typhimurium* strains (TA98, TAl00, TA1535, TA1537, and TA1538) were tested with or without S9 rat liver homogenate and with a pre-incubation period. PFDA concentrations ranged from 33.3 to 10, 000 µg/plate.	No increase in the number of reverent colonies was observed with PFDA in any of the tester strains in the presence or absence of S9 metabolic activation.	There is no evidence of PFDA mutagenicity in *S. typhimurium* strains.	Godin et al. (1992); Myhr et al. (1990)
Ames assay	*S. typhimurium* strains (TA98 and TA1535) were incubated with PFDA (1 to 100 g/plate) with or without S9.	Test results were negative in the two strains tested irrespective of the presence of S9.	There is no evidence of PFDA mutagenicity in *S. typhimurium* strains.	Kim et al. (1998)
Ames assay	*S. typhimurium* strains (TA98 and TA100) and *E. coli* strain (WP2 *uvrA* pKM101) in the presence or absence of S9. Concentrations of PFDA were 0−10,000 µg/plate.	Test results were negative in all bacterial strains irrespective of the presence of S9.	There is no evidence of PFDA mutagenicity in *S. typhimurium* and *E. coli* strains.	NTP (2005)
Genotoxicity studies in mammalian cells – in vitro				
Mutagenicity assay	L5178Y mouse-lymphoma cells were treated with PFDA (0.01–500 µg/mL) for 24 h and plated in the presence of selective agents to evaluate mutation frequency (ouabain, excess thymidine, methotrexate, cytosine arabinoside and thioguanine) and in non-selective medium to evaluate survival.	Mutagenicity tests showed no significant results in any of the selective systems.	There is no evidence of PFDA mutagenicity in L5178Y cells.	Rogers et al. (1982)
CHO/HGPRT forward mutation assay	Chinese hamster ovary (CHO) cells were treated with PFDA concentrations ranging from 0.005 to 0.5 mg/mL with or without S9.	The results were negative for PFDA-mediated induction of forward mutations in the HGPRT locus in CHO cells under conditions of S9 metabolic activation and nonactivation.	There is no evidence of PFDA mutagenicity in CHO cells in the HGPRT forward mutation assay.	Godin et al. (1992); Myhr et al. (1990)

Test	Materials and methods	Results	Conclusions	References
Cytogenetic assays in CHO cells	CHO cells were treated with PFDA to evaluate induction of sister chromatic exchange (SCE) and chromosomal aberrations with or without S9. PFDA concentrations of 0.167 to 5,000 µg/mL were tested in the SCE assays and 7.50 to 201 µg/mL were used in the chromosomal aberration assay.	The results of the SCE assay were negative in the presence or absence of S9 metabolic activation. PFDA did induce chromosomal aberrations at 151 and 201 µg/mL but only under conditions of metabolic S9 activation. Cytotoxicity was observed at a concentration of 201 µg/mL in the chromosomal aberration assay.	Induction of chromosomal aberrations provides evidence of clastogenic activity of PFDA in combination with S9. PFDA did not cause DNA damage in the SCE assay.	Godin et al. (1992); Myhr et al. (1990)
In vitro transformation of BALB/C-3T3 cells	BALB/C-3T3 mouse cells were treated with PFDA at doses of 40.0 to 650 µg/mL with or without S9.	PFDA failed to significantly increase morphological transformation in BALB/C-3T3 cells in the presence or absence of S9 metabolism.	There is no evidence of malignant transformation with PFDA in cultured BALB/C-3T3 mouse cells.	Godin et al. (1992)
DNA damage (double-strand breaks)	Human gastric adenocarcinoma AGS and SGC cell lines treated with PFDA (concentration not specified).	PFDA induced double-strand DNA breaks, reduced DNA repair activity, altered expression of DNA repair gene pathways (e.g., NHEJ), inhibited apoptosis via p53 downregulation and affected chemotherapy sensitivity of human gastric cells.	PFDA can cause double strand DNA damage in vitro by altering DNA repair mechanisms.	Liu et al. (2019a)
DNA damage (strand breaks and oxidative damage [8OHdG])	Primary hepatocytes isolated from male C57BL/6 mice and exposed to PFDA at doses of 0.1, 1, 10, 100 µM.	PFDA increased DNA strand breaks and levels of 8OHdG and ROS in primary mouse hepatocytes (statically significant only at highest dose for ROS but there was a dose-response gradient).	There is evidence of oxidative DNA damage with PFDA in vitro exposure.	Xu et al. (2019b)
Genotoxicity studies in mammalian species – in vivo				
Unscheduled DNA synthesis (UDS) and S-phase induction assays	Adult male F344 rats were treated by oral gavage with a dose of PFDA (5.5 to 44.0 mg/kg) and primary hepatocyte cultures were prepared ~15–48 h after treatment to examine nuclear labeling.	PFDA was found to be inactive in the UDS assays but induced a significant increase in the number of S-phase cells at doses ≥11.0 mg/kg.	S-phase induction provides some in vivo evidence of genotoxicity with PFDA.	Godin et al. (1992); Myhr et al. (1990)

Test	Materials and methods	Results	Conclusions	References
Oxidative DNA damage (8OHdG)	Male Fischer F344 rats were treated with PFDA (0.01% or 10 mg/kg-d) via the diet for 14 d. DNA was isolated from the liver and kidney of rats after treatment for analysis of 8OHdG formation.	8OHdG levels were significantly increased by PFDA treatment in rat liver but no effects were seen in the kidney.	PFDA (10/mg/kg-d) caused oxidative DNA damage in rat liver after repeated dose exposure via the diet.	(Takagi et al., 1991)
Oxidative DNA damage (8OHdG)	Female Sprague Dawley rats were treated with a dose of 10 mg/kg PFDA via i.p. once a week for a 2- or 8-week period. DNA was isolated from rat liver after treatment for analysis of 8OHdG formation.	8OHdG levels were not significantly affected by PFDA treatment in the two time points analyzed.	PFDA (1.4 mg/kg-d) did not cause oxidative DNA damage in rat liver after repeated dose exposure via i.p. administration.	Kim et al. (1998)
Micronucleus assay	Male and female Sprague Dawley rats (5/group) were exposed daily to PFDA by oral gavage at doses of 0, 0.156, 0.312, 0.625, 1.25 and 2.5 (males only) mg/kg for 28 d.	Test results were negative for the increase in frequency of micronucleated polychromatic or normochromatic erythrocytes in rat blood.	There is no evidence of PFDA (0.156–2.5 mg/kg-d) genotoxicity in the erythrocyte micronucleus assay.	NTP (2012)
DNA damage (double-strand breaks)	Mice were exposed to PFDA via drinking water (dosing regimen was not specified)	PFDA induced double-strand DNA breaks in mouse gastric cells.	PFDA can cause double strand DNA damage in vivo.	Liu et al. (2019a)

CA = chromosomal aberration; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; LD50 = median lethal dose; ROS = reactive oxygen species; S-D = Sprague Dawley.

Evidence integration

The available evidence to evaluate the potential for PFDA exposure to lead to the development of any cancer type consists of sparse and minimally informative studies in humans and animals and limited mechanistic information from genotoxicity studies. Specifically, the single *low* confidence study of prostate cancer (reporting an association that was not statistically significant) in exposed humans, as well as the single, *low* confidence null study in rats with poor sensitivity due to short-term duration are of limited utility for drawing a conclusion regarding potential carcinogenicity with PFDA exposure. The results from genotoxicity studies were mostly null, although a few studies provided some evidence of potential genotoxic effects in response to PFDA (i.e., clastogenic effects in CHO cells, S-phase induction in rat hepatocytes, double strand DNA breaks in human and mouse gastric cells and oxidative DNA damage in primary mouse hepatocytes). Considering evidence for all potential cancer types across the available human, animal and mechanistic studies and based on the EPA cancer guidelines (U.S. EPA, 2005), the evidence base is judged to be ***inadequate to assess the carcinogenic potential*** of PFDA in humans.

4. SUMMARY OF HAZARD IDENTIFICATION CONCLUSIONS

4.1. SUMMARY OF CONCLUSIONS FOR NONCANCER HEALTH EFFECTS

The available ***evidence indicates*** hazards likely exist with respect to the potential for liver, immune, developmental, and male and female reproductive effects in humans, given sufficient PFDA exposure conditions[12]. Additionally, the available ***evidence suggests*** that PFDA exposure might also have the potential to cause cardiometabolic and neurodevelopmental effects in humans given sufficient exposure conditions. These judgments were derived primarily from epidemiological studies and studies in experimental animals, the latter exposed to PFDA during short-term (7–28 days) and developmental (GD 6–15) oral exposures. On the other hand, there is ***inadequate evidence*** for urinary, endocrine, and other health effects to determine the potential for health hazards in humans with PFDA exposure. A summary of the justifications for the evidence integration judgments for each of the main hazard sections is provided below.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause liver effects in humans, given sufficient exposure conditions[12], is based on concordant effects for increased liver weight, alterations in levels of serum biomarkers of liver injury (ALT, AST, ALP, bile salts/acids, bilirubin and blood proteins), and some evidence of hepatocyte degenerative or necrotic changes that provide support for the adversity of PFDA-induced liver toxicity reported in *high and medium* confidence studies in rats and mice exposed to PFDA doses ≥0.156 mg/kg-day. Although associations between serum ALT levels and PFDA exposure in epidemiological studies of adults were observed, the epidemiology evidence overall is uncertain due to unexplained inconsistency in the results for other clinical markers and a lack of clear evidence of adversity. Mechanistic studies in rodents and limited evidence from in vitro studies and animal models considered more relevant to humans provide support for the biological plausibility and human relevance of the apical effects observed in animals and suggest a possible PPARα-dependent and independent MOA for PFDA-induced liver toxicity.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause immunosuppression in humans, given sufficient exposure conditions[12], is based on *moderate* human evidence of immunosuppression primarily from two *medium* confidence studies in children and one *low* confidence study in adults at levels of 0.3 ng/mL (median exposure in studies observing an adverse effect). Although some evidence for coherent immunomodulatory responses

[12] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

consistent with immunosuppression (decreases in phagocytic activity of liver microphages, spleen cell counts and immune organ weights and immune organ histopathology) was identified in short-term, *high, and medium* confidence studies in rats and mice at ≥0.089 mg/kg-day, the animal evidence overall is uncertain. Issues with overt organ and general systemic toxicity pose limitations with respect to the interpretation of the animal evidence. Although possible effects of hypersensitivity-related responses were reported in one epidemiological study and one high-exposure study in mice (21.4 mg/kg-day), outstanding uncertainties remain to draw specific conclusions for this outcome.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause developmental toxicity, given sufficient exposure conditions[13], is based primarily on consistent findings of dose-dependent decreases in fetal weight in mice gestationally exposed to PFDA doses ≥0.5 mg/kg-day, supported by evidence of decreased birth and childhood weight from studies of exposed humans in which PFDA was measured during pregnancy. The conclusion is further supported by coherent epidemiological evidence for biologically related effects (e.g., decreased birth length).

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause potential adverse effects to the male reproductive system in humans, given sufficient exposure conditions, is based on a coherent pattern of effects on sperm counts, testosterone levels, and male reproductive histopathology and organ weights at doses ≥0.625 mg/kg-day in adult rats exposed for 28 days (*high* confidence for most endpoints evaluated). Although the MOA for PFDA-induced male reproductive effects is unknown, a few acute i.p. and in vitro rodent studies suggest a possible mechanism via disruption of Leydig cell function and impaired steroidogenesis. Evidence from a *medium* confidence epidemiological study reported non-statistically significant decreases in testosterone levels and altered sperm parameters that are coherent with the effects observed in animals. Although these findings were imprecise, the study had limited sensitivity to observe an effect and no conflicting evidence was identified from studies of similar confidence.

The hazard identification judgment that the ***evidence indicates*** PFDA exposure is likely to cause female reproductive toxicity in humans given sufficient exposure conditions is based primarily on the results of a *high* confidence study in rats showing biologically coherent effects on uterus weight and the estrous cycle after oral exposure to PFDA at ≥1.25 mg/kg-day for 28 days. Although human studies are available for examining associations between PFDA and female reproductive toxicity (e.g., fecundity), the results were mostly null, possibly due to their low sensitivity for observing effects.

[13] The "sufficient exposure conditions" are more fully evaluated and defined for the identified health effects through dose-response analysis in Section 5.

The hazard identification judgment that the ***evidence suggests*** PFDA exposure has the potential to cause cardiometabolic effects in humans given sufficient exposure conditions[14] is based primarily on associations between PFDA and serum lipids, adiposity, cardiovascular disease, and atherosclerosis in a few epidemiological studies. However, evidence is largely inconsistent across studies, which adds considerable uncertainty. Evidence in experimental animals from a *high* confidence rat study was *indeterminate*.

The hazard identification judgment that the ***evidence suggests*** PFDA exposure has the potential to cause neurodevelopmental effects in humans given sufficient exposure conditions[15] is based on associations between PFDA exposure and outcomes related to attention and behavior, although there is high degree of uncertainty due to inconsistencies and imprecision in the results. No relevant animal studies were available.

Finally, there is ***inadequate evidence*** to evaluate the potential for PFDA exposure to cause effects on the endocrine system, urinary system, and other health outcomes in adult humans (i.e., respiratory, digestive, dermal, musculoskeletal, and hematological systems, and nonspecific clinical chemistry). The available data from human and/or animal studies for these health outcomes was largely limited or lacked consistency and coherence. Further, the absence of studies examining the potential for effects of PFDA exposure on the thyroid in developing organisms, or on mammary glands, represent data gaps in light of associations observed for other PFAS, such as PFBS, PFOA and PFOS (ATSDR, 2018b; U.S. EPA, 2018), see Table 4-1 below.

Table 4-1. Hazard conclusions across published EPA PFAS human health assessments

Health Outcome	EPA PFAS Assessments[a,b]					
	PFDA	PFBA	PFBS	GenX Chemicals	PFOA[c]	PFOS[c]
Thyroid	-	+	+	-[d]	Human: + Animal: +/-	Human: +/- Animal: +/-
Liver	+	+	-	+	Human: + Animal: +	Human: - Animal: +
Developmental	+	+	+	+/-	Human: + Animal: +	Human: + Animal: +
Reproductive	+	-	-	+/-	Human: - Animal: +/-	-[d]
Immunotoxicity	+	-	-	+/-	Human: + Animal: +	Human: +/- Animal: +

[14] Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advance for dose-response analysis in Section 5).

[15] Given the uncertainty in this judgment and the available evidence, this assessment does not attempt to define what might be the "sufficient exposure conditions" for developing these outcomes (i.e., these health effects are not advance for dose-response analysis in Section 5).

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

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

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

[c] The U.S. EPA (2016b) and U.S. EPA (2016a) PFOA and PFOS assessments did not use structured language to summarize the noncancer hazard conclusions. The presentation in this table was inferred from the hazard summaries found in the respective assessments; however, this is for comparison purposes only and should not be taken as representative of the conclusions from these assessments. Those interested in the specific noncancer hazard conclusions for PFOA and PFOS must consult the source assessments.

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

[e] Data available for PFDA includes neurodevelopmental outcomes in humans.

4.2. SUMMARY OF CONCLUSIONS FOR CARCINOGENICITY

Given the limited scope and utility of the available evidence across human, animals and genotoxicity studies, the evidence is judged to be insufficient to determine whether PFDA exposure (via any exposure route) might affect the development of any specific cancer types. In accordance with EPA cancer guidelines (U.S. EPA, 2005) a weight of evidence descriptor of ***inadequate to assess the carcinogenic potential*** is assigned for PFDA.

4.3. CONCLUSIONS REGARDING SUSCEPTIBLE POPULATIONS AND LIFE STAGES

Understanding of potential areas of susceptibility to the identified human health hazards of PFDA can help to inform expectations of variability in responses across individuals, as well as uncertainties and confidence in candidate toxicity values (see Section 5.2). The available human and animal studies indicate that early life represents a susceptible lifestage for the effects of PFDA exposure. Two medium confidence studies reported immune effects (i.e., decreased antibody response) in children exposed to PFDA during gestation and childhood (Grandjean et al., 2017b) and (Grandjean et al., 2017a; Grandjean et al., 2012). Additionally, developmental effects (i.e., fetal growth restriction, gestational duration, postnatal growth and spontaneous abortion) were reported in multiple high quality studies (Buck Louis et al., 2018; Gyllenhammar et al., 2018; Meng et al., 2018; Lind et al., 2017a; Swedish Environmental Protection Agency, 2017; Valvi et al., 2017; Woods et al., 2017; Bach et al., 2016; Kwon et al., 2016; Lenters et al., 2016; Wang et al., 2016; Robledo et al., 2015). The strongest and most consistent evidence was observed for fetal growth restriction. Potentially coherent with these epidemiological observations, effects in developing rodents (decreased fetal body weight, skeletal variations, decreased live fetuses per litter) after maternal exposure also support the potential for early life susceptibility. Young individuals may also be susceptible to PFDA-induced male reproductive effects. Although no animal studies and only a few human studies are available examining reproductive effects in early lifestages (i.e., pubertal development and anogenital distance), effects on sperm motility and testosterone were consistently reported in exposed human and rodent adults (NTP, 2018; Zhou et al., 2016; Joensen et al., 2013). Given the potential for PFDA to impair androgen function, boys exposed during critical developmental lifestages may be susceptible as exposure during gestation and early postnatal life stages could result in agenesis of the male reproductive system and/or infertility.

Although inconclusive, some effects on thyroid hormone homeostasis were observed in adult rats (NTP, 2018). Although no studies are available that assessed the effect of PFDA on thyroid hormones in developing organisms, young individuals exposed during gestation, early childhood and puberty may be a susceptible population given that T3 and T4 levels play critical roles in bone growth and brain development (O'Shaughnessy et al., 2019) at these lifestages (i.e., both pregnancy and early life). PFDA was also observed to disrupt estrous cyclicity in female

rats with potential implications for impaired fertility (NTP, 2018). Therefore, although the current evidence does not explicitly address the potential for a linkage between these observations and impaired fertility in women, women of reproductive age may also be susceptible to the effects of PFDA exposure.

5. DERIVATION OF TOXICITY VALUES

5.1. NONCANCER AND CANCER HEALTH EFFECT CATEGORIES CONSIDERED

The available ***evidence indicates*** that oral exposure to PFDA is likely to cause adverse hepatic, immune, developmental, and male and female reproductive effects in humans given sufficient exposure conditions[3], based on epidemiological and animal toxicity studies. This section aims to characterize the dose levels associated with these identified hazards and derive toxicity values as presented below. Additionally, the available ***evidence suggests*** PFDA exposure might have the potential to cause cardiometabolic and neurodevelopmental effects in humans given sufficient PFDA exposure conditions[4] based on a limited number of epidemiological studies; however, the results are considered too uncertain to support the derivation of toxicity values. For all other health effects (i.e., endocrine, urinary, hematology, special senses [eye and harderian gland], dermal and musculoskeletal systems), the ***evidence is inadequate*** to assess the hazard potential; therefore, these endpoints were not considered for the derivation of toxicity values.

There are no available studies to inform the potential for PFDA to cause adverse health effects via inhalation exposure, therefore, the derivation of reference concentrations (RfC) is precluded (see Section 5.2.4). Likewise, evidence pertaining to the evaluation of carcinogenicity was considered ***inadequate to assess carcinogenic potential*** of PFDA in humans, precluding the derivation of cancer toxicity values via any exposure route (see Section 5.3).

5.2. NONCANCER TOXICITY VALUES

The noncancer toxicity values (i.e., RfDs) derived in this section are estimates of an exposure for a given duration to the human population (including susceptible subgroups and/or life stages) that are likely to be without an appreciable risk of adverse health effects (Section 1.2.1). The RfD derived in Section 5.2.1 corresponds to chronic, lifetime exposure and is the primary focus of this document. In addition, a less-than-lifetime toxicity value (referred to as a "subchronic RfD") is derived in Section 5.2.2. This subchronic RfD can be useful for certain decision purposes (e.g., site-specific risk assessments with less-than-lifetime exposures). Both the lifetime and subchronic RfD include organ/system-specific RfDs (osRfDs) associated with each health effect considered for point of departure (POD) derivation, as supported by the available data. These toxicity values might be useful in some contexts (e.g., when assessing the potential cumulative effects of multiple chemical exposures occurring simultaneously). Section 5.2.3 summarizes that no information exists to inform the potential toxicity of inhaled PFDA or to derive an inhalation reference concentration (RfC).

5.2.1. Oral Reference Dose (RfD) Derivation

Study/Endpoint Selection

As outlined in the sections below, data sufficient to support dose-response analyses for oral PFDA exposure were available for all identified human health hazards (see Section 4.1): hepatic, immune, developmental, and male and female reproductive effects. Rationales for study selection and the specifics of RfD calculations, as well as the determination of confidence in quantitative estimates are detailed in this section.

The following general considerations were used to prioritize studies for estimating points of departure (PODs) for potential use in toxicity value derivation. Dependent on the evidence for each identified hazard, *high* or *medium* confidence human studies that were deemed influential to the hazard conclusions and suitable for dose-response analysis were prioritized for POD derivation and compared to PODs derived from animal data when possible. Human studies were available for developmental and immunotoxicity effects. For other health effects (i.e., hepatic, and male and female reproductive effects), only evidence from animal studies was considered influential for hazard identification and, therefore, these data were prioritized for dose-response assessment. Given the lack of comprehensive subchronic or chronic animal studies, *medium* and *high* confidence short-term studies in animals of longer exposure duration (e.g., 28 days versus 7 or 14 days) and with exposure levels near the lower dose range of doses tested across the evidence base were preferred along with *medium* or *high* confidence animal studies evaluating exposure during development. These types of *medium* and *high* confidence human and animal studies increase the confidence in the resultant RfD because they represent data with lower risk of bias and reduce the need for low-dose and exposure duration extrapolation (see Appendix C.1.1,).

A summary of endpoints and rationales considered for toxicity value derivation is presented below.

Hepatic effects

The hazard conclusions for PFDA-induced liver effects are based primarily on *moderate* evidence from short-term animal studies (see Section 3.2.1). In humans, an association between PFDA exposure and ALT levels in the blood was identified, but there was considerable uncertainty due to inconsistent results for other clinical markers. As such, only animal studies were considered for dose-response analysis. The database of animal studies examining liver effects includes several short-term studies in rats and mice (Wang et al., 2020; Frawley et al., 2018; NTP, 2018; Yamamoto and Kawashima, 1997; Kawashima et al., 1995; Permadi et al., 1993; Takagi et al., 1992, 1991; Harris and Birnbaum, 1989). In particular, two *high* confidence studies in S-D rats gavaged with PFDA for 28-days were prioritized for the derivation of candidate values because they included several hepatic endpoints that together provided coherent evidence of liver toxicity with PFDA exposure across histopathology, organ weights and/or clinical chemistry (Frawley et al., 2018; NTP, 2018) (see Table 5-1). Additionally, these studies had the longest exposure duration (28 days) and

examined the lower range of PFDA doses (dose range of observed effects is 0.156–2.5 mg/kg-day) across the available studies examining hepatic effects.

PFDA induced changes in serum liver biomarkers, hepatocyte lesions and increased liver weights in rats across the two 28-day studies (Frawley et al., 2018; NTP, 2018). Although some of the individual changes have the potential to represent adaptive responses (e.g., increased liver weights and hypertrophy), the constellation of coherent liver effects, most notably consistent effects across multiple serum biomarkers of hepatocyte and biliary injury and histological findings of structural hepatocyte degeneration (necrosis), provide clear evidence of adversity (see "Consideration for potentially adaptive versus adverse responses" under Section 3.2.1 for more details). Alterations in the levels of serum enzymes such as ALT, AST and ALP and other functional biomarkers (bile salt/acids, bilirubin, and blood proteins [albumin, globulin, and total protein]) were reported in the 28-day study that evaluated clinical chemistry (NTP, 2018). Increases in AST and ALP levels were consistent across sexes and dose groups and generally occurred at lower doses that did not induce significant body weight changes or other general systemic effects (0.156–0.625 mg/kg-day PFDA). Similarly, dose-related increases in relative liver weights were reported in male and female rats at ≥0.125 mg/kg-day across the two 28-day studies (Frawley et al., 2018; NTP, 2018). As discussed in Section 3.2.1, relative liver weight is generally preferred over absolute liver weight; as information on the former were available, changes in absolute liver weight were not considered for dose-response analyses. Since there is no clear indication of sex-specific differences in sensitivity with respect to PFDA-induced liver effects in the available animal toxicity studies, data for both male and female S-D rats for these endpoints were advanced for dose-response modeling.

Corroborative hepatocyte lesions such as cytoplastic alterations and vacuolization, hypertrophy and necrosis were reported in rats at higher doses (≥0.625 mg/kg-day) across the two 28-day studies prioritized for dose-response analysis (Frawley et al., 2018; NTP, 2018). The histopathological observations showed a clear progression in severity across lesions and dose groups. These findings provide additional support for the adversity of the progressive effects on the liver with PFDA exposure but were not prioritized for dose-response analysis due to the presence of more sensitive liver endpoints (i.e., serum AST and ALP levels, and relative liver weight; see Table 5-1).

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

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased serum ALT	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male and female	No	Dose-dependent effects were only observed in females and occurred at higher doses compared to other liver findings

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased serum AST	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male and female	Yes	Dose-dependent effects were consistent across sexes and concordant with liver weight and liver histopathology findings
Increased serum ALP	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male and female	Yes	Effects were consistent across sexes and dose groups and concordant with liver weight and liver histopathology findings.
Other serum biomarkers (increased bile salts/acids and bilirubin, and decreased albumin and globulin)	NTP (2018); *high* confidence	Gavage, 28 days	S-D rat, male and female	No	Effects were mostly consistent across sexes but occurred at higher doses compared to other liver findings
Hepatocyte lesions	NTP (2018); *high* confidence (cytoplasmic alterations and vacuolization, hypertrophy, and necrosis)	Gavage, 28 days	S-D rat, male and female	No	Effects were consistent across sexes and studies but occurred at higher doses compared to other liver findings
	Frawley et al. (2018); *high* confidence (necrosis)	Gavage, 28 d	S-D rat, male	No	
Increased relative liver weight	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male and female	Yes	Dose-dependent effects were consistent across studies, cohorts, sexes and were concordant with serum biomarker and liver histopathology findings. There was no reason to prioritize one dataset over the other.
	Frawley et al. (2018); *high* confidence	Gavage, 28 d	S-D rat, female (included 3 experimental cohorts)	Yes	

Immune Effects

As described in Section 3.2.2, the strongest evidence for immune effects was from epidemiological studies that provided *moderate* evidence of immunosuppression (Shih et al., 2021; Timmermann et al., 2021; Grandjean et al., 2017b; Grandjean et al., 2017a; Kielsen et al., 2016; Grandjean et al., 2012); thus, this outcome was prioritized for dose-response analysis and studies of hypersensitivity (which collectively provided *slight* human evidence) were not considered. Given the uncertainties with the animal data described in Section 3.2.2 that would be expected to strongly impact quantitative estimates (e.g., influence of systemic toxicity), only the human data were considered for the derivation of PODs.

The two *medium* confidence epidemiology studies of antibody response following vaccination providing the primary support for the hazard judgment were conducted in different birth cohorts of the Faroe Islands population (see Table 5-2). These studies include measures of PFDA exposure taken perinatally (pregnancy week 32 to 2 weeks postpartum), at 18 months, and at 5, 7, and 13 years, and measures of antibody levels at 5, 7, and 13 years for both diphtheria and tetanus. The relevant etiologic window of exposure for this outcome is not known. Although there were some heterogeneous results (see Section 3.2.2), the direction of association across these combinations of different timings of exposure and outcome measurement were generally consistent, indicating immunosuppression (i.e., decreased antibody response with higher exposure). However, selecting the most informative exposure-outcome combination(s) for POD derivation is complicated by the lack of a clear etiologic window. In a follow-up publication without new data, the study authors performed benchmark dose modeling for a subset of the data presented in Grandjean et al. (2012), specifically antibody levels at age 7 and PFDA concentrations at age 5, and antibody levels at age 5 (prebooster) and perinatal PFDA concentrations (Budtz-Jørgensen and Grandjean, 2018b). These were selected by the authors due to the strong inverse associations observed and the results were considered reasonably representative of the study results overall. After review of the BMD methods and additional modeling details (Budtz-Jørgensen and Grandjean, 2018b) for completeness and appropriateness (see Appendix C.1 "Benchmark Dose Response Modeling Results from Human Studeis," EPA utilized their analytic regression results for this assessment.

Budtz-Jørgensen and Grandjean (2018a) fit multivariate models of PFDA measured at age 5 years, against $\log_2$-transformed anti-tetanus antibody concentrations measured at the 7 year-old examination controlling for sex, exact age at the 7 year-old examination, and booster type at age 5 years. Three model shapes of PFDA were evaluated by Budtz-Jørgensen and Grandjean (2018a): a linear model, a piecewise-linear model with a knot at the median, and a logarithmic function. Ultimately, the linear model was found to have the best fit. In the absence of a clear definition of an adverse effect for a continuous endpoint like antibody concentrations, a default BMR of one SD change from the control mean may be selected, as suggested in EPA's Benchmark Dose Technical Guidance Document (U.S. EPA, 2012a). A lower BMR can also be used if it can be justified on a biological and/or statistical basis. Regression coefficients for PFDA as the only PFAS in the model were used to estimate the BMD and BMDL for a BMR of one standard deviation (SD) change in $\log_2$-transformed anti-tetanus antibody concentration and for a BMR of ½ standard deviation (SD) change in $\log_2$-transformed anti-tetanus antibody concentration (see Appendix C.1.1 for details). Budtz-Jørgensen and Grandjean (2018a) also fit multivariate models of PFDA controlling for both PFOS and PFOA and BMD and BMDL estimates from those results were also derived in Appendix C.1.1.

Statistically, the Technical Guidance additionally suggests that studies of developmental effects can support lower BMRs, and BMRs of ½ SD (or BMRs of 5% rather than 10%) are routinely

applied to rodent developmental toxicity study endpoints due to the sensitive lifestage. Biologically, a BMR of ½ SD is considered a reasonable choice as anti-tetanus antibody concentrations prevent against tetanus, which is a rare, but severe and sometimes fatal infection, with a case-fatality rate in the U.S. of 13% during 2001–2008 (Liang et al., 2018). The case-fatality rate can be more than 80% for early lifestage cases (Patel and Mehta, 1999). Selgrade (2007) suggests that specific immuno-toxic effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards – which has the potential to be a more adverse effect than just a single immuno-toxic effect. Thus, decrements in the ability to maintain effective levels of tetanus antitoxins following immunization may be indicative of wider immunosuppression in these children exposed to PFDA. Taken together, the severity of this indicator of developmental immunosuppression and the sensitive lifestage is interpreted to support the use of a BMR of ½ SD

A blood concentration for tetanus antibodies of 0.1 IU/mL is sometimes cited in the tetanus literature as a 'protective level' and (Grandjean et al., 2017b) noted that the Danish vaccine producer Statens Serum Institut recommended the 0.1 IU/mL "cutoff" level "to determine whether antibody concentrations could be considered protective;" and Galazka and Kardymowicz (1989)mentions the same concentration, but Galazka et al. (1993)argues:

> "*The amount of circulating antitoxin needed to ensure complete immunity against tetanus is not known for certain. Establishment of a fixed level of tetanus antitoxin does not take into consideration variable conditions of production and adsorption of tetanus toxin in the anaerobic area of a wound or a necrotic umbilical stump. A given serum level could be overwhelmed by a sufficiently large dose of toxin. Therefore, there is no absolute protective level of antitoxin and protection results when there is sufficient toxin-neutralizing antibody in relation to the toxin load* (Passen and Andersen, 1986)."

As a check, EPA evaluated how much extra risk would have been associated with a BMR set at a cutoff value of 0.1 IU/mL. Using the observed distribution of tetanus antibodies at age 7 years in $\log_2$(IU/mL), EPA calculated that 2.8% of those values would be below the cutoff value of 0.1 IU/mL. A BMR of ½ SD resulted in 7.9% of the values being below that cutoff which is 5.1% extra risk and shows that the generic guidance that a BMR of ½ SD can provide a reasonably good estimate of 5% extra risk.

Table 5-2. Endpoints considered for dose-response modeling and derivation of points of departure for immune effects in humans

Endpoint	Study reference and confidence	POD derived?	Notes
Antibody concentrations for diphtheria and tetanus	Grandjean et al. (2012) [Birth cohort 1997–2000 with follow-up to age 7] and (Grandjean et al., 2017a) [Birth cohort 1997–2000 with follow-up to age 13]; Grandjean et al. (2017b) [Birth cohorts from 1997–2000 & 2007–2009 with follow-up to age 5]; *medium* confidence	No	Effect was generally coherent with epidemiological evidence for other antibody effects. However, while these results contribute to understanding the hazard for PFDA, the analytic models in these specific publications used log-transformed exposure and log-transformed outcome variables and such log-log models cannot be used for BMD calculations and thus PODs were not derived.
Antibody concentrations for diphtheria and tetanus	Budtz-Jørgensen and Grandjean (2018a); Birth cohorts 1997–2000 & 2007–2009 using different analyses of combined data from Grandjean et al. (2012) and (2017a) *medium* confidence	Yes	Effect was large in magnitude and generally coherent with epidemiological evidence for other antibody effects. Results were based on analytic models using log-transformed outcome and untransformed exposure which were suitable for BMD calculations and POD derivations (see Appendix C.1.1 for more details on BMD modeling results).

Developmental effects

Uncertainties in the human evidence of developmental effects resulted in a judgment of slight (see Section 3.2.3); however, the database includes several well-conducted *medium* and *high* confidence epidemiological studies reporting birth weight deficits of varying magnitude in male or female neonates or both. Birth weight deficits (and several other developmental endpoints) were generally larger and more consistent among studies that sampled maternal serum later in pregnancy including postpartum measures. This suggests that those samples may be most prone to potential bias from changing pregnancy hemodynamics, but the complex patterns of influence due to pregnancy hemodynamics are not completely understood. Nevertheless, the apparent influence of pregnancy hemodynamics introduces considerable uncertainty in the interpretation of these associations of PFDA-induced developmental effects and was a major contributing factor in the overall evidence integration judgement for this health effect (see Section 3.2.3). Despite these concerns regarding sample timing, decreased birth weight was the focus of dose-response analysis, given the accuracy in measurement of the endpoint, and the abundance of high-quality studies. There is considerably less uncertainty related to pregnancy hemodynamics in studies based on maternal serum samples collected during the first trimester.

Twenty-eight epidemiology studies (8 high and 10 medium confidence) evaluated associations between PFDA and fetal growth restriction, including 26 studies examining mean birth weight. Given the abundance of high confidence studies, low and medium confidence studies were not considered for POD derivation; thus, four *high* confidence studies were considered as they provided consistent evidence of associations within the overall population and across both sexes. Among the eight high confidence studies detailed in Table 5-3, two studies Buck Louis et al. (2018);

Bach et al. (2016) were not considered further, as they did not find evidence of an inverse association between PFDA exposures and mean birth weight in the overall population. Two studies were not advanced because they reported vastly different findings across the sexes Lind et al. (2017a); Wang et al. (2016) with no clear biological explanation for this inconsistency (see discussion in Section 3.2.3).

Three of the four remaining studies examined PFDA during trimester three Luo et al. (2021); Yao et al. (2021); Valvi et al. (2017) and one examined PFDA across trimesters one and two (Wikström et al., 2020).Two high confidence studies Valvi et al. (2017) and Wikström et al. (2020) were selected for dose-response quantification. In the (Wikström et al., 2020) study, 96% of samples were collected during the first trimester and the remaining during the early weeks of the second trimester; sensitivity analyses showed no differences when trimester two samples excluded. The Valvi et al. (2017) has a unique design that may increase study sensitivity by sampling all participants during the same gestational week (i.e., 34). These two studies had a low overall risk of bias and reliable exposure measurements with sufficient exposure contrasts (PFDA median/interquartile ranges: 0.26/0.15 and 0.28/0.16 ng/mL, respectively for Wikström et al. (2020); Valvi et al. (2017)) and other characteristics that allowed for adequate study sensitivity to detect associations (see Table 5-4). As noted above, the Valvi et al. (2017) and Wikström et al. (2020) studies selected for dose-response quantification reported results consistent in magnitude that allowed the consideration of sex-specific and overall population results. A limitation of the Valvi et al. (2017) study advancing to dose-response is that it did not have early trimester samples (trimester 3 only) and may be prone to some potential bias due to pregnancy hemodynamics (see more details in Appendix F). Despite these important concerns regarding sample timing, as noted above, derivation of a POD(s) for developmental outcomes using the Valvi, 2017 study was considered potentially informative to toxicity value derivation for birth weight effects reported by (Wikström et al., 2020).

The one available *high* confidence animal study that examined developmental toxicity in mice treated with PFDA (Harris and Birnbaum, 1989) provided moderate evidence of developmental toxicity (see Section 3.2.3). Several endpoints from this study were considered to be suitable for POD derivation (see Table 5-5) and for comparison to PODs derived from the human studies. Harris and Birnbaum (1989) reported developmental effects in C57BL/6N mice treated either on GD 10–13 (0–32 mg/kg-day) or GD 6–15 (0–12.8 mg/kg-day). Harris and Birnbaum (1989) reported statistically significant changes for increased % resorptions per litter and decreased number of live fetuses GD 6–15 component of the study. However, these effects were not considered for dose-response analysis because their interpretation is confounded by overt maternal toxicity (i.e., mortality) observed at the same dose. Statistically significant and dose-dependent decreases in fetal body weight were also observed in both the GD 10–13 and the GD 6–15 experiments. Data for decreased fetal body weight from the GD 6–15 experiment were prioritized for dose-response analysis over data from the GD 10–13 experiment, since the former

experiment encompasses a larger developmental window. Statistically significant and dose-dependent increases in variations (i.e., delayed braincase and phalanges ossification and absence of fifth sternebrae) were also reported, but there were methodological concerns and uncertainty regarding the adversity of these endpoints (see Section 3.2.3) that precluded their consideration for dose-response analysis.

Table 5-3. Mean Birth Weight deficit studies considered for dose-response modeling and derivation of points of departure for developmental effects in humans

Study reference and confidence	Population-Overall Population, Sex-specific and All Births vs. Term Births only	PFDA Biomarker Sample Timing	POD derived?	Notes
Valvi et al. (2017); *high* confidence	Overall Population; Sex-specific; All Births	Trimester 3	Yes	Effect was large in magnitude and coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., decreased postnatal growth and birth length).
Wikström et al. (2020), *high* confidence	Overall Population; Sex-specific; All Births	Trimesters 1-2 (94% in T1)	Yes	Effect was statistically significant, large in magnitude, and coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., decreased postnatal growth and birth length).
Luo et al. (2021), *high* confidence	Overall Population; Term Births	Trimester 3	No	Effect size was statistically significant and moderate in magnitude. Results are coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., preterm birth, postnatal growth, and other fetal growth measures such as birth length).
Yao et al. (2021), *high* confidence	Overall Population; Sex-specific; All Births	Trimester 3	No	Effect size was moderate in magnitude. Results are coherent with findings in mice and epidemiological evidence for other biologically related effects (e.g., preterm birth, postnatal growth, and other fetal growth measures such as birth length).
Wang et al. (2016); *high* confidence	Sex-specific; Term Births	Trimester 3	No	Study reported sex-specific findings that were not consistent across male and female neonates.
Bach et al. (2016); *high* confidence	Sex-specific; Term Births	Trimester 1	No	Study reported sex-specific findings that were not consistent across male and female neonates.
Buck Louis et al. (2018), *high* confidence	Overall Population; Term Births	Trimester 2	No	Study did not detect inverse associations between mean birth weight and PFDA.
Lind et al. (2017a), *high* confidence	Sex-specific; All Births	Trimester 1	No	Study reported sex-specific findings that were not consistent across male and female neonates.

Table 5-4. Endpoints considered for dose-response modeling and derivation of points of departure for developmental effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Increased % resorptions per litter	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	Effect was observed at the same dose as significant maternal mortality.
Decreased live fetuses per litter	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	Effect was observed at the same dose as significant maternal mortality.
Decreased fetal body weight	Harris and Birnbaum (1989); *medium* confidence	Gavage, GD 10–13	C57BL/6N mouse, male and female	No	Fetal body weight data from GD 10–13 was not advanced in lieu of the more sensitive data available from GD 6–15.
Decreased fetal body weight	Harris and Birnbaum (1989); *medium* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	Yes	Effect displayed a dose-response trend and was coherent with other developmental changes in mice and humans.
Skeletal variations (i.e., delayed braincase ossification; absence of fifth sternebrae; delayed phalanges ossification)	Harris and Birnbaum (1989); *high* confidence	Gavage, GD 6–15	C57BL/6N mouse, male and female	No	The adversity and interpretation of these effects is unclear (see Section 3.2.3)

Male reproductive effects

The hazard conclusions for PFDA-induced male reproductive effects are driven by *moderate* evidence from a single, *high* confidence study in rats gavaged for 28 days (NTP, 2018). The available evidence from human studies was *indeterminate* (see Section 3.2.4); thus, there was no further consideration of these human studies for POD derivation.

The single, 28-days study in adult male rats examining reproductive effects was considered *low* confidence for sperm evaluations based on potential reduced sensitivity due to inadequate exposure duration. Otherwise, the study would have been considered high confidence for sperm measures and was considered *high* confidence for other, related male reproductive endpoints. Thus, the coherent results across multiple measures, including sperm evaluations, in this well-conducted study provide support for advancing the study for dose-response modeling. Effects in male rats included significant decreases in testicular and epididymal sperm counts at doses ≥1.25 mg/kg-day (NTP, 2018). Although there are concerns over exposure sensitivity for sperm evaluations, the alterations in sperm counts are supported by concordant effects for histopathology and organ weight measures in the testis and epididymis evaluated. The decreases in absolute

epididymal sperm counts (but not testicular sperm counts) displayed a dose-response gradient and thus were prioritized for POD derivation (see Table 5-5).

A consistent pattern of mild degenerative changes was detected in the testes and epididymis of exposed rats at the two highest doses (NTP, 2018). These doses were associated with moderate body weight decreases (21–38%) but concerns over potential confounding with overt systemic toxicity were mitigated by mechanistic evidence suggesting that male reproductive effects are only affected by severe changes in body weight (72%; see Mechanistic studies and supplemental information in Section 3.2.4). Increased incidence of Leydig cell atrophy was observed at doses ≥1.25 mg/kg-day, which is consistent with reductions in spermatogenesis and serum testosterone levels reported in this same 28-day rat study and with mechanistic evidence that suggests PFDA targets Leydig cells and disrupts steroidogenesis (see Mechanistic studies and supplemental information in Section 3.2.4). As such, this endpoint was selected for dose-response modeling (see Table 5-5). Other corroborative histopathological lesions (germinal epithelium degeneration, seminiferous tubule spermatid retention, epididymal duct germ cell exfoliation and hypospermia in the epididymis) were not advanced, as these lesions occurred mostly in the high-dose group (2.5 mg/kg-day) and had low to medium incidence rates (10–40% compared to 0-10% for controls). Finally, decreases in absolute testicular and epididymal weights and serum testosterone levels identified in rats were also advanced for POD derivation. Absolute weights are the preferred measure for testis and epididymis as these organs appeared to be conserved even with body weight changes (Creasy and Chapin, 2018; U.S. EPA, 1996b). The changes in organ weights and testosterone levels demonstrated a dose-response effect and were concordant with other male reproductive findings occurring at similar doses (≥1.25 mg/kg-day) (NTP, 2018).

Table 5-5. Endpoints considered for dose-response modeling and derivation of points of departure for male reproductive effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Decreased testicular sperm counts	NTP (2018); *low* confidence	Gavage, 28 d	S-D rat, male	No	Effects provide corroborative evidence of male reproductive toxicity but were not dose dependent.
Decreased absolute epididymis sperm counts (cauda)	NTP (2018); *low* confidence due to concern for potential insensitivity	Gavage, 28 d	S-D rat, male	Yes	Effects displayed a dose-response pattern and were coherent with other male reproductive findings
Leydig cell atrophy	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male	Yes	Effects were coherent with other male reproductive findings and mechanistic evidence supporting biological plausibility

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Other histopathological lesions in the testes and epididymis	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male	No	Effects provide corroborative evidence of male reproductive toxicity but were less sensitive compared to other findings
Decreased serum testosterone levels	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male	Yes	Effects displayed a dose-response pattern and were coherent with other male reproductive system findings
Decreased absolute testis weight	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male	Yes	
Decreased absolute epididymis weight (cauda and whole)	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, male	Yes	

Female reproductive effects

The available human evidence was judged to be *indeterminate* and thus these data were not considered for dose-response analysis (see Section 3.2.5). Only one animal study (NTP, 2018) evaluated female reproductive effects due to PFDA exposure; the study was evaluated as *high confidence* for all endpoints examined and provided *moderate* evidence for female reproductive toxicity. The NTP (2018) study reported reproductive effects in female rats exposed to PFDA (doses of 0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg-day) via gavage for 28 days (see Table 5-6). Statistically significant dose-dependent changes were observed for the number of days spent in estrus and diestrus and for absolute and relative uterus weights; these endpoints were advanced for POD derivation. Although Bailey et al. (2004) provided guidance on the preferred measure (relative or absolute) for many organs (e.g., liver), both relative and absolute uterus weight were carried forward for POD derivation because it is unclear which is the preferred measure for this organ. Endpoints related to estrous cyclicity were also advanced for POD derivation. Under normal conditions, the estrus stage is highlighted by sexual receptivity (Goldman et al., 2007). PFDA was shown to decrease the number of days spent in estrus in female rats, which could result in decreased opportunities for mating and ultimately in reductions or delays in fertility. PFDA was also reported to cause a continuous state of diestrus (NTP, 2018). Per the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment, "Persistent diestrus indicates temporary or permanent cessation of follicular development and ovulation, and thus at least temporary infertility"; please refer to Section 3.2.5 for a more detailed discussion. Whereas the study authors also reported increased testosterone in female rats, this effect was not considered further because its biological relevance to the development of PFDA-induced female reproductive toxicity is unclear.

Table 5-6. Endpoints considered for dose-response modeling and derivation of points of departure for female reproductive effects in animals

Endpoint	Study reference and confidence	Exposure route and duration	Test strain, species, and sex	POD derived?	Notes
Decreased estrus time	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Increased diestrus time	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Decreased absolute and relative uterus weight	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, female	Yes	Effect displayed a dose-response trend and was coherent with other female reproductive changes.
Increased testosterone	NTP (2018); *high* confidence	Gavage, 28 d	S-D rat, female	No	The toxicological significance of this effect in females for the purposes of this assessment is unclear.

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

Consistent with EPA's *Benchmark Dose Technical Guidance* (U.S. EPA, 2012a), the BMD and 95% lower confidence limit on the BMD (BMDL) were estimated using a BMR selected to represent a minimal, biologically significant level of change. The BMD technical guidance (U.S. EPA, 2012a) sets up a hierarchy by which BMRs are selected, with the first and preferred approach using a biological or toxicological basis to define what minimal level of response or change is biologically significant. If that biological or toxicological information is lacking, the BMD technical guidance recommends alternative BMRs, specifically a BMR of 1 standard deviation (SD) from the control mean for continuous data or a BMR of 10% extra risk (ER) for dichotomous data (see Appendix D for more details). In cases when a biological or toxicological basis to define what minimal level of response or change is biologically significant is lacking, a BMR of less than 1 SD is also considered when there are concerns about the severity of the effect, or effects occur in a sensitive lifestage. The BMRs selected for dose-response modeling of PFDA-induced health effects are listed in Table 5-7 along with the rationale for their selection.

Table 5-7. Benchmark response levels selected for BMD modeling of PFDA health outcomes

Endpoint	BMR	Rationale
Liver effects		
Increased serum enzymes in adult rats (ALT and ALP)	1 standard deviation	No information is readily available that allows for determining a minimally biologically significant response.

Endpoint	BMR	Rationale
		The BMD Technical Guidance (U.S. EPA, 2012a) recommends a BMR based on 1 standard deviation (SD) for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.
Increased relative liver weight in adult rats	10% relative deviation	A 10% increase in liver weight is considered a minimally biologically significant response level in adult animals and has been used as the BMR for benchmark dose modeling in prior IRIS assessments.
Immune effects		
Decreased antibody concentrations for diphtheria and tetanus in children	½ standard deviation	Diphtheria and tetanus are serious and sometimes fatal infections. Immunomodulatory effects observed in children may be broadly indicative of developmental immunosuppression impacting these children's ability to protect against a range of immune hazards. In addition, childhood represents a sensitive lifestage. Given the potential severity of this outcome, a BMR of both 1 SD and ½ SD were considered (see additional discussion in Appendix C.1.1). Ultimately, it was concluded that a BMR of ½ SD is best supported based on the severity of the outcome and the sensitive lifestage.
Developmental effects		
Decreased birth weight in humans	5% extra risk of exceeding adversity cutoff (hybrid approach)	A 5% extra risk is commonly used for dichotomous developmental endpoints as recommended by Benchmark Dose Technical Guidance (U.S. EPA, 2012a). For birth weight, a public health definition of low birth weight exists, and the hybrid approach was used to estimate the dose at which the extra risk of falling below that cut-off equaled 5%.
Decreased fetal weight in mice	5% relative deviation	A 5% change was used because the developmental effects were observed during a sensitive lifestage. A 5% change in markers of growth/development in gestational studies (e.g., fetal weight) is considered a minimally biologically significant response level and has been used as the BMR for benchmark dose modeling in prior IRIS assessments (U.S. EPA, 2012b, 2004, 2003).
Male reproductive effects		
Increased Leydig cell atrophy in adult rats	10% extra risk	No information is readily available that allows for determining a minimally biological significant response. A 10% ER is recommended as the standard BMR for dichotomous endpoints in the absence of information for a biologically based BMR (U.S. EPA, 2012a).
Decreased epididymal sperm counts in adult rats	1 standard deviation	No information is readily available that allows for determining a minimally biological significant response. The BMD Technical Guidance (U.S. EPA, 2012a) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.
Decreased serum testosterone in adult rats		
Decreased testicular weight in adult rats		

Endpoint	BMR	Rationale
Decreased epididymal weight in adult rats		
Female reproductive effects		
Decreased estrus time in adult rats	5% relative deviation	Given that the PFDA-induced alterations in estrous cyclicity are possible indicators of infertility, which is an outcome of serious concern to the human population, a BMR of 5% RD is selected for these effects. Further support for the BMR of 5% RD is provided by the large magnitude of these effects. Specifically, PFDA induced a continuous state of diestrus in 100% of rats at the highest dose tested.
Increased diestrus time in adult rats		
Decreased absolute and relative uterus weight in adult rats	1 standard deviation	No information is readily available that allows for determining a minimally biologically significant response. The BMD Technical Guidance (U.S. EPA, 2012a) recommends a BMR based on 1 SD for continuous endpoints when biological information is not sufficient to identify an appropriate BMR.

Where modeling was feasible, the estimated BMDLs were used as points of departure (PODS, see Table 5-7). Further details, including the modeling output and graphical results for the model selected for each endpoint, can be found in Appendix C. Where dose-response modeling was not feasible, or adequate modeling results were not obtained, NOAEL or LOAEL values were identified based on biological rationales when possible and used as the POD. NOAELs and LOAELs were determined based on the dose at which biologically significant changes were identified, which takes precedence over statistical significance. For example, for relative liver weight, a 10% change is generally viewed as a biologically significant level of change, taking into consideration the study-specific variability. If no biological rationale for selecting the NOAEL/LOAEL is available, statistical significance was used as the basis for selection. The PODs (based on BMD modeling or NOAEL/LOAEL selection) for the endpoints advanced for dose-response analysis are presented in Table 5-7.

Application of data-derived extrapolation factors for animal-human extrapolation of PFDA toxicological endpoints and dosimetric interpretation of epidemiological endpoints

Table 5-8 displays the POD and estimated HED PODs for liver, immune, developmental, and male and female reproductive endpoints from animal and/or human studies selected for the derivation of candidate values. Given that the available studies tested the free acid form of PFDA, normalization from a salt to the free acid using a molecular weight conversion was not performed, but formulas for providing such conversions are included in later tables.

Table 5-8. PODs considered for the derivation of PFDA candidate values

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD type/model	POD (mg/kg-day)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-day)
Liver effects						
Increased AST	28-d study (NTP, 2018); *high* confidence	SD rat, male	$BMDL_{1SD}$, Hill CV	0.123	--	1.16×10^{-3}
		SD rat, female	NOAEL[c] (1% increase)	0.625	--	4.00×10^{-3}
Increased ALP		SD rat, male	NOAEL[d] (9% increase)	0.156	--	1.47×10^{-3}
		SD rat, female	NOAEL[c] (14% increase)	0.156	--	1.00×10^{-3}
Increased relative liver weight		SD rat, male	$BMDL_{10RD}$, Hill CV	0.170	--	1.60×10^{-3}
		SD rat, female	$BMDL_{10RD}$, Hill CV[(e)]	0.112	--	7.17×10^{-4}
	28-day study (Frawley et al., 2018); *high* confidence	SD rat, female (histopathology study cohort)	$BMDL_{10RD}$, Exp2 CV	0.222	--	1.42×10^{-3}
		SD rat, female (MPS study cohort)	$BMDL_{10RD}$, Linear CV	0.187	--	1.20×10^{-3}
		SD rat, female (TDAR study cohort)	NOAEL[c] (2% increase)	0.125	--	8.00×10^{-4}
Immune effects (developmental)						
Decreased serum anti-tetanus antibody concentrations in children at age 7 yrs and PFDA measured at age 5 yrs	Budtz-Jørgensen and Grandjean (2018a); Grandjean et al. (2012); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	--	4.11×10^{-4}	1.07×10^{-8}
Decreased serum anti-diphtheria antibody concentrations at age 7 yrs and PFDA concentrations at age 5 yrs	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	--	4.07×10^{-4}	1.06×10^{-8}
Decreased serum anti-tetanus antibody	Grandjean et al. (2012);	Human, male and female	$BMDL_{1/2SD}$ Linear	--	7.02×10^{-4}	1.83×10^{-8}

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD type/model	POD (mg/kg-day)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-day)
concentrations at age 5 yrs and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Budtz-Jørgensen and Grandjean (2018a); *medium* confidence					
Decreased serum anti-diphtheria antibody concentrations at age 5 yrs and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	$BMDL_{1/2SD}$ Linear	--	2.57×10^{-4}	6.68×10^{-9}
Developmental effects						
Decreased birth weight	Valvi et al. (2017); *high* confidence[f]	Human, male and female	$BMDL_{5RD}$, Hybrid	--	2.8×10^{-4}	7.3×10^{-9}
	Valvi et al. (2017); *high* confidence[f]	Human, male	$BMDL_{5RD}$, Hybrid	--	2.2×10^{-4}	5.7×10^{-9}
	Valvi et al. (2017); *high* confidence[f]	Human, female	$BMDL_{5RD}$, Hybrid	--	2.4×10^{-4}	6.2×10^{-9}
	(Wikström et al., 2020); *high* confidence[g]	Human, male and female[h]	$BMDL_{5RD}$, Hybrid	--	3.7×10^{-4}	9.6×10^{-9}
	(Wikström et al., 2020); *high* confidence[g]	Human, male	$BMDL_{5RD}$, Hybrid	--	3.3×10^{-4}	8.6×10^{-9}
	(Wikström et al., 2020); *high* confidence[g]	Human, female	$BMDL_{5RD}$, Hybrid	--	3.1×10^{-4}	8.1×10^{-9}
Decreased fetal body weight	Developmental study (GD 6-15) (Harris and Birnbaum, 1989); *medium* confidence	C57BL/6N mouse, male and female	NOAEL(c) (4% decrease)	1	--	1.18×10^{-2}
Male reproductive effects						
Decreased cauda epididymis sperm count	28-d study (NTP, 2018); *low* confidence	SD rat, male	$BMDL_{1SD}$, Exp3 CV	0.963	--	9.07×10^{-3}

Endpoint	Study/ Confidence	Strain/ Species/Sex	POD type/model	POD (mg/kg-day)	POD internal concentration[a] (mg/L)	POD_{HED}[b] (mg/kg-day)
Increased Leydig cell atrophy	28-day study (NTP, 2018); *high* confidence		NOAEL[d] (0% change)	0.625	--	5.89×10^{-3}
Decreased serum testosterone			NOAEL[d] (25% decrease)	0.625	--	5.89×10^{-3}
Decreased absolute testis weight			$BMDL_{1SD}$, Linear CV	1.074	--	1.01×10^{-2}
Decreased absolute cauda epididymis weight			$BMDL_{1SD}$, Linear CV	0.582	--	5.48×10^{-3}
Decreased absolute whole epididymis weight			$BMDL_{1SD}$, Linear NCV	0.546	--	5.14×10^{-3}
Female reproductive effects						
Decreased number of days spent in estrus	28-d study (NTP, 2018); *high* confidence	SD rat, female	$BMDL_{5RD}$, Linear CV	0.128	--	1.77×10^{-3}
Increased number of days spent in diestrus			$BMDL_{5RD}$, Exp2 CV	0.200	--	2.76×10^{-3}
Decreased relative uterus weight			NOAEL[c] (12% increase)	0.625	--	8.63×10^{-3}
Decreased absolute uterus weight			NOAEL[c] (12% increase)	0.625	--	8.63×10^{-3}

[a] Blood concentration PODs determined from human epidemiological analyses.

[b] For PODs based on animal toxicity studies, PODHED = POD × DDEF, where the DDEF is taken from Table 3-4 based on the species, sex and endpoint being extrapolated. For POD internal concentrations (PODint; i.e., PODs from human epidemiological studies), PODHED = POD × CLH, with CLH = 2.6 x 10-5 L/kg-d. For details, see Approach for pharmacokinetic modeling of PFDA in rats and humans.

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

[d] After visual inspection, data were not considered amenable for BMD modeling due to obvious non-monotonicity in the dose-response; therefore, a NOAEL approach was used instead.

[e] Highest dose group was dropped to allow for adequate model fit.

[f] Trimester 3 maternal biomarker samples.

[g] 96% of samples during the first trimester and the remaining during the early weeks of the second trimester; sensitivity analyses showed no differences when trimester 2 samples excluded.

[h] Sex-specific results were available for both males and females separately; these were consistent in magnitude with the overall result.

Derivation of Candidate Lifetime Toxicity Values for the RfD

Under EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) and *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994), five possible areas of uncertainty and variability were considered in deriving the candidate values for PFDA. The identified potential areas of susceptibility to PFDA exposure-induced health effects, including in children and possibly in women of reproductive age (see Section 4.3), can help inform UF value selection and, subsequently, confidence in toxicity values. An explanation of these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate POD_{HED} values are listed In Table 5-9 below. For liver and male and female reproductive effects, quantitative information is limited to studies in which animals were exposed for ≤28 days. For each of these identified hazards, very little information is available to assess the extent to which the specific changes caused by PFDA exposure for 28 days might be expected to worsen with PFDA exposure for a lifetime. Separately, human equivalent PODs for these endpoints were much less sensitive (several orders of magnitude) than the PODs for developmental and immune effects from the epidemiology studies (see Table 5-9). As such, for liver, male reproductive, and female reproductive effects, derivation of candidate lifetime values was not attempted given the high degree of uncertainty associated with using PODs from a 28-day rodent study to protect against effects observed in a chronic setting. However, these endpoints were considered for the derivation of the subchronic RfD (see Section 5.2.2).

Developmental effects observed in mice from the Harris and Birnbaum (1989) study, albeit observed after exposure during a sensitive lifestage, were not considered for derivation of a candidate lifetime value. Specifically, given the availability of PODs for developmental effects from *high* confidence human studies that were observed to be more sensitive than the POD from the rodent study (by 6–7 orders of magnitude; see Table 5-10), the available human data were given preference. It is important to note that the (Valvi et al., 2017) study was not considered for the derivation of candidate toxicity values for developmental effects given the limitations described above. However, the PODs determined from the (Valvi et al., 2017)study are informative for the PODs and resulting RfDs for developmental effects based on birth weight data from the (Wikström et al., 2020) study.

Table 5-9. Uncertainty factors for the development of the candidate lifetime toxicity values for PFDA

UF	Value	Justification
UF_A	1	A UF_A of 1 is applied to developmental and immunological effects observed in humans.
UF_H	10	A UF_H of 10 is applied for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFDA exposure in humans.

UF	Value	Justification
UF_S	1	A UF_S of 1 is applied to developmental delays (i.e., decreased birth body weight) [Wikström et al. (2020); and reduced antibody responses in children Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a). The developmental period is recognized as a susceptible lifestage when exposure during a time window of development is more relevant than lifetime exposure in adulthood (U.S. EPA, 1991). Additional considerations for the UF_S for immune effects are discussed below.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. BMDLs were available for both the developmental and immune effects in the epidemiology studies advanced for candidate value derivation.
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of *high/medium* confidence short-term studies in rodents and a *high* confidence developmental study in mice. The database for PFDA also includes several *high/medium* confidence epidemiological studies most informative for immune and developmental effects, which are sensitive effects of PFDA exposure. However, uncertainties remain regarding the lack of studies examining effects with long-term exposure in adults—including in women of reproductive age (which may have increased susceptibility), studies of potential multi-generational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity after PFDA exposure during development. In all, the data are too sparse to conclude with certainty that the quantified developmental effects are likely to be the most sensitive; thus, a UF_D of 1 was not selected. However, a UF_D of 10 was also not selected give the availability of data from well-conducted studies on a range of health outcomes in multiple species, including sensitive evaluations of developmental and immune endpoints in humans. See discussion below for additional details.
UF_C	See Table 5-10	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

As described in EPA's *A Review of the Reference Dose and Reference Concentration Processes* (U.S. EPA, 2002) the interspecies uncertainty factor (UF_A) is applied to account for extrapolation of animal data to humans, and accounts for uncertainty regarding the pharmacokinetic and pharmacodynamic differences across species. The datasets considered for derivation of candidate lifetime values were from human studies, so a $UF_A = 1$ was applied to all PODs after the application of dosimetric approaches for estimation of HEDs as described above.

For immune effects, both a duration extrapolation uncertainty factor (UFs) = 3 and a value of UFs = 1 were considered to account for extrapolation from less than chronic data, ultimately selecting a UFs = 1. A UFs=10 was not considered as the developmental period is recognized as a susceptible lifestage for these types of effects and therefore exposure during this time window can be considered more relevant than exposure in adulthood (U.S. EPA, 1991). The reduced antibody responses were measured in children 5–7 years of age. The HED calculations used for these immune effects assume chronic exposure, so an RfD based on them will assure that serum PFDA levels remain below the POD irrespective of exposure duration. Also, development is recognized as a sensitive period for effects on immune system responses. According to the WHO/IPCS Immunotoxicity Guidance for Risk Assessment, developmental immunotoxicity encompasses the prenatal, neonatal, juvenile and adolescent lifestages and should be viewed differently from the immune system of adults from a risk assessment perspective (IPCS, 2012). Special considerations

for developmental immunotoxicity include increased dose sensitivity, potential for effects to become permanent even after cessation of exposure, broader spectrum of adverse effects and "rewiring of the immune system" (IPCS, 2012), which indicates a greater health risk for early-life exposures to immunotoxicants compared to adults. Given PFDA's long half-life and the expectation that the children and their mothers have been exposed to elevated levels of PFDA for many years, the observed effects on immune response are considered to be the result of a cumulative, prolonged exposure to the subjects from conception until the age when the response was evaluated. Further, the consequences of perturbed immune system function (in this case, suppressed antibody responses leading potentially to increased disease) during development are expected to be generally more severe and longer lasting than those that manifest in healthy adults. Taken together, the observed immune effects in children considered to be the result of prolonged exposure to PFDA and the enhanced susceptibility of the developmental immune system to chemical pollutants, attenuate concerns of potentially increased sensitivity with longer-term exposures. As such, a UFs =1 rather than a UFs = 3 was applied for immune effects in children. Uncertainties regarding possible more sensitive latent effects of these impacts on the immune system during early-life exposures leading to unpredictable outcomes later in life, for example in other susceptible lifestages of reduced immunocompetence such as pregnancy and most notably old age, are addressed as part of the justification for selecting a database uncertainty factor (UF_D) > 1, as discussed below.

For PFDA, both a UF_D = 10 and a UF_D = 3 were considered due to the limited database (e.g., the lack of a two-generation developmental/reproductive toxicity study) and a UF_D = 3 ultimately was applied. Typically, the specific study types lacking in a chemical's database that influence the value of the UF_D to the greatest degree are developmental toxicity and multigenerational reproductive toxicity studies. The PFDA database does include a *medium* confidence (Harris and Birnbaum, 1989) developmental toxicity study in mice. Despite its quality, however, that study fails to cover potential transgenerational impacts of longer-term exposures evaluated in a two-generation study. The 1994 *Reference Concentration Guidance* (U.S. EPA, 1994) and 2002 *Reference Dose Report* (U.S. EPA, 2002); (U.S. EPA, 2002) support applying a UF_D in situations when such a study is missing. The 2002 *Reference Dose Report* (U.S. EPA, 2002); (U.S. EPA, 2002) states that "[i]f the RfD/RfC is based on animal data, a factor of 3 is often applied if either a prenatal toxicity study or a two-generation reproductive study is missing." Consideration of the PFDA, PFBA (a short-chain perfluoroalkyl carboxylic acid),[16, 17] PFBS (a short-chain

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

[17]IRIS Toxicological Review of Perfluorobutanoic Acid (PFBA, CASRN 375- 22-4) and Related Salts (U.S. EPA, 2022).

perfluoroalkane sulfonic acid with a 4-carbon backbone),[18] PFHxA (a short-chain perfluoroalkyl carboxylic acid), and PFHxS (a long-chain perfluoroalkane sulfonic acid)[19] databases together, however, diminish the concern that the availability of a multigenerational reproductive study would result in reference values far below those currently derived for PFDA. Although limited in their ability to assess reproductive health or function, measures of possible reproductive toxicity occurred at doses equal to or higher than those that resulted in effects in other organ systems (e.g., thyroid, liver) when measured after exposure to PFDA for 28 days (NTP, 2019). Similar results were observed for the animal databases for PFOA and PFOS indicating reproductive effects were not uniquely sensitive markers of toxicity for these long-chain PFAS (ATSDR, 2018b). Further, no notable male or female reproductive effects were observed in epidemiological or toxicological studies investigating exposure to PFHxS (MDH, 2019). Therefore, considering the limited chemical-specific information alongside information gleaned from structurally related compounds, the lack of a multigenerational reproductive study is not considered a major concern relative to UF_D selection for PFDA.

The lone animal developmental study (Harris and Birnbaum, 1989) for PFDA also did not evaluate postnatal developmental effects. Effects on postnatal development (e.g., delayed eye opening; reduced postnatal growth) have been observed in rodents exposed to other long-chain PFAS such as PFOA (ATSDR, 2018b). Overall, the available information on potential PFDA-induced postnatal developmental effects is sparse, introducing uncertainty as to whether more sensitive developmental effects of PFDA might occur and may be of concern relative to UF_D selection.

Another gap in the PFDA database is the lack of measures of thyroid toxicity in gestationally exposed offspring or after longer-than-28-day PFDA exposures, and the lack of a developmental neurotoxicity study. Thyroid hormones are critical in myriad physiological processes and must be maintained at sufficient levels during times of brain development in utero and after birth. Although no PFDA-specific data on thyroid hormone levels following gestational exposure are available, effects on thyroid hormone homeostasis were observed in a study in adult rats exposed to PFDA for 28 days (NTP, 2018), and disrupted thyroid signaling has been shown to be a consequence of exposure to other PFAS (U.S. EPA, 2021b). Therefore, anticipating that potentially sensitive effects due to PFDA exposure also could have been observed had thyroid hormone levels been measured in the Harris and Birnbaum (1989) developmental study, or in longer-term studies, is reasonable. Thus, the lack of data for PFDA-induced effects on thyroid levels in developing animals or with prolonged exposure or data on potential thyroid dependent neurodevelopmental effects is a source of uncertainty.

[18] Human health toxicity values for perfluorobutane sulfonic acid (CASRN 375-73-5) and related compound potassium perfluorobutane sulfonate (CASRN 29420-49-3)(U.S. EPA, 2021b)
[19] Health Based Guidance for Water: Toxicological Summary for: Perfluorohexane sulfonate (PFHxS), MDH (2019)

Lastly, the potential for sensitive effects following long-term exposure durations represents an area of uncertainty for the PFDA database. While the potential for more sensitive effects is mitigated mostly by the availability of very sensitive PODs (compared to other PODs) for developmental effects from human studies, there are no comprehensive subchronic and chronic animal studies available for PFDA. The longest exposure study treated mice for 30–49 days via drinking water but tested only one high-PFDA dose (6.6 mg/kg-day) and evaluated limited endpoints (body weight and survival) (Wang et al., 2020). No chemical-specific information is available to judge the degree to which the existing endpoints in the PFDA Toxicological Review would be more sensitive with extended durations. Given that the PODs used to derive candidate values were from studies of developmental exposure, this uncertainty cannot be fully addressed through the application of a UF_S. Specifically, for immune effects, there is a lack of epidemiological studies or studies in animals examining the effects of PFDA exposures that encompass later developmental periods (e.g., late childhood and adolescence) or other potentially susceptible lifestages such as pregnancy and old age. In addition, the available studies include limited or no evaluation of immunotoxicity categories other than immunosuppression, namely sensitization and allergic response, and autoimmunity and autoimmune disease.

Given the residual concerns for potentially more sensitive effects outlined above, a database uncertainty factor is considered necessary. Specifically, a value of 3 was selected for the UF_D to account for the uncertainty surrounding the lack of an evaluation of postnatal or multigenerational effects in animals, specific investigations of potential effects on thyroid function after developmental exposure or neurodevelopmental effects, and comprehensive long-term studies in multiple species.

The uncertainty factors described in Table 5-9 and the text above were applied and the resulting candidate values are shown in Table 5-10. The candidate values are derived by dividing the POD_{HED} by the composite uncertainty factor as shown below.

Candidate values for PFDA= $POD_{HED} \div UFc$

Table 5-10. Candidate values for PFDA

Endpoint	Study/ Confidence	Strain/ Species/ Sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Immune effects (developmental)										
Decreased serum anti-tetanus antibody concentration in children at age 7 yrs and PFDA measured at age 5 yrs	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.07×10^{-8}	1	10	1	1	3	30	4×10^{-10}
Decreased serum anti-diphtheria antibody levels at age 7 yrs and PFDA concentrations at age 5 yrs	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.06×10^{-8}	1	10	1	1	3	30	4×10^{-10}
Decreased serum anti-tetanus antibody levels at age 5 years and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.83×10^{-8}	1	10	1	1	3	30	6×10^{-10}
Decreased serum anti-diphtheria antibody levels at age 5 yrs and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	6.68×10^{-9}	1	10	1	1	3	30	2×10^{-10}
Developmental effects										
Decreased birth weight	(Wikström et al., 2020) *high* confidence	Human, male and female	9.6×10^{-9}	1	10	1	1	3	30	3×10^{-10}
	(Wikström et al., 2020) *high* confidence	Human, male	8.6×10^{-9}	1	10	1	1	3	30	3×10^{-10}
	(Wikström et al., 2020) *high* confidence	Human, female	8.1×10^{-9}	1	10	1	1	3	30	3×10^{-10}

[a]The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

5.2.2. Selection of Lifetime Toxicity Value(s)

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

From among the candidate values presented in Table 5-10, organ/system-specific RfDs (osRfDs) are selected for the individual organ systems identified as hazards in Section 3. The osRfD values selected were associated with decreased serum antibody concentrations in children for immune effects and decreased birth weight for developmental effects. The confidence decisions about the studies, evidence base, quantification of the POD, and overall osRfD are fully described in Table 5-11, along with the rationales for selecting those confidence levels. In deciding overall confidence, confidence in the evidence base is prioritized over the other confidence decisions. The overall confidence in the osRfD for immune effects is medium, and the confidence in the osRfD for developmental effects is medium-low. Selection of the overall RfD is described in the following section.

Table 5-11. Confidence in the organ/system-specific (osRfDs) for PFDA

Confidence categories	Designation	Discussion
Immune (developmental) osRfD = 4×10^{-10} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) was rated as *medium* primarily due to relatively limited PFDA exposure contrasts, which can decrease study sensitivity in general. (HAWC link). Given that the results in this study were statistically *significant*, EPA concluded that while there were potential study sensitivity concerns at the evaluation stage, the results clearly showed that those concerns were not borne out, and confidence in this study to derive an osRfD was judged to be *high*.
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for immune effects is medium based on consistent findings of reduced antibody responses from two *medium* confidence birth cohort studies (Grandjean et al., 2012); (Grandjean et al., 2017a); (Grandjean et al., 2017b) and a low confidence study in adults (Kielsen et al., 2016). Short-term studies in animals of high/medium confidence provide supportive evidence of immunosuppression after PFDA exposure (Frawley et al., 2018); (NTP, 2018). Some residual uncertainties regarding unexplained inconsistency and potential confounding by other co-occurring PFAS from epidemiological studies and issues with concomitant overt target organ and systemic toxicity in animal studies lower confidence in the available evidence for this hazard. Other limitations include the lack of epidemiological studies or long-term/chronic studies in animals examining effects on the immune system across different developmental life stages and immunotoxicity categories, including sensitization and allergic response and autoimmunity and autoimmune disease.

Confidence categories	Designation	Discussion
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium.* The POD is based on BMD modeling at the lower end of the range of the observed data and a $BMDL_{1/2SD}$ estimate that is associated with a small degree of uncertainty due to potential confounding by PFOA (see Appendix D.1.1 for more details). The POD for decreased tetanus antibodies at age 7 yrs was judged to be *medium* confidence based on a good model fit and was supported by the nearly identical POD for decreased diphtheria antibodies at age 7 yrs. Both PODs support the osRfD. An estimate for human clearance was applied to estimate the POD_{HED} using PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above). There is also uncertainty as to the most sensitive window of vulnerability with respect to the exposure/outcome measurement timing (BMDs/BMDLs were estimated from PFDA levels measured at age 5 or perinatally and anti-tetanus antibody concentrations measured at age 7 or 5); (Grandjean et al., 2017b) reported that *estimated* PFDA "concentrations at 3 mo and 6 mo showed the strongest inverse associations with antibody concentrations at age 5 yrs, particularly for tetanus." Thus, it is possible that adverse effects during infancy could be more sensitive than between ages 5 and 7 yrs.
Overall confidence in osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is driven by *medium* confidence in the evidence base for immune effects, the quantification of the POD, and the study used for BMD modeling.
Developmental osRfD = 3×10^{-10} mg/kg-d		
Confidence in study[a] used to derive osRfD	*Medium*	Confidence in the Wikström et al. (2020) study for hazard identification was rated as *high* (HAWC link) for developmental effects. The study was selected for dose-response analysis due to low overall risk of bias and reliable exposure measurements which had sufficient exposure contrasts and other characteristics that allowed for adequate study sensitivity to detect associations. The Wikström et al. (2020) study demonstrated associations consistent in magnitude for boys, girls, and the overall population. Overall, mean birth weight was considered the most precise and accurate endpoint and not anticipated to be subject to much error. This study was advanced for dose-response analysis, given no presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). Wikström et al. (2020) also adjusted for sample timing in their multivariate models and show no differences in models also restricted to trimester 1 samples only. Some uncertainty remains on the potential for confounding by other PFAS (concern primarily for PFNA) which were not examined in this study. Given the potential quantitative impact of this uncertainty, confidence in the use of this study for dose-response analysis was judged as *medium* rather than *high*.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for developmental effects is *medium*. There was consistent evidence for reduced birth weight among multiple human studies, including *high* quality studies. However, unlike the Wikström et al. (2020) study used here and noted above, some uncertainty remains in many studies given the predominance of associations that were detected for studies with later pregnancy sampling. The human database also showed some coherence across different measures of fetal growth restriction. In animals, the lone developmental study reported effects on fetal growth that are coherent with effects observed in humans. Some residual uncertainty regarding potential confounding by other co-occurring PFAS from epidemiological studies lowers confidence in the available evidence for this hazard.
Confidence in quantification of the PODHED	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on a BMD hybrid approach within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is driven by *medium-low* confidence in the evidence base for developmental effects (i.e., fetal growth restriction).

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

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

Organ/system-specific and overall RfD values for PFDA selected in the previous section are summarized in Table 5-12.

Table 5-12. Organ/System-specific and overall lifetime RfDs for PFDA

System	Toxicity Value	Basis	POD_{HED} (mg/kg-d)	UF_C	osRfD or RfD (mg/kg-d)	Confidence
Immune (developmental)	osRfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yrs and PFDA measured at age 5 yrs	1.07×10^{-8} based on $BMDL_{½\ SD}$ from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a)	30	4×10^{-10}	*Medium*
Developmental	osRfD	Decreased birth weight in males and females	9.6×10^{-9} based on $BMDL_{5\%RD}$ from (Wikström et al., 2020)	30	3×10^{-10}	*Medium-low*
Immune /developmental	Overall lifetime RfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yrs and PFDA measured at age 5 yrs Decreased birth weight in males and females	1.07×10^{-8} based on $BMDL_{½\ SD}$ from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) 9.6×10^{-9} based on $BMDL_{5\%RD}$ from (Wikström et al., 2020)	30	4×10^{-10}	*Medium*

From the identified human health effects of PFDA and derived osRfDs for immune and developmental effects (see Table 5-12), an overall ***RfD of 4 × 10⁻¹⁰ mg/kg-day based on decreased serum antibody concentrations and decreased birth weight in humans*** was selected. As described in Table 5-12, confidence in the RfD is *medium*, based on *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental (Wikström et al., 2020) endpoints using BMD modeling. This RfD is considered to be representative of both immune and developmental effects given the close proximity (~1.5-fold) of the developmental and immune

PODs and resulting osRfDs and that both critical effects are observed during the developmental period. There is a slight difference in the immune and developmental osRfDs due to numerical rounding in the RfD calculation (immune osRfD = $1.07 \times 10^{-8}/30 = 3.6 \times 10^{-10} = 4 \times 10^{-10}$; developmental osRfD = $9.6 \times 10^{-9}/30 = 3.2 \times 10^{-10} = 3 \times 10^{-10}$). Although the value associated with the immune osRfD is slightly higher than the developmental osRfD, this value is chosen as the representative overall RfD given that it is higher confidence (*medium* vs *medium-low* for the developmental osRfD) and is considered appropriate given the definition of the RfD being a value with uncertainty of up to an order of magnitude. Selection of this overall RfD is presumed to be protective of all other potential health effects in humans, based on the currently available evidence. Finally, the immune osRfD and developmental osRfD are based on effects observed in males and females indicating that the overall RfD would be protective for both sexes.

Overall, the immune and developmental endpoints from epidemiological studies of PFDA were preferentially advanced for the derivation of candidate lifetime values. For immune effects, osRfDs were derived for decreased serum antibody levels (for both diphtheria and tetanus) in children (male and female) at different timing of exposure and outcome measurement combinations, specifically antibody levels at age 7 and PFDA concentrations at age 5, and antibody levels at age 5 and perinatal PFDA concentrations (Budtz-Jørgensen and Grandjean, 2018a) (see Table 5-8). The toxicity value (osRfD) for immune effects of 4×10^{-10} mg/kg-day was based on deleterious effects observed in children showing decreased antibody concentrations for both tetanus and diphtheria at age 7 years related to serum PFDA concentrations measured at age 5 years. The PODs for decreased tetanus and diphtheria antibody concentrations were nearly identical ($BMDL_{1/2SD[HED]}$ of 1.07×10^{-8} mg/kg-day for tetanus and 1.06 mg/kg-day for diphtheria) and were close to the PODs for other outcome-exposure combinations (see Table 5-10), which further supports the selected osRfD. Although both tetanus and diphtheria are rare in the U.S., the findings that PFDA exposure reduced antibody responses may be broadly indicative of developmental immunosuppression impacting overall immune function in these children. The lowest serum PFDA concentration measured at age 5 years was 0.05 ng/mL and the 10th% was 0.2 ng/mL (Grandjean and Bateson, 2021) so the estimated $BMD_{½SD}$ (0.411 ng/mL) for this endpoint in the single PFAS model is well within the observed range. No information was available to judge the fit of the model in the range of the BMDLs (see Appendix C.1.1 for more details).

For developmental effects, given that the candidate toxicity values are identical (see Table 5-10), the osRfD of 3×10^{-10} mg/kg-day (BMDL5RD[HED] of 9.6×10^{-9} mg/kg-day) based on reduced birth weight in males and females from the Wikström et al. (2020) study was selected. Although this osRfD is not based on the lowest POD for reduced birth weight from the (Wikström et al., 2020) study, it is more representative of the general human population (males and females combined) than the comparisons in males or females only. There is some uncertainty with PODs considered from the Valvi et al. (2017) study because it is not based on early sampling and may be prone to bias from pregnancy hemodynamics to some unknown degree. As discussed in Appendix F,

there is only one developmental study (Gyllenhammar et al., 2018) for PFDA that collected and was able to analyze maternal hemodynamics data such as GFR and/or albumin. This study did not report any evidence of confounding following statistical adjustment of different GFR measures for any of the PFAS examined, which is consistent with no demonstrated confounding by either GRR (Manzano-Salgado et al., 2017); (Whitworth et al., 2012) or albumin (Sagiv et al., 2018) for other PFAS examined in other studies. However, existing meta-analyses for both PFOA (Steenland et al., 2018) and PFOS (Dzierlenga et al., 2020) only detected birth weight deficits for later trimester sampling (e.g., beyond trimester 1). A similar detailed analysis was precluded for PFDA given that there are only two studies that examined any first trimester measures. Overall, there was limited evidence of any patterns of larger birth weight associations with sample timing for PFDA, but possible associations could not be evaluated further given limited available data as well as disparate exposure measures, distributions, and contrasts being examined. In contrast, the Wikström et al. (2020) study was prioritized for RfD derivation as it was a high confidence study that sampled maternal plasma in the first and second trimester thereby reducing uncertainty relating to pregnancy hemodynamics. Further confidence in the osRfD derived from the (Wikström et al., 2020) study is provided by the fact that the PODs from the (Wikström et al., 2020) and (Valvi et al., 2017) studies are relatively close (see Table 5-8 above). While not presented in this Toxicological Review, additional birth weight studies were BMD modeled to provide a sensitivity analysis for the comparison of birth weight effects; please see Table C-8 of the Supplemental Appendices. These studies are either medium confidence and/or have later trimester sampling and thus not considered in the dose-response analysis. The PODs from these birth weight studies are relatively close (varying by ~3-fold), providing further confidence in using the POD from the (Wikström et al., 2020) study for RfD derivation. In addition to the quantitative implications, the close proximity of the BMDLs from a multitude of birth weight studies increases the confidence in deriving osRfDs despite slight evidence of developmental effects in humans.

5.2.3. Subchronic Toxicity Values for Oral Exposure (Subchronic Oral Reference Dose [RfD]) Derivation

In addition to providing an RfD for lifetime exposure in health systems, this document also provides an RfD for less-than-lifetime ("subchronic") exposures. Datasets considered for the subchronic RfD were based on endpoints advanced for RfD derivation in Table 5-8. Given that the developmental and immune effects were observed in humans exposed to PFDA during susceptible lifestages (postnatal growth/development and immune system effects in children at ages 5–7), these endpoints were also considered for the derivation of candidate subchronic values, applying identical uncertainty factors to those used for the lifetime candidates values (see Table 5-14 below).

Similar to the derivation of the lifetime RfD, the developmental effects observed in mice from the Harris and Birnbaum (1989) study were not advanced for the derivation of candidate subchronic values. The developmental PODs from human studies are 6–7 orders of magnitude more sensitive than the POD from the rodent study (see Table 5-9), and were, therefore, prioritized. In

addition, endpoints for hepatic, male reproductive toxicity, and female reproductive toxicity observed in the 28-day rodent study (NTP, 2018) were considered for the derivation of subchronic toxicity values. As compared to the large uncertainty in extrapolating the available 28-day studies to lifetime PFDA exposure in the context of the RfD, it was considered reasonable to try to extrapolate the 28-day study data for the purposes of deriving subchronic candidate values.

The use of animal data for hepatic, male reproductive, and female reproductive endpoints required the application of different uncertainty factors than those used for developmental and immune effects in humans and can be found in Table 5-13.

Table 5-13. Uncertainty factors for the development of the candidate subchronic values for PFDA

UF	Value	Justification
UF_A	1 3	A UF_A of 1 is applied to developmental and immunological effects observed in epidemiological studies. A UF_A of 3 is applied to account for uncertainty in characterizing the pharmacokinetic and pharmacodynamic differences between mice or rats and humans following oral PFDA exposure. Aspects of the cross-species extrapolation of pharmacokinetic processes have been accounted for by using a DDEF to convert internal doses in rodents to administered doses in humans using evidence on clearance; however, some residual pharmacokinetic uncertainty remains as does the potential for pharmacodynamic differences. Availability of chemical-specific data justify the selection of a UF of 3 for PFDA. See discussion below for more details.
UF_H	10	A UF_H of 10 is applied for interindividual variability in humans in the absence of quantitative information on potential differences in pharmacokinetics and pharmacodynamics relating to PFDA exposure in humans.
UF_S	1 10	A UF_S of 1 is applied to developmental delays (i.e., decreased birth body weight) Wikström et al. (2020); and reduced antibody responses in children (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012).The developmental period is recognized as a susceptible life stage when exposure during a time window of development is more relevant than subchronic exposure (U.S. EPA, 1991). A UF_S of 10 is applied to liver, male reproductive, and female reproductive effects in adult animals (increased AST levels, decreased epididymis weight and decreased number of days in estrus, respectively) because of the short exposure duration (28 d) and the presumption that effects would worsen with longer exposures. See discussion below for more details.
UF_L	1	A UF_L of 1 is applied for LOAEL-to-NOAEL extrapolation when the POD is a BMDL or a NOAEL. All PODs considered for candidate subchronic values were BMDLs.

UF	Value	Justification
UF_D	3	A UF_D of 3 is applied to account for deficiencies and uncertainties in the database. Although limited, the evidence base in laboratory animals consists of *high/medium* confidence short-term studies in rodents and a *high* confidence developmental study in mice. The database for PFDA also includes several *high/medium* confidence epidemiological studies most informative for immune and developmental effects. However, uncertainties remain regarding the lack of studies of potential multi-generational effects, and studies of postnatal development, neurotoxicity, and thyroid toxicity during developmental lifestages. In all, the data are too sparse to conclude with certainty that the quantified developmental effects are likely to be the most sensitive; thus, a UF_D of 1 was not selected. However, a UF_D of 10 was also not selected give the availability of data from well-conducted studies in multiple species, including developmental and short-term rodent studies examining a range of potentially sensitive health outcomes and sensitive evaluations of developmental and immune endpoints in humans.
UF_C	See Table 5-11 and Table 5-15	Composite Uncertainty Factor = $UF_A \times UF_H \times UF_S \times UF_L \times UF_D$

As described above under *Derivation of Candidate Lifetime Toxicity Values for the RfD*, and in (U.S. EPA, 2002), five possible areas of uncertainty and variability were considered in deriving the candidate subchronic values for PFDA. In general, the explanations for these five possible areas of uncertainty and variability and the values assigned to each as a designated UF to be applied to the candidate POD_{HED} values are listed above and in Table 5-13, including the UF_D which remained at 3 due to data gaps discussed previously in the derivation of the lifetime RfD. One UF that differs between subchronic and chronic RfDs is that for effects (i.e., decreased fetal body weight, increase AST levels, decreased whole epididymis weight and decreased estrus time) observed in rodents a UF_A of 3 was applied to account for pharmacokinetic and pharmacodynamic differences between rodents and humans following oral PFDA exposure. As is usual in the application of this uncertainty factor, the pharmacokinetic uncertainty is mostly addressed through the application of an adjustment factor, in this case, chemical-specific dosimetric data for estimating human equivalent doses (see Approach for pharmacokinetic extrapolation of PFDA among rats, mice, and humans). This leaves some residual uncertainty around the pharmacokinetics and the uncertainty surrounding differences in pharmacodynamic differences between animals and humans. Typically, a UF_A of 3 is applied for this uncertainty when either $BW^{3/4}$ scaling or chemical-specific information is used for dose extrapolation. This is the case for developmental, male reproductive and female reproductive endpoints. For the liver endpoint, available mechanistic and supplemental information is considered further in determining the most appropriate value for the UF_A to account for the uncertainty.

Evidence from in vitro studies suggest that PFDA interacts with several human receptor pathways relevant to its mechanism of hepatotoxicity, including PPARα. PFDA can bind and activate PPARα in vitro, but reduced sensitivity towards the human PPARα versus other mammalian isoforms (i.e., mouse and Baikal seal) is apparent (Ishibashi et al., 2019);(Wolf et al., 2012);(Wolf et al., 2008) and similar findings have been demonstrated for some other

perfluorinated compounds. If PPARα were the only operant MOA for noncancer effects in the liver, this observation might support reducing the remaining portion of the UF_A to 1, as it could be argued that humans are not as sensitive as wild-type rats to the hepatic effects of PFDA exposure (note: without evidence to the contrary, as mentioned in the previous paragraph, the toxicodynamic portion of this UF is typically assigned a value of 3 assuming responses manifest in humans could be more sensitive than those observed in animals). Although PPARα appears to be an important mechanism of PFDA-induced liver toxicity in animals and reduced sensitivity in PPAR activation in humans compared to rodents has been suggested, available evidence for PFDA in PPARα null mice, human in vitro assays and in vivo animal models more relevant to humans with respect to PPARα sensitivity (i.e., guinea pigs and Syrian hamsters) suggest that liver effects occur, at least in part, independent of PPARα (see Summary of mechanistic studies for PFDA in Section 3.2.1). A plausible PPARα-dependent and independent MOA for liver effects is also supported by studies in null and humanized animal models of structurally related long-chain PFAS [C7–C9] (see Evidence for other PFAS in Section 3.2.1), which are mostly lacking for PFDA (a few studies in null mice but no humanized models). Considering the remaining uncertainty in additional MOAs that appear active in PFDA-induced liver effects, and the relative contribution of these MOAs to toxicity in humans as compared to rodents, uncertainties surrounding a potential multifaceted MOA for PFDA-induced liver effects, the value of 3 was selected for the UF_A for the purposes of deriving candidate subchronic toxicity values for hepatic effects.

EPA states that for "short-term and longer-term reference values, the application of a UF analogous to the subchronic-to-chronic duration UF also needs to be explored, as there may be situations in which data are available and applicable, but they are from studies in which the dosing period is considerably shorter than that for the reference value being derived" (U.S. EPA, 2002). This is the case for hepatic, male reproductive and female reproductive endpoints derived from the 28-day NTP (2018) study. Although there is no chemical-specific information to evaluate the potential for increased sensitivity with exposures longer than 28-days (e.g., a 90-day subchronic study), the following considerations are outlined to inform the application of the UF_S for duration extrapolation. (U.S. EPA, 2002)

With regards to female reproductive toxicity, PFDA-induced effects on estrous cyclicity were observed to be of large magnitude in the 28-day study. Specifically, PFDA induced a continuous state of diestrus in 100% of rats treated at the highest dose tested (2.5 mg/kg-day) by Day 21 (by Day 9 of the sixteen days in which vaginal cytology was assessed) (NTP, 2018). Based on these data, it is possible that PFDA-induced effects on estrous cyclicity could become more sensitive or lead to more severe downstream effects like infertility with longer exposure durations. For male reproductive effects, the study duration (28 days) was insufficient to cover the entire period of spermatogenesis in rats (~8 weeks), raising concerns about reduced sensitivity for some of the endpoints evaluated and selected for POD derivation (i.e., sperm evaluations). For liver effects, increases in relative liver weights demonstrated a time dependency across short-term

exposures. Relative liver weight increased by 17–56% at 1.15–10 mg/kg-day in rats exposed for 7–14 days and by 12–127% at 1–16 mg/kg-day in mice exposed during gestion (GD 10–13 and 6–15). Similar magnitudes of liver weight increases were achieved in rodents after 28-day exposure but at lower PFDA doses (10–102% at 0.125–2.5 mg/kg-day in rats and 16–81% at 0.089–0.71 mg/kg-day in mice). The limited data for liver weight suggest potential increase in sensitivity with increasing duration, although there is no information on how liver weight or other sensitive liver endpoints (increased AST and ALP levels) are impacted by longer-term exposures (>28 days). Considering the potential for some health effects (prolonged diestrus, sperm measures and increased liver weight) to worsen with increasing duration and the large uncertainty associated with the lack of any chemical-specific data on whether the effects observed in the short-term study worsen after subchronic exposure, a UFs of 10 is selected for the purposes of deriving candidate subchronic toxicity values from the 28-day toxicity data.

The uncertainty factors described in Table 5-13 and the text above were applied and the resulting candidate subchronic values are shown in Table 5-14. The candidate values are derived by dividing the PODHED by the composite uncertainty factor as shown below.

Candidate values for PFDA = $POD_{HED} \div UFc$

Table 5-14. Candidate values for deriving the subchronic RfD for PFDA

Endpoint	Study/ Confidence	Strain/ Species/ Sex	POD_{HED} (mg/kg-d)	UF_A	UF_H	UF_S	UF_L	UF_D	UF_C	Candidate value (mg/kg-d)[a]
Immune effects (developmental)										
Decreased serum anti-tetanus antibody concentrations in children at age 7 yrs and PFDA measured at 5 yrs	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.07×10^{-8}	1	10	1	1	3	30	4×10^{-10}
Decreased serum anti-diphtheria antibody concentrations at age 7 yrs and PFDA concentrations at age 5 yrs	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.06×10^{-8}	1	10	1	1	3	30	4×10^{-10}
Decreased serum anti-tetanus antibody concentrations at age 5 yrs and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	1.83×10^{-8}	1	10	1	1	3	30	6×10^{-10}
Decreased serum anti-diphtheria antibody concentrations at age 5 yrs and perinatal (pregnancy week 32–2 wks postpartum) PFDA concentrations	Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a); *medium* confidence	Human, male and female	6.68×10^{-9}	1	10	1	1	3	30	2×10^{-10}
Developmental effects										
Decreased birth weight	Wikström et al. (2020); *high* confidence	Human, male and female	9.6×10^{-9}	1	10	1	1	3	30	3×10^{-10}
	Wikström et al. (2020); *high* confidence	Human, male	8.6×10^{-9}	1	10	1	1	3	30	3×10^{-10}
	Wikström et al. (2020); *high* confidence	Human, female	8.1×10^{-9}	1	10	1	1	3	30	3×10^{-10}
Liver effects										
Increased AST		SD rat, male	1.16×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}

	28-d study NTP (2018); *high* confidence	SD rat, female	4.00×10^{-3}	3	10	10	1	3	1,000	4×10^{-6}
Increased ALP		SD rat, male	1.47×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
		SD rat, female	1.00×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
Increased relative liver weight		SD rat, male	1.60×10^{-3}	3	10	10	1	3	1,000	2×10^{-6}
		SD rat, female	7.17×10^{-4}	3	10	10	1	3	1,000	7×10^{-7}
	28-d study Frawley et al. (2018); *high* confidence	SD rat, female (histopathology study cohort)	1.42×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
		SD rat, female (MPS study cohort)	1.20×10^{-3}	3	10	10	1	3	1,000	1×10^{-6}
		SD rat, female (TDAR study cohort)	8.00×10^{-4}	3	10	10	1	3	1,000	8×10^{-7}
Male reproductive effects										
Decreased cauda epididymis sperm count	28-d study NTP (2018); *low* confidence	SD rat, male	9.07×10^{-3}	3	10	10	1	3	1,000	9×10^{-6}
Increased Leydig cell atrophy	28-d study NTP (2018); *high* confidence		5.89×10^{-3}	3	10	10	1	3	1,000	6×10^{-6}
Decreased serum testosterone			5.89×10^{-3}	3	10	10	1	3	1,000	6×10^{-6}
Decreased absolute testis weight			1.01×10^{-2}	3	10	10	1	3	1,000	1×10^{-5}
Decreased absolute cauda epididymis weight			5.48×10^{-3}	3	10	10	1	3	1,000	5×10^{-6}
Decreased absolute whole epididymis weight			5.14×10^{-3}	3	10	10	1	3	1,000	5×10^{-6}
Female reproductive effects										
Decreased number of days spent in estrus	28-day study NTP (2018); *high* confidence	SD rat, female	1.77×10^{-3}	3	10	10	1	3	1,000	2×10^{-6}
Increased number of days spent in diestrus			2.76×10^{-3}	3	10	10	1	3	1,000	3×10^{-6}

Decreased relative uterus weight			8.63×10^{-3}	3	10	10	1	3	1,000	9×10^{-6}
Decreased absolute uterus weight			8.63×10^{-3}	3	10	10	1	3	1,000	9×10^{-6}

[a]The candidate values for different salts of PFDA would be calculated by multiplying the candidate value for the free acid of PFDA by the ratio of molecular weights. For example, for the ammonium salt the ratio would be: $\frac{MW\ ammonium\ salt}{MW\ free\ acid} = \frac{531}{514} = 1.033$. This same method of conversion can be applied to other salts of PFDA, such as the potassium or sodium salts, using the corresponding molecular weights.

Selection of Subchronic Toxicity Value(s)

As described above, candidate subchronic values for several health effects associated with PFDA exposure were derived. The subchronic osRfD values selected were associated with decreased serum antibody concentrations for developmental immune effects, decreased birth weight for developmental effects, increased relative liver weight for liver effects, decreased whole epididymis weight for male reproductive effects and increased number of days spent in diestrus for female reproductive effects. As discussed earlier, these subchronic osRfDs may be useful for certain decision purposes (i.e., site-specific risk assessments with less-than-lifetime exposures). Confidence in each subchronic osRfD is described in Table 5-15 and this considers confidence in the study used to derive the quantitative estimate, the overall health effect, specific evidence base, and quantitative estimate for each subchronic osRfD.

Table 5-15. Confidence in the subchronic organ/system specific RfDs (subchronic osRfDs) for PFDA

Confidence categories	Designation[a]	Discussion
Immune (developmental) subchronic osRfD = 4×10^{-10} mg/kg-d		
Confidence in study used to derive the subchronic osRfD	*High*	Confidence in Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) was rated as *medium* primarily due to relatively limited PFDA exposure contrasts, which can decrease study sensitivity in general. (HAWC link). Given that the results in this study were statistically significant, EPA concluded that while there were potential study sensitivity concerns at the evaluation stage, the results clearly showed that those concerns were not borne out, and confidence in this study to derive an osRfD was judged to be *high*.
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for immune effects is *medium* based on consistent findings of reduced antibody responses from 2 *medium* confidence birth cohort studies (Grandjean et al., 2012); (Grandjean et al., 2017a); (Grandjean et al., 2017b) and a *low* confidence study in adults (Kielsen et al., 2016). Short-term studies in animals of *high/medium* confidence provide supportive evidence of immunosuppression after PFDA exposure (Frawley et al., 2018); (NTP, 2018). Some residual uncertainties regarding unexplained inconsistency and potential confounding by other co-occurring PFAS from epidemiological studies and issues with concomitant overt target organ and systemic toxicity in animal studies lower confidence in the available evidence for this hazard. Other limitations include the lack of epidemiological studies or long-term/chronic studies in animals examining effects on the immune system across different developmental life stages and

Confidence categories	Designation[a]	Discussion
		immunotoxicity categories, including sensitization and allergic response and autoimmunity and autoimmune disease.
Confidence in the quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium.* The POD is based on BMD modeling at the lower end of the range of the observed data and a $BMDL_{1/2SD}$ estimate that is associated with a small degree of uncertainty due to potential confounding by PFOA (see Appendix D.1.1 for more details). The POD for decreased tetanus antibodies at age 7 yrs was judged to be *medium* confidence based on a good model fit and was supported by the nearly identical POD for decreased diphtheria antibodies at age 7 yrs. Both PODs support the osRfD. A health-protective estimate for human clearance was applied to estimate the POD_{HED} using PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above). There is also uncertainty as to the most sensitive window of vulnerability with respect to the exposure/outcome measurement timing (BMDs/BMDLs were estimated from PFDA levels measured at age 5 or perinatally and anti-tetanus antibody concentrations measured at age 7 or 5); (Grandjean et al., 2017b) reported that *estimated* PFDA "concentrations at 3 mo and 6 mo showed the strongest inverse associations with antibody concentrations at age 5 yrs, particularly for tetanus." Thus, it is possible that adverse effects during infancy could be more sensitive than between ages 5 and 7 yrs.
Overall confidence in subchronic osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is driven by *medium* confidence in the evidence base for immune effects, the quantification of the POD, and the study used for BMD modeling.
Developmental subchronic osRfD = 3×10^{-10} mg/kg-d		
Confidence in study[a] used to derive osRfD	*Medium*	Confidence in the Wikström et al. (2020) study for hazard identification was rated as *high* (HAWC link) for developmental effects. The study was selected for dose-response analysis due to low overall risk of bias and reliable exposure measurements which had sufficient exposure contrasts and other characteristics that allowed for adequate study sensitivity to detect associations. The Wikström et al. (2020) study demonstrated associations consistent in magnitude for boys, girls, and the overall population. Overall, mean birth weight was considered the most precise and accurate endpoint and not anticipated to be subject to much error. This study was advanced for dose-response analysis, given no presumed impact of pregnancy hemodynamics given the early sampling (96% from trimester 1). Wikström et al. (2020) also adjusted for sample timing in their multivariate models and show no differences in models also restricted to trimester 1 samples only. Some uncertainty remains on the potential for confounding by other PFAS (concern primarily for PFNA) which were not examined in this study. Given the potential quantitative impact of this uncertainty, confidence in the use of this study for dose-response analysis was judged as *medium* rather than *high*.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for developmental effects is *medium*. There was consistent evidence for reduced birth weight among multiple human studies, including *high* quality studies. However, unlike the Wikström et al. (2020) study used here and noted above, some uncertainty remains in many studies given the predominance of associations that were detected for studies with later pregnancy sampling. The human database also showed some coherence across different measures of fetal growth restriction. In animals, the lone developmental study reported effects on fetal growth that are coherent with effects observed in humans.

Confidence categories	Designation[a]	Discussion
		Some residual uncertainty regarding potential confounding by other co-occurring PFAS from epidemiological studies lowers confidence in the available evidence for this hazard.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on a BMD hybrid approach within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in osRfD	*Medium-low*	The overall confidence in the osRfD is *medium* and is driven by *medium-low* confidence in the evidence base for developmental effects (i.e., fetal growth restriction).
Liver subchronic osRfD = 7×10^{-7} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in the NTP (2018) study was rated *high* based on good or adequate ratings for most study quality domains (HAWC link) and characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium*	Confidence in the evidence base for liver effects is *medium*. Coherent liver effects for histopathology, serum biomarkers and organ weights were observed across short-term rodent studies (primarily two *high* confidence 28-d studies) that are supported by mechanistic studies of biological plausibility and possible human relevance. Uncertainties remain due to the absence of longer-term toxicity studies (28 d) and limited information from available epidemiological studies and in vivo models to characterize the role of PPARα and other signaling pathways in the mechanisms of hepatotoxicity of PFDA in both humans and animals.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within (at the lower end) the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium*	The overall confidence in the osRfD is *medium* and is primarily driven by *medium* confidence in both the evidence base supporting this hazard and the quantification of the POD using BMD modeling of data from a *high* confidence study.
Male reproductive subchronic osRfD = 5×10^{-6} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High-medium*	Confidence in the NTP (2018) study was rated *high-medium* (HAWC link) since most of male reproductive measures were rated as high, including the basis for the subchronic osRfD (decreased whole epididymis weight), with the exception of sperm measures which suffered from insensitivity due to short-term exposure. This is supported by the study evaluation results (good or adequate ratings for most study quality domains) and characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for male reproductive effects is *medium* to *low*. Coherent effects across several relevant measures, including, sperm parameters, histopathology, serum testosterone levels and organ weights

Confidence categories	Designation[a]	Discussion
		were observed in a *high* confidence 28-d rat study. The findings are supported by coherent evidence from a limited number of epidemiological and mechanistic studies. In spite of the available evidence, some outstanding uncertainties in the database remain, including the absence of longer-term exposure studies (>28 d), developmental or multigenerational studies that evaluate effects in both adults and developing humans and animals. Given these evidence base uncertainties, it is likely that this osRfD is under-protective of all male reproductive effects.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is primarily driven by the *medium-low* confidence in the evidence base. The *high* confidence in the study and *medium* confidence in the quantification of the POD does not fully mitigate the uncertainties associated with *medium-low* confidence in the evidence base.
Female reproductive subchronic osRfD = 3×10^{-6} mg/kg-d		
Confidence in study[a] used to derive osRfD	*High*	Confidence in the NTP (2018) study is *high* (HAWC link) given the study evaluation results (i.e., rating of *good* in all evaluation categories) and characteristics that make it suitable for deriving toxicity values, including the relevance of the exposure paradigm (route, duration, and exposure levels), use of a relevant species, and the study size and design.
Confidence in evidence base supporting this hazard	*Medium-low*	Confidence in the evidence base for female reproductive effects is *medium-low*. There were consistent and coherent effects on uterus weight and the estrous cycle in a single *high* confidence study. Despite the available evidence, limitations of the evidence base for female reproductive effects include the lack of informative human studies and the lack of a subchronic study in animals as well as lack of studies that examined the effect of PFDA on female fertility and pregnancy outcomes in exposed animals. There are also no developmental or multigenerational studies that evaluated effects in both adults and developing humans and animals. Given these evidence base uncertainties, it is likely that this osRfD is under-protective of all female reproductive effects.
Confidence in quantification of the POD_{HED}	*Medium*	Confidence in the quantification of the POD and osRfD is *medium* given the POD was based on BMD modeling within the range of the observed data and dosimetric adjustment was based on PFDA-specific pharmacokinetic information, the latter of which involves some residual uncertainty (see discussion on Uncertainty in the pharmacokinetic modeling of PFDA above).
Overall confidence in the subchronic osRfD	*Medium-low*	The overall confidence in the osRfD is *medium-low* and is primarily driven by the *medium-low* confidence in the evidence base. The *high* confidence in the study and *medium* confidence in the quantification of the POD does not fully mitigate the uncertainties associated with *medium-low* confidence in the evidence base.

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

Selection of Subchronic RfD and Confidence Statement

Organ/system-specific and overall subchronic RfD values for PFDA selected in the previous section are summarized in Table 5-16.

Table 5-16. Organ/system-specific and overall subchronic RfDs for PFDA

System	Toxicity Value	Basis	POD_{HED} (mg/kg-d)	UF_C	osRfD (mg/kg-d)	Confidence
Immune (developmental)	Subchronic osRfD	Decreased serum antibody concentrations for both tetanus and diphtheria in children at age 7 yrs and PFDA measured at age 5 yrs	1.07×10^{-8} based on $BMDL_{½SD}$ from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a)	30	4×10^{-10}	*Medium*
Developmental	Subchronic osRfD	Decreased birth weight in males and females	9.6×10^{-9} based on $BMDL_{5\%RD}$ from Wikström et al. (2020)	30	3×10^{-10}	*Medium-low*
Liver	Subchonic osRfD	Increased liver weight in SD female rats	7.17×10^{-4} based on $BMDL_{10\%RD}$ from NTP (2018)	1,000	7×10^{-7}	*Medium*
Male reproductive	Subchronic osRfD	Decreased absolute whole epididymis weight in SD rats	5.14×10^{-3} based on $BMDL_{1SD}$ from NTP (2018)	1,000	5×10^{-6}	*Medium-low*
Female reproductive	Subchronic osRfD	Increased number of days spent in diestrus in SD rats	2.76×10^{-3} based on $BMDL_{5\%RD}$ from NTP (2018)	1,000	3×10^{-6}	*Medium-low*
Immune/developmental	Overall subchronic RfD	Decreased antibody concentrations for both tetanus and diphtheria in children at age 7 yrs and PFDA measured at age 5 yrs Decreased birth weight in males and females	1.07×10^{-8} based on $BMDL_{½ SD}$ from Grandjean et al. (2012); Budtz-Jørgensen and Grandjean (2018a) 9.6×10^{-9} based on $BMDL_{5\%RD}$ from (Wikström et al., 2020)	30	4×10^{-10}	*Medium*

From the identified subchronic osRfDs (see Table 5-16), an overall **subchronic *RfD of 4 × 10^{-10} mg/kg-day based on decreased serum antibody concentrations and decreased birth weight in humans*** was selected. As described in Table 5-15, confidence in the RfD is *medium*, based on *medium* confidence in the immune osRfD (the developmental osRfD was *medium-low* confidence), noting that there was *medium* confidence in the quantification of the PODs for both immune (Budtz-Jørgensen and Grandjean, 2018a); (Grandjean et al., 2012) and developmental (Wikström et al., 2020) endpoints using BMD modeling. This RfD is considered to be representative of both immune and developmental effects given the close proximity (~1.5-fold) of the developmental and immune PODs and resulting osRfDs and that both critical effects are observed during the developmental period (see Section 5.2 for more details).

As described above, the toxicity value of 4×10^{-10} mg/kg-day for decreased serum antibody concentrations for both diphtheria and tetanus at age 7 and PFDA measured at age 5 was selected for immune effects Budtz-Jørgensen and Grandjean (2018a); Grandjean et al. (2012); and the toxicity value of 3×10^{-10} mg/kg-day based on reduced birth weight from the Wikström et al. (2020) study was selected for developmental effects.

The PODs calculated in Table 5-9 from 28-day studies in rodents were selected for each health effect for the derivation of the candidate subchronic toxicity values based on several considerations, including whether there is an endpoint with less uncertainty and/or greater sensitivity, and whether the endpoint is protective of both sexes and all life stages.

For liver effects, the toxicity value of 7×10^{-7} mg/kg-day (BMDL10RD[HED] of 7.17×10^{-4} mg/kg-day) for increased liver weight in female rats in the NTP (2018) study was selected as the liver osRfD because it is a reliable marker of hepatotoxicity and represents a more sensitive reference value than other liver endpoints considered for dose-response modeling (see Table 5-8). For male reproductive effects, endpoints with a high confidence rating (i.e., increased Leydig cell atrophy, decreased serum testosterone, decreased testis weight, and decreased epididymis weight [whole and cauda]) were prioritized over endpoints which suffered from potential sensitivity issues due to short-term study exposure (i.e., decreased epididymal sperm counts). Since the PODs for the prioritized endpoints were similar (HEDs ranging from 5.14×10^{-3}–1.01×10^{-2}) and consistent with mechanistic evidence that suggest PFDA targets Leydig cells and causes decreased steroidogenesis and androgen deficiency (see section 3.2.4), the most sensitive POD based on a BMDL1SD(HED) of 5.14×10^{-3} mg/kg-day for decreases in whole epididymis weights was selected for derivation, resulting in a subchronic toxicity value of 5×10^{-6} mg/kg-day for male reproductive effects. Lastly, the osRfD of 3×10^{-6} mg/kg-day (BMDL5RD[HED] of 2.76×10^{-3} mg/kg-day) based on increased number of days spent in diestrus was selected for female reproductive effects given its association with infertility as provided by the U.S. EPA's Guidelines for Reproductive Toxicity Risk Assessment. This endpoint is also supported by concomitant decreases in estrus time (BMDL5RD[HED] of 1.77×10^{-3} mg/kg-day), for which the association with infertility is less clear.

The subchronic osRfDs for liver, male reproductive and female reproductive effects derived from short-term animal data were several orders of magnitude higher than the subchronic osRfDs for immune and developmental effects in humans; therefore, they were not considered to be sufficiently protective for consideration in the selection of the overall subchronic RfD. Also, in the case of male and female reproductive effects, confidence in the respective osRfDs was lower compared to the immune osRfD (medium-low versus medium) due to deficiencies in the evidence base for these health effects.

5.2.4. Inhalation Reference Concentration (RfC) Derivation

No studies examining inhalation effects of short-term, subchronic, chronic or gestational exposure for PFDA in humans or animals have been identified, precluding the derivation of an RfC.

5.3. CANCER TOXICITY VALUES

Considering the limitations in the evidence base across human, animal, and mechanistic studies of PFDA (see Section 3.3) and in accordance with the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 2005), EPA concluded that the evidence is ***inadequate to assess carcinogenic potential*** of PFDA in humans. The lack of adequate carcinogenicity data for PFDA precludes the derivation of quantitative estimates of either oral (oral slope factor, OSF) or inhalation (inhalation unit risk; IUR) exposure.

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